TECAN.

Instructions for Use – Reference Guide

SPARK

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30246429 03

WARNING: Carefully read and follow the instructions provided in this document before operating the instrument.

Notice

Every effort has been made to avoid errors in text and diagrams; however, Tecan Austria GmbH assumes no responsibility for any errors, which may appear in this publication.

It is the policy of Tecan Austria GmbH to improve products as new techniques and components become available. Tecan Austria GmbH therefore reserves the right to change specifications at any time with appropriate validation, verification, and approvals.

We would appreciate any comments on this publication.

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Copyright Information

CE Declaration of Conformity

See the last page of these Instructions for Use.

Area of Application – Intended Use

See chapter 2.2 Intended Use (Hardware and Software).

About the Instructions for Use

Original Instructions. This document describes the SPARK multifunctional microplate reader. It is intended as reference and instructions for use. This document describes how to:

- Install the instrument
- Operate the instrument
- Clean and maintain the instrument

Remarks on Screenshots

The version number displayed in screenshots may not always be the one of the currently released version. Screenshots are replaced only if content related to the application has changed.

Trademarks

The following trademarks are either trademarks or registered trademarks in major countries of Tecan Group Ltd., Männedorf, Switzerland:

- Spark®
- Spark® Cyto
- SparkControl™
- Spark-Stack™
- NanoQuant Plate™
- Image Analyzer™
- Te-Cool™
- Tecan®
- TECAN - Logo®

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Warnings, Cautions, and Notes

The following types of notices are used in this publication to highlight important information or to warn the user of a potentially dangerous situation:

NOTE: Gives helpful information.

CAUTION: Indicates a possibility of instrument damage or data loss if instructions are not followed.

WARNING: Indicates the possibility of severe personal injury, loss of life or equipment damage if the instructions are not followed.

WARNING: This symbol indicates the possible presence of biologically hazardous material. Proper laboratory safety precautions must be observed.

WARNING: This symbol indicates the possible presence of flammable materials and a risk of fire. Proper laboratory safety precautions must be observed.

WARNING: Negative environmental impacts associated with the treatment of waste (WEEE).

- Do not treat electrical and electronic equipment as unsorted municipal waste.
- Collect waste electrical and electronic equipment separately.

WARNING: Indicates laser. Do not stare into the beam!

FOR CALIFORNIA RESIDENTS ONLY:

WARNING: This product can expose you to chemicals such as lead which is known to the State of California to cause cancer and birth defects or other reproductive harm.
For more information go to: www.P65warnings.ca.gov/product.

Symbols

	CE conformity marking
	United Kingdom Conformity Assessed marking shows that the labeled product is following the applicable regulation in Great Britain.
	Date of manufacture
	Manufacturer
	Catalogue number
	Consult Instructions for Use
	China RoHS symbol
	Serial number
	Single use only
	TÜV SÜD Mark
	USB symbol
	Use by date
	WEEE symbol

Table of Contents

1 Safety

1.1 Introduction

- Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.
- Read and understand all information in the Instructions for Use. Failure to read, understand, and follow the instructions in this document may result in damage to the product, injury to operating personnel or poor instrument performance.
- Observe all WARNING and CAUTION statements in this document.
- Never open the instrument while it is plugged into a power source.
- Never force a microplate into the instrument.
- Observe proper laboratory safety precautions, such as wearing protective clothing (gloves, lab coat, safety glasses, etc.) and using approved laboratory safety procedures.

CAUTION: To ensure the optimal operation of the SPARK, an annual maintenance procedure must be performed by a Tecan service engineer.

WARNING: Read and understand all information in the Instructions for Use. Failure to read, understand, and follow the instructions in this document may result in damage to the product, injury to operating personnel or poor instrument performance.

It is assumed that the instrument operators, because of their vocational experience, are familiar with the necessary safety precautions for handling chemicals and biohazardous substances.

Adhere to the following laws and guidelines:

- National industrial protection law
- Accident prevention regulations
- Safety data sheets of the reagent manufacturers

WARNING: Depending on the applications, parts of the instrument may come in contact with biohazardous/infectious material. Make sure that only qualified personnel operate the instrument. In case of service or when relocating or disposing of the instrument, always disinfect the instrument according to the instructions given in this manual.

WARNING: Do not open the instrument! Only Tecan authorized service technicians are allowed to open the instrument. Removing or breaking the warranty seal voids the warranty.

2 General Description

2.1 Instrument

The SPARK is a multifunctional microplate reader platform and is robotic compatible.

2.2 Intended Use (Hardware and Software)

The SPARK microplate multimode reader with a modular design is intended for use in research laboratories. Depending on the configuration, the instrument is intended for the measurement and data analysis of absorbance, fluorescence, time resolved fluorescence, fluorescence polarization and luminescence of biological and non-biological samples, as well as for the acquisition and analysis of bright field and fluorescence images.

Additionally, the reader is suited for both endpoint and kinetic measurements with either single or multi-labels. The SPARK is equipped with SparkControl software for reader control and data reduction.

Users must evaluate this instrument and any associated data reduction packages with their specific assays to ensure specified performance characteristics of the assay are met. The performance characteristics of the reader have not been validated for specific assays.

The SPARK multimode reader is for research use only.

CAUTION: A system validation by the operating authority is required. It is the responsibility of any operating authority to ensure that the SPARK has been validated for every specific assay used on the instrument.

2.3 User Profile

2.3.1 Professional User – Administrator Level

The administrator is a person who has suitable technical training and corresponding skills and experiences. If the product is used as intended, the person is able to recognize and avoid dangers.

The administrator has extensive skills and is able to instruct the end user or the routine user in assay protocols in connection with a Tecan product within the bounds of the intended use.

Computer application skills and good English skills are required.

2.3.2 End User or Routine User

The end user or routine user is a person who has suitable technical training and corresponding skills and experiences. If the product is used as intended, the person is able to recognize and avoid dangers.

Computer application skills and good language skills in the respective national language at the installation site and English are required.

2.3.3 Service Technician

The service technician is a person who has suitable technical training and corresponding skills and experiences. If the product needs to be serviced or maintained, the person is able to recognize and avoid dangers.

Computer application skills and good English skills are required.

NOTE: Training dates, their duration and frequency are available at your customer support. Address and phone number can be found on the web: http://www.tecan.com/customersupport

2.4 Multifunctionality

The fully equipped SPARK is able to perform the following measurement techniques (for detailed information, see chapter 5 SPARK Platform).

- Absorbance
- Absorbance Scan
- Absorbance Cuvette
- Absorbance Scan Cuvette
- Fluorescence Intensity Top (FRET)
- Fluorescence Intensity Bottom
- Time Resolved Fluorescence (TRF, TR- FRET)
- Fluorescence Scan
- Fluorescence Polarization
- Inject and Read (Injection incl. Fluorescence Intensity Bottom)
- Luminescence (Glow Type, Flash Type and Multicolor)
- Luminescence Scan
- Alpha Technology
- Bright field Imaging (Cell Counting, Cell Confluence) or
- Fluorescence Imaging (CYTO configurations)

The instrument can be equipped with up to two injectors, a heater/stirrer, and a microplate stacker. Special functionalities (such as cell counting, gas supply and lid lifting, temperature control - heating and cooling - and humidity control) support cell based studies in particular.

2.4.1 SPARK CYTO Configurations

All instruments equipped with Fluorescence Imaging are denoted as SPARK CYTO and are available in four different configurations designed for the needs of various customers from academia to biopharma:

SPARK CYTO 100	SPARK CYTO 300	SPARK CYTO 400	SPARK CYTO 500	SPARK CYTO 600
	Absorbance (Standard)	Absorbance (Standard)	Absorbance (Enhanced)	Absorbance (Enhanced)
	Absorbance Scan	Absorbance Scan	Absorbance Scan	Absorbance Scan
Fluorescence Imaging	Fluorescence Intensity Top (Standard, Filter)	Fluorescence Intensity Top (Enhanced, Monochromator)	Fluorescence Intensity Top (Enhanced, Filter)	Fluorescence Intensity Top (Enhanced, Fusion Optics)
	Fluorescence Intensity Bottom (Standard, Filter)	Fluorescence Intensity Bottom (Enhanced, Monochromator)	Fluorescence Intensity Bottom (Enhanced, Filter)	Fluorescence Intensity Bottom (Enhanced, Fusion Optics)
		Fluorescence Intensity Scan		Fluorescence Intensity Scan
	TRF and TR-FRET (Filter)	TRF and TR-FRET (Monochromator)	TRF and TR-FRET (Filter)	TRF and TR-FRET (Enhanced, Fusion Optics)
		Fluorescence Polarization	Fluorescence Polarization	Fluorescence Polarization
	Luminescence (Standard, Multicolor)	Luminescence (Standard, Multicolor)	Luminescence (Enhanced, Multicolor)	Luminescence (Enhanced, Multicolor)
	Luminescence Scan	Luminescence Scan	Luminescence Scan	Luminescence Scan
				Alpha Technology

The characteristics of the module options given in the table above are described in chapter 5 SPARK Platform.

All CYTO configurations come with environmental control:

- Temperature Control (up to 42 °C)
- CO_2 and O_2 Control
- Integrated Lid Lifter

Additionally, the following optional functionalities are available for all CYTO configurations:

- Injectors
- Stacker
- Humidity Cassettes

CAUTION: SPARK CYTO is equipped with an internal USB drive which contains instrument specific calibration data for the optimal quality of fluorescence images. This drive is visible in the File Explorer under the name "USB DISK" or “SPARK CYTO”. Do not eject or modify it to avoid a potential loss of functionality for SparkControl versions 4.0 or higher.

2.5 Microplate Requirements

Any common microplate ranging from 1 to 384-/1536-well formats compliant to the following ANSI/SBS standards can be measured with any of the above measurement techniques.

- ANSI/SBS 1-2004 (footprint dimensions)
- ANSI/SBS 2-2004 (height dimensions)
- ANSI/SBS 3-2004 (bottom outside flange dimensions)
- ANSI/SBS 4-2004 (well positions)

The SPARK supports microplates up to 384 wells; advanced modules support microplates up to 1536 wells.

Exceptions are as follows:

- Performing a Cell Counting measurement, using the Cell Module, requires cells in suspension in disposable Cell Chips.
- A Cell Confluence measurement, using the Cell Module, can be performed in the following plate formats and flasks: 6- to 96-well transparent microplates, 96- well black with transparent bottom microplates and RoboFlask. Additionally, a valid bottom-thickness must be defined in the Plate Definition Files.
- Fluorescence Imaging must be performed only in the following plate formats: 6- to 384-well transparent, 96- to 384-well black with transparent bottom. Additionally, a valid bottom-thickness must be defined in the Plate Definition Files.

The supported range of plate heights is 10 mm (without lid) up to 24.5 mm (including lid). For bottom measurements, the elevation of the bottom of the well relative to the supporting plate rim must not be larger than 5.5 mm.

In addition to the above mentioned microplate formats, cuvettes in an adapter, the Tecan NanoQuant Plate, the Tecan MultiCheck plate and the Tecan Adapter for Cell Chips can be used with limitations for selected measurement techniques.

CAUTION: Tecan Austria GmbH has taken great care when creating the Plate Definition Files (.pdfx) that are delivered with the instrument.

Tecan Austria has taken every precaution to ensure that plate heights and well depths are correct according to the defined plate type. These parameters are used to determine the minimum distance between the top of the plate and the ceiling of the measurement chamber. Additionally, Tecan Austria adds a very small safety gap to prevent any damage that may occur to the measurement chamber as a result of small changes in plate height. This has no effect on the performance of the instrument.

Make sure that the selected plate definition file corresponds to the currently used microplate, so that the safety gap is correctly calculated, otherwise the instrument could become damaged.

NOTE: For instruments with the Spark-Stack module, additional microplate requirements apply, see chapter 14.2 Microplate Requirements for the Spark-Stack.

2.5.1 Filling Volumes/Smooth Mode

CAUTION: The following microplates can be processed only with the subsequent filling volumes:

- 1-well plates: <= 15000 µl
- 4-well plates: <= 4500 µl
- 6-well plates: <= 2000 µl
- 12-well plates: <= 1200 µl
- 24-well plates: <= 1000 µl
- 48-well plates: <= 400 µl
- 96-well plates: <= 200 µl
- 384-well plates: <= 100 µl
- 1536-well plates: <= 10 µl

Larger filling volumes can lead to an overflow of liquids, which can result in cross-contamination. Additionally, the spillover can cause damage to the device (e.g. contamination of the optics and the centering clamp).

If the working volume in the plate definition file (pdfx) is smaller than the above defined volumes the smaller filling volumes must be used to avoid spilling (e.g. Corning 384-well plates have a working volume of only 80 µl).

For fluids that have a lower viscosity than aqueous solutions, the filling volume should additionally be optimized during method validation.

Smooth mode slows down the plate transport movements. **Smooth mode** is activated by selecting the appropriate checkbox in the **Plate** strip. Larger filling volumes than the ones defined above may be possible when **Smooth mode** is selected; however, the maximum filling volumes for each plate type and application must be optimized during method validation.

CAUTION: The maximum filling volumes for each plate type and application must be optimized even if **Smooth mode** is used.

Smooth mode is selected by default if a plate format with less than 96-wells is selected in the measurement method. **Smooth mode** is not available when using the onboard **Retract/Eject** button to move the plate in/out.

CAUTION: Smooth mode is not available when using the onboard **Retract/Eject** button to move the plate in/out.

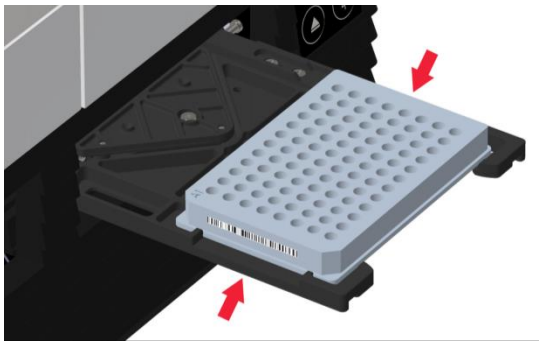
NOTE: The Filling Volumes/Smooth Mode parameters listed above also apply to microplates suitable for use with the Spark-Stack module, e.g. 6- to 1536-well formats (see chapter 14.2 Microplate Requirements for the Spark-Stack).

2.5.2 Microplates with Barcode

The SPARK multimode reader may be optionally equipped with a barcode reader mounted on the left or right side of the plate transport. For example, for a 96-well microplate, apply the barcode on the left (A) or right (H) side of the microplate (see picture below), depending on which side the barcode reader is mounted.

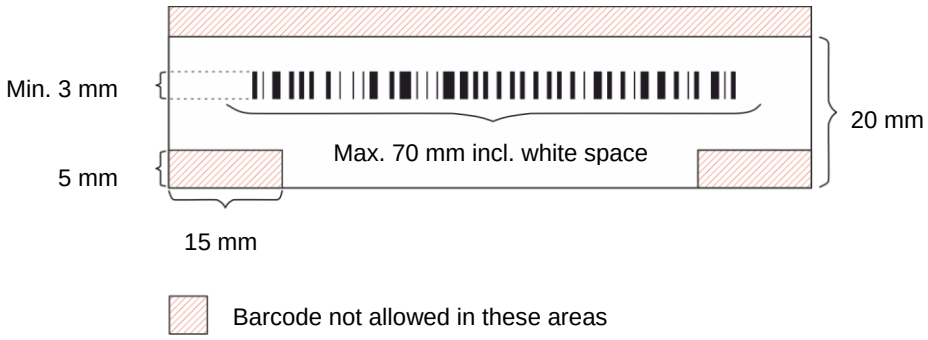
The minimum height of the barcode is 3 mm. At the start and end of the barcode, 2 mm of white space is required. The maximum length of the barcode is 70 mm including the white space at each end. The barcode must be mounted on the short side of the microplate with a minimum distance of 15 mm from the front as well as the back edge and 5 mm above the lower edge of the microplate.

Microplate on the plate carrier:



Apply the barcode on the left or right side of the microplate.

Side view of the microplate:



CAUTION: Yellowed, dirty, folded, wet, or damaged barcode labels must not be used. The adhesive labels must be flat and without peeled edges. We recommend assuring the quality of the barcodes, by means of a local Standard Operating Procedure (SOP).

CAUTION: The barcode is not readable when hidden by the plate lid.

The specified barcode types are:

- CODE 39
- UPC A
- UPC E
- EAN 8
- EAN 13
- CODE 128
- CODE 2/5 Interleaved
- CODABAR
- CODE 93

2.6 Onboard Control Buttons

The SPARK has onboard control buttons to simplify some common tasks.

	An **On/Off** button is available on the front to easily switch the instrument on and off.
	The **Onboard-Start** button is used to start favorite SparkControl Methods directly from the instrument. It can also be used to stop a measurement, confirm user-defined user interventions, and to continue kinetic measurements already paused via the software.
	The **Retract/Eject** button allows microplates to be inserted or removed from the instrument without software activation.
	The **Eject Filter** button is used to move out the filter slides. The filter slides are moved in automatically at insertion.

NOTE: For functionality of onboard Control Buttons in combination with the installed microplate stacker module, see chapter 14 Spark-Stack Microplate Stacker Module.

2.7 Instrument LEDs

The SPARK is equipped with multi-color LEDs to optically signal the operation/activity state of the instrument. The table below gives an overview of possible signals that define which functionalities (Onboard Control buttons) are available at which instrument state.

Led Status	Instrument State	Onboard Control Buttons		
		Retract/ Eject	Eject Filter	Onboard-Start
-	OFF	O	O	O
-	STANDBY (5V)	O	O	O
BLUE	IDLE (not connected to SparkControl)	X	X	X
MAGENTA	IDLE (connected to SparkControl)	X	X	X
GREEN	RUN	O	O	X
RED BLINKING	ERROR	O	O	O
YELLOW BLINKING	USER INTERACTION	X	O	X
GREEN BLINKING	PAUSE	X	O	X
5x CYAN BLINKING	ACTION NOT POSSIBLE	O	O	O

Table of LED states and functionalities.
O = function not available.
X = function available.

2.8 Rear View

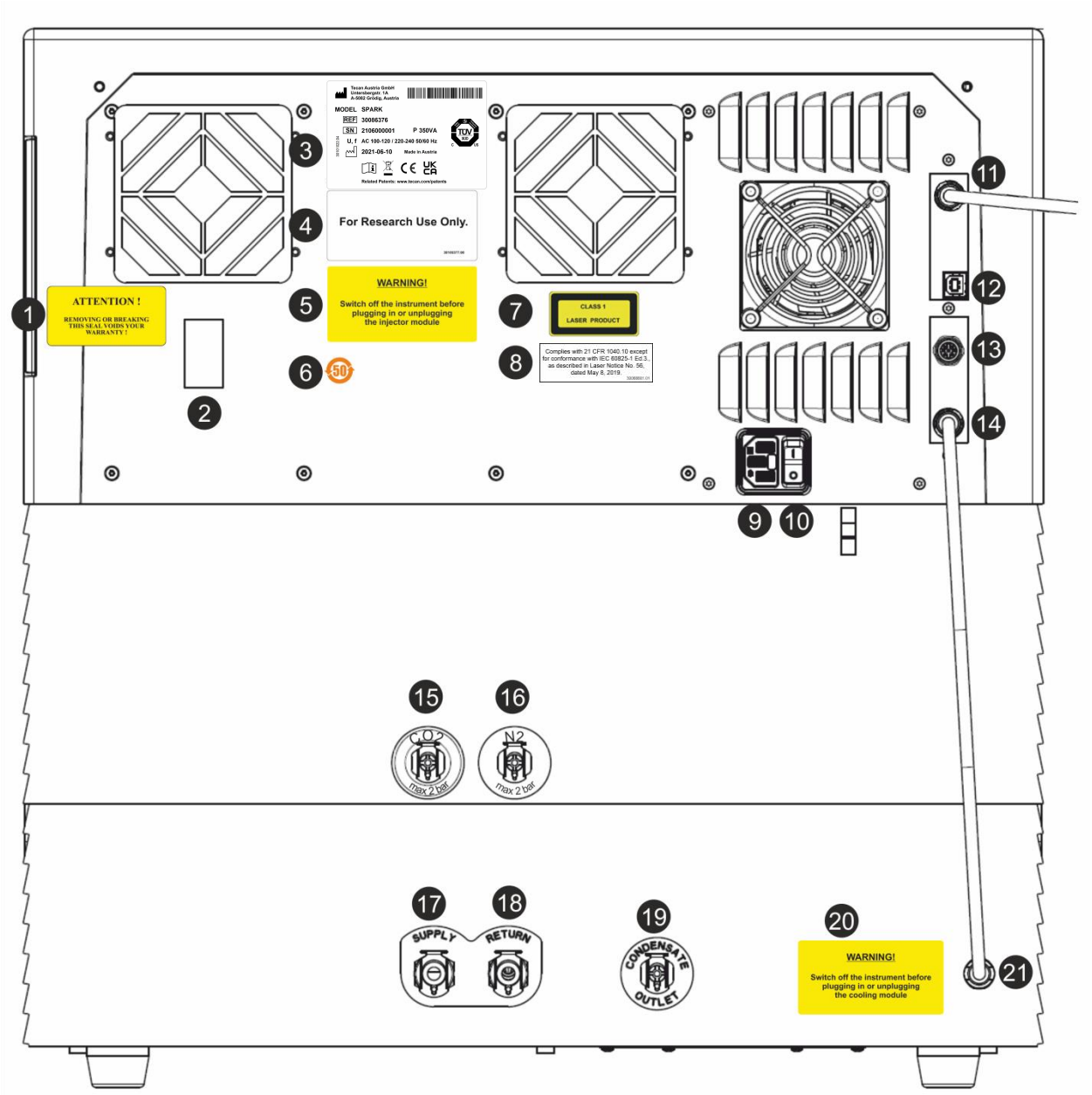
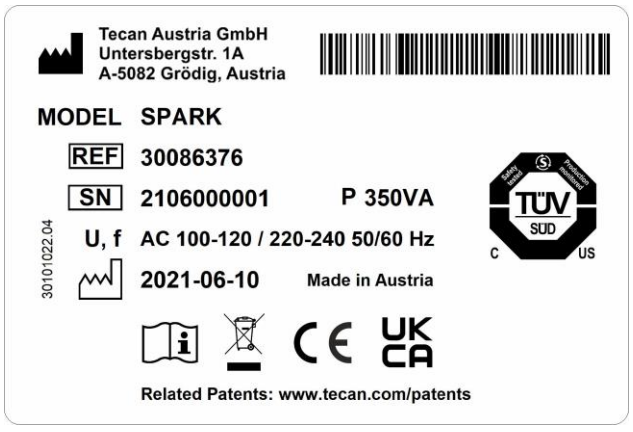


Figure 1: Rear view of the instrument

NOTE: This figure is only an example. The labels on the instrument depend on options installed and destination country.

1	Warranty Label: ATTENTION ! REMOVING OR BREAKING THIS SEAL VOIDS YOUR WARRANTY! (also on bottom of instrument)
2	Temperature sensor cover
3	Name Plate (example)
4	Label: For Research Use Only.
5	Label: WARNING! Switch off the instrument before plugging in or unplugging the injector module
6	Label: China RoHS symbol
7	Label: Class 1 Laser Product
8	Label: Complies with 21 CFR 1040.10 except for conformance with IEC 60825-1 Ed.3, as described in Laser Notice No. 56, dated May 8, 2019.
9	Main Power Socket
10	Main Power Switch
11	USB 3.0 connection for camera
12	USB connection
13	Injector connection
14	CAN Cable to integrated cooling module (Te-Cool)
15	CO_2 connection (max 2 bar)
16	N_2 connection (max 2 bar)
17	Supply: liquid cooling
18	Return: liquid cooling
19	Condensate outlet
20	Label: WARNING! Switch off the instrument before plugging in or unplugging the cooling module
21	CAN Cable to Instrument

Example Name Plate

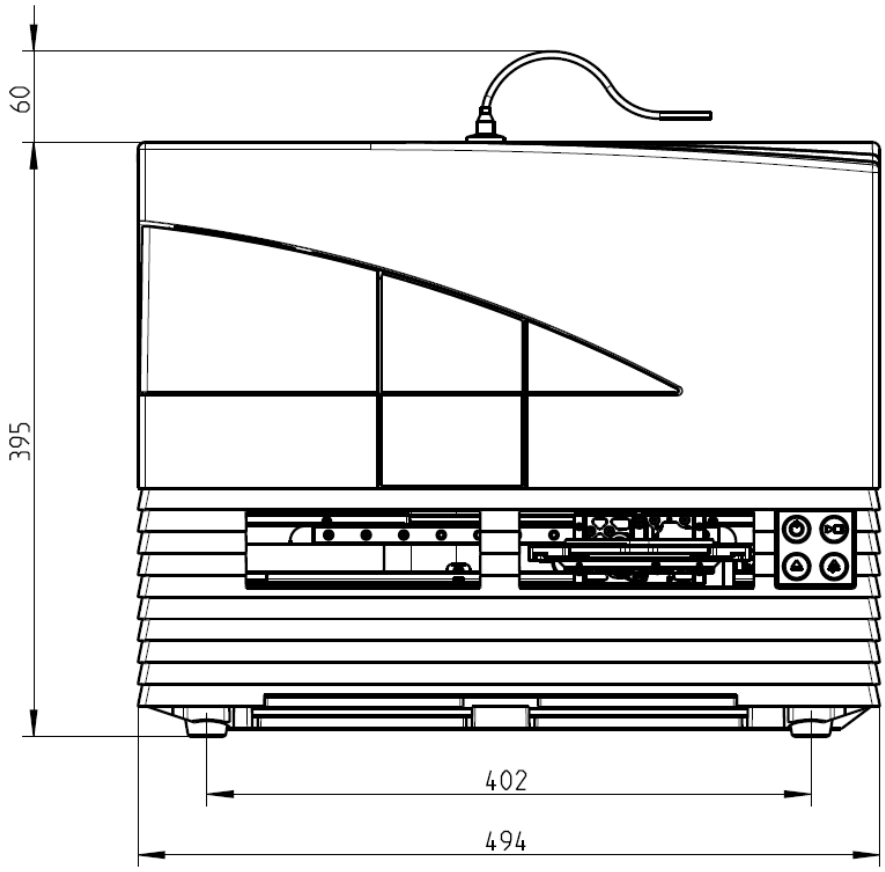


Contents of the name plate (e.g. model name and article number) may vary depending on the specific model.

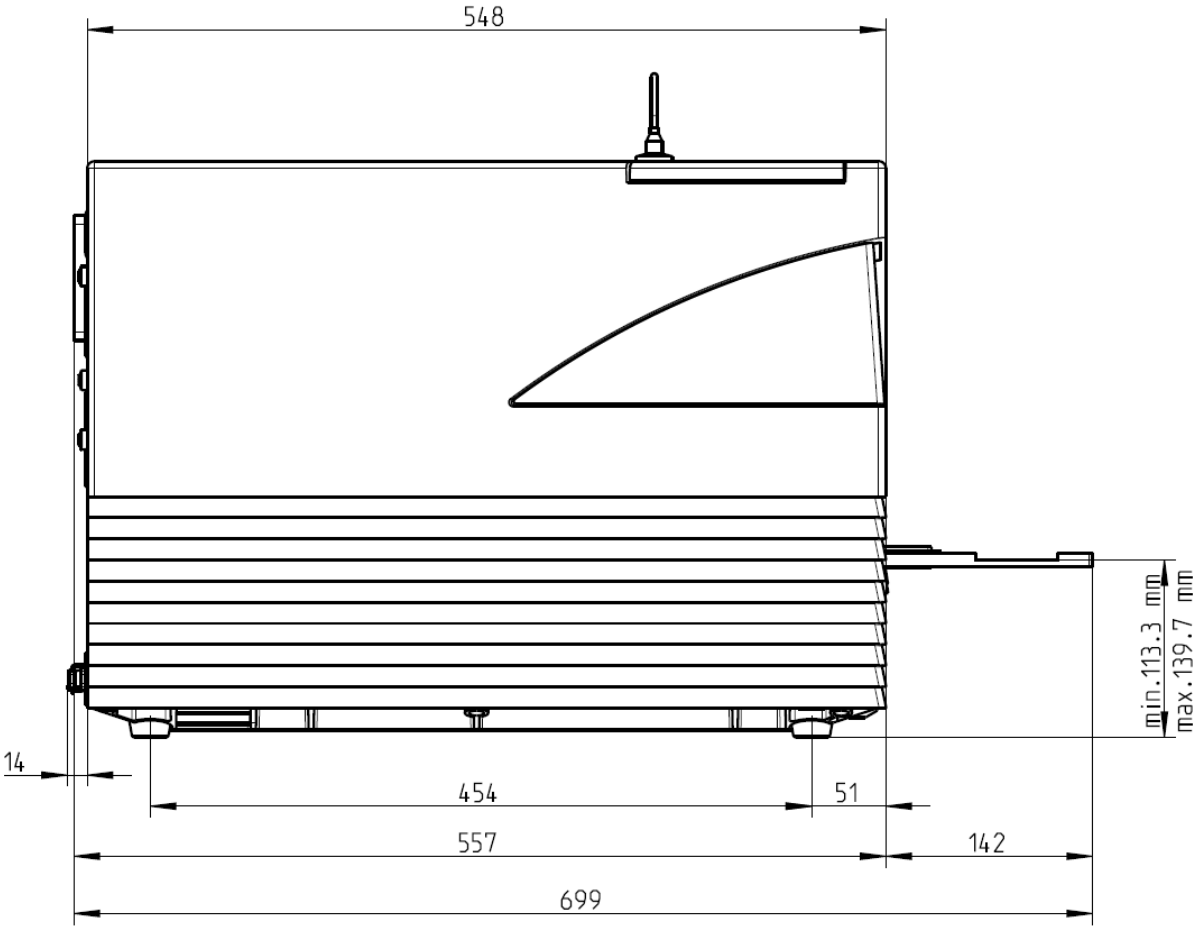
2.9 Instrument Dimensions

All dimensions are given in millimeters.

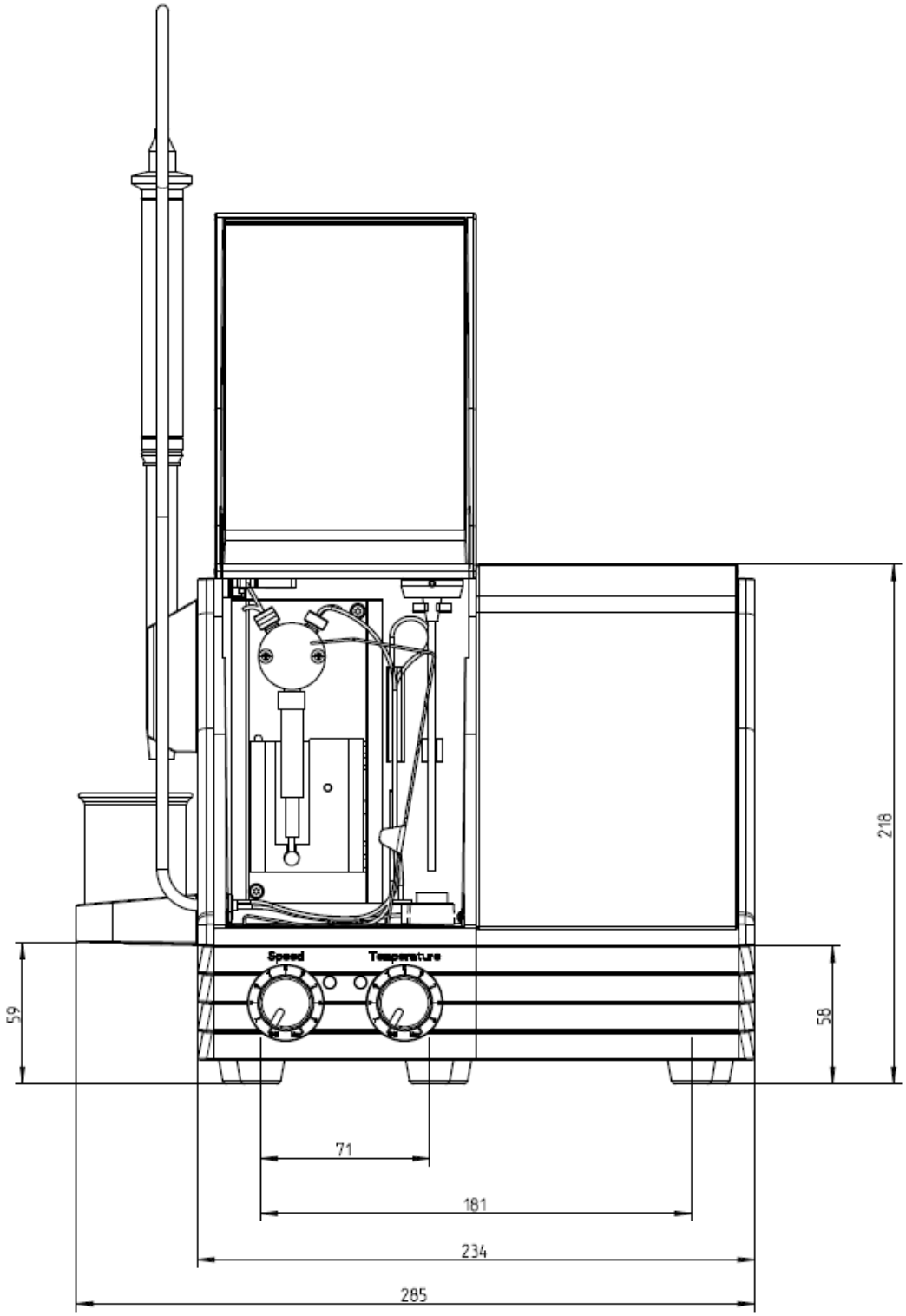
Instrument - Front View



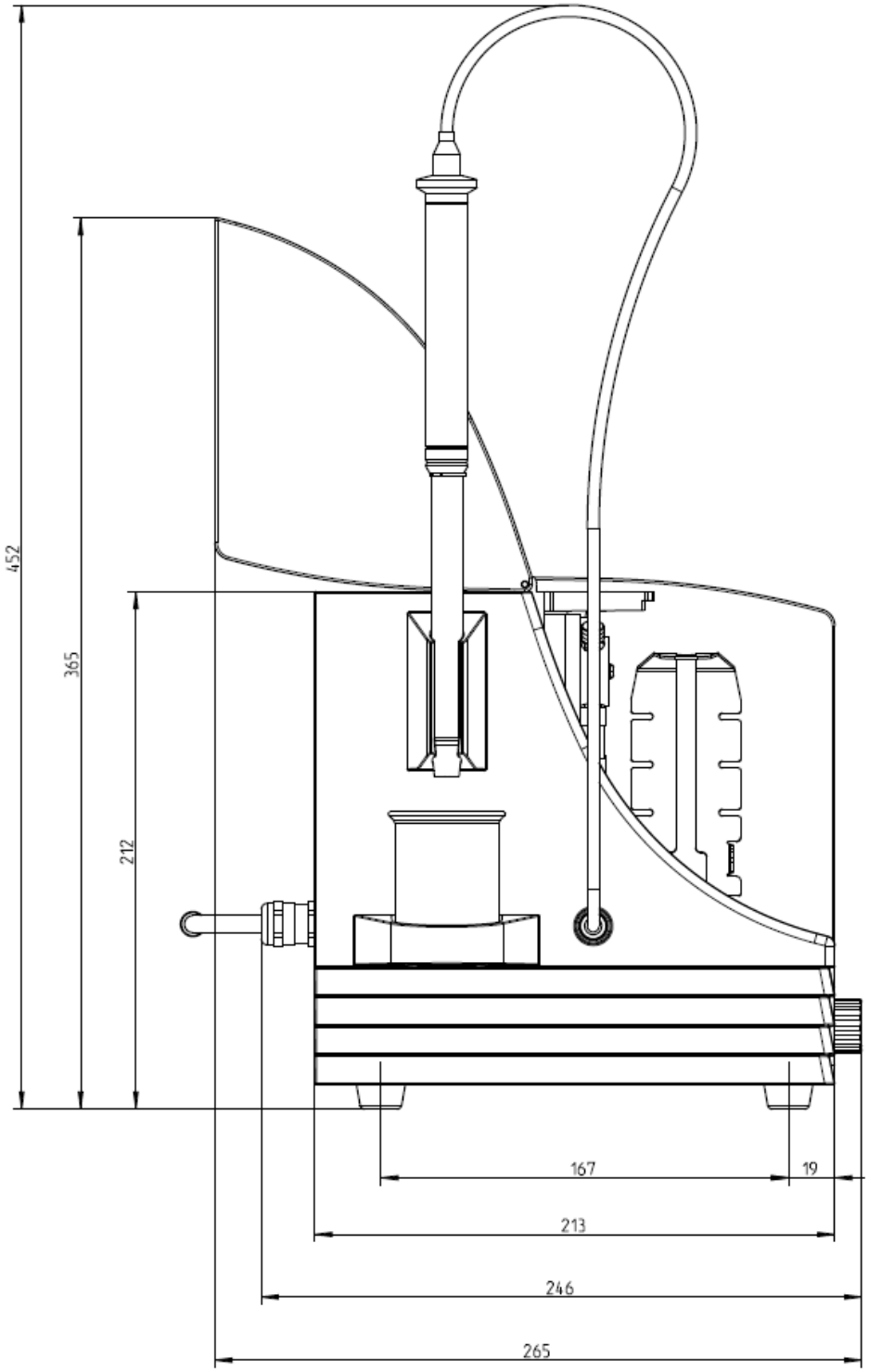
Instrument - Side View



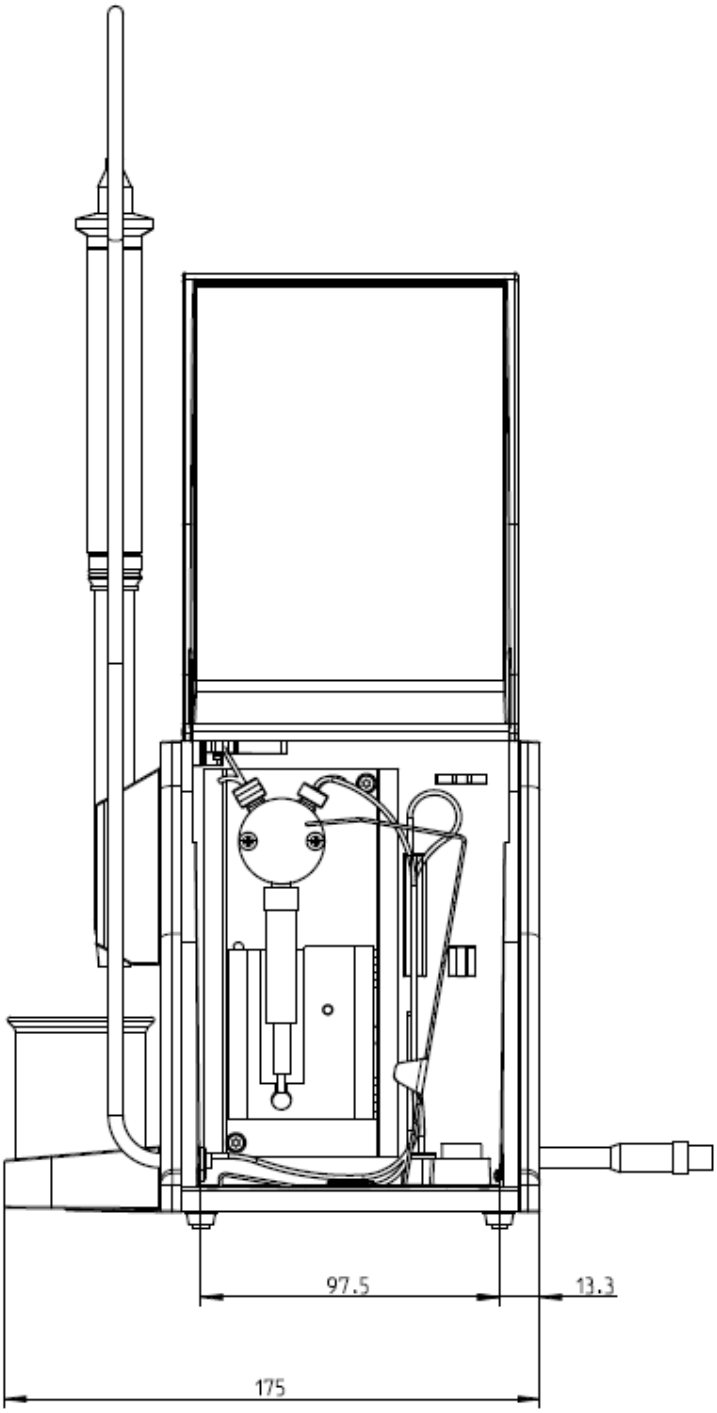
Two Syringe Injector with Heater Stirrer - Front View



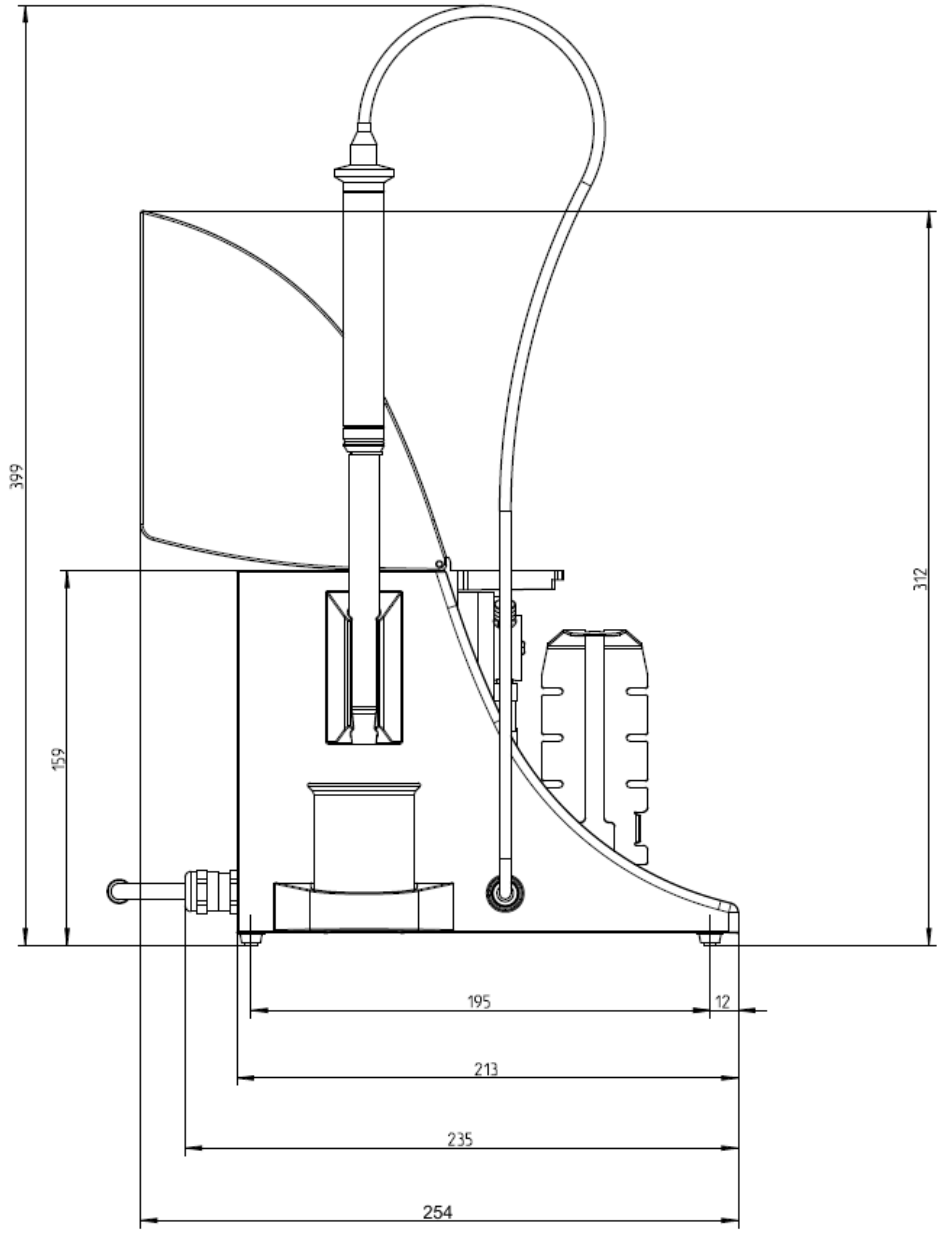
Two Syringe Injector with Heater Stirrer - Side View



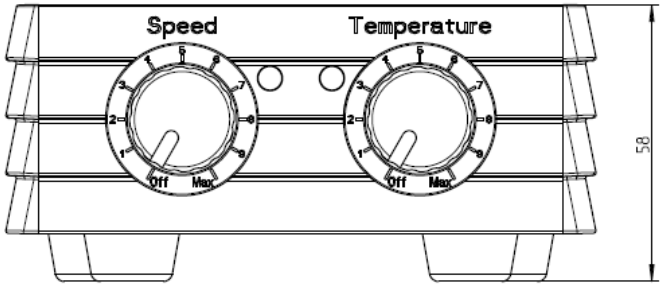
Injector Box - Front View



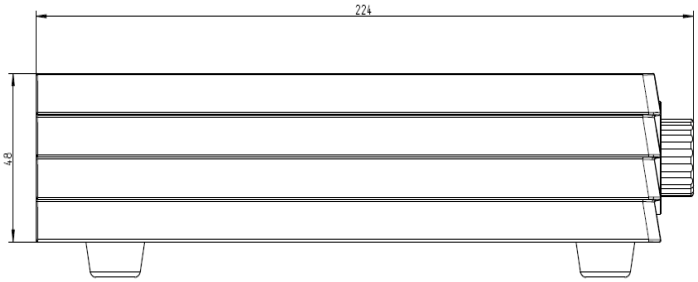
Injector Box - Side View



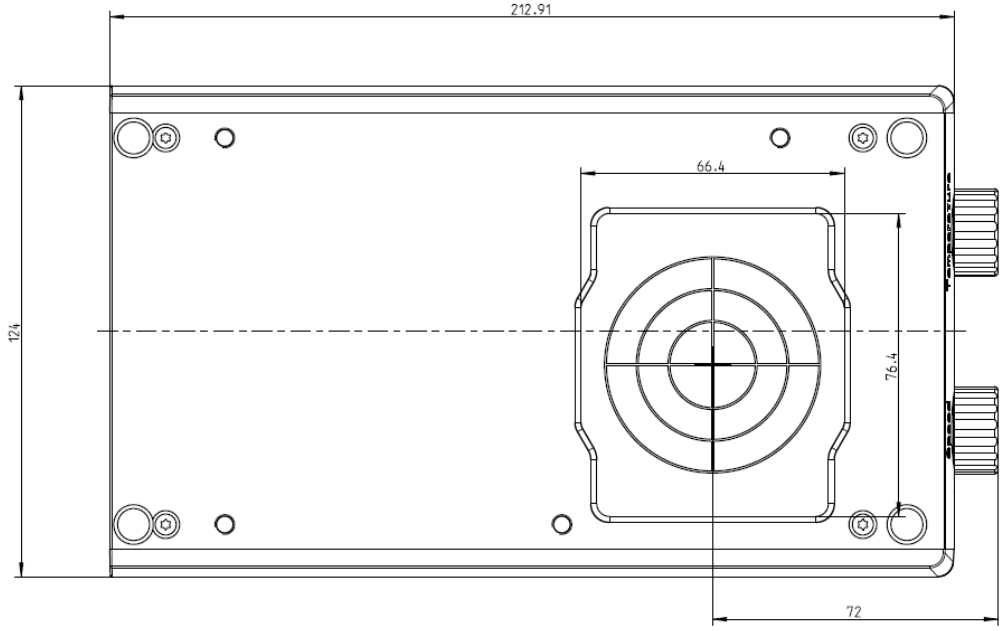
Heater/Stirrer - Front View



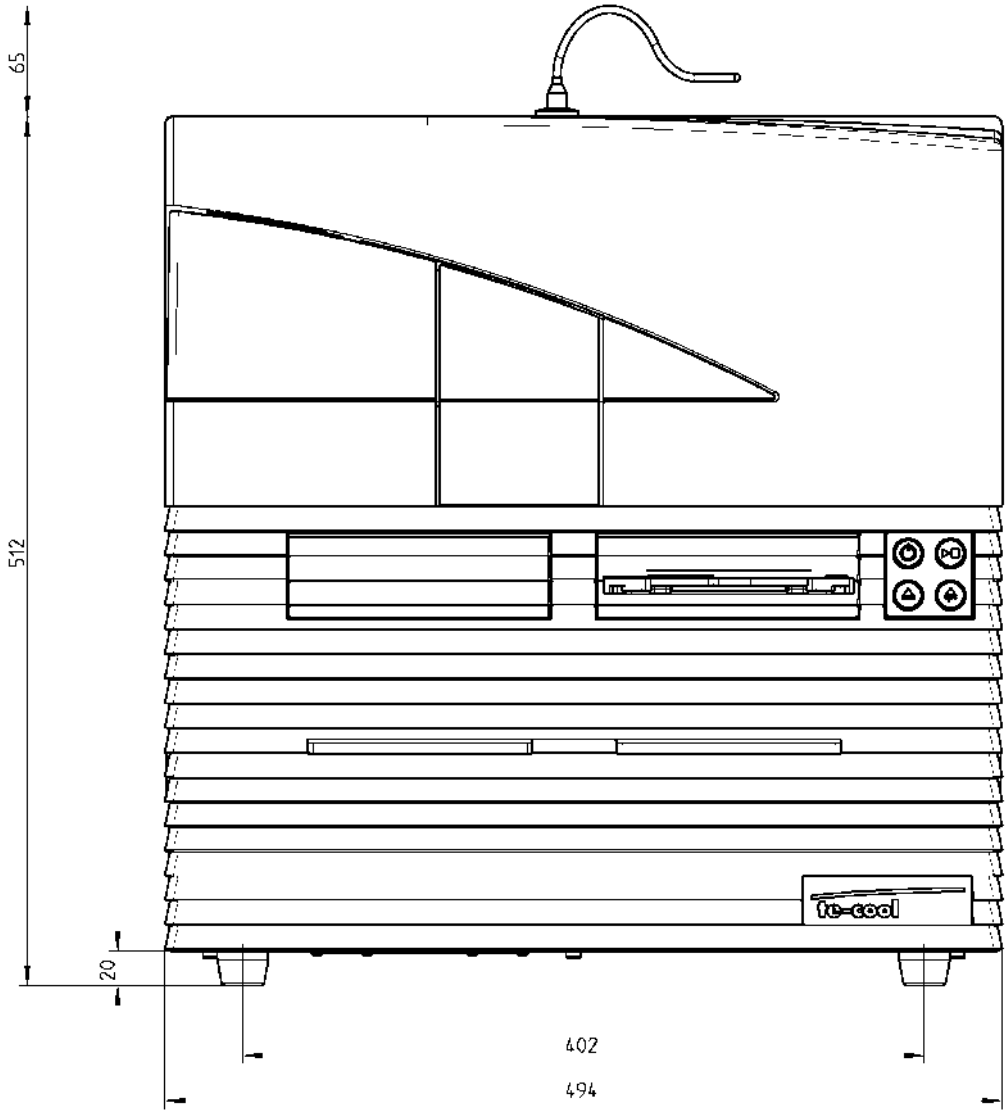
Heater/Stirrer - Side View



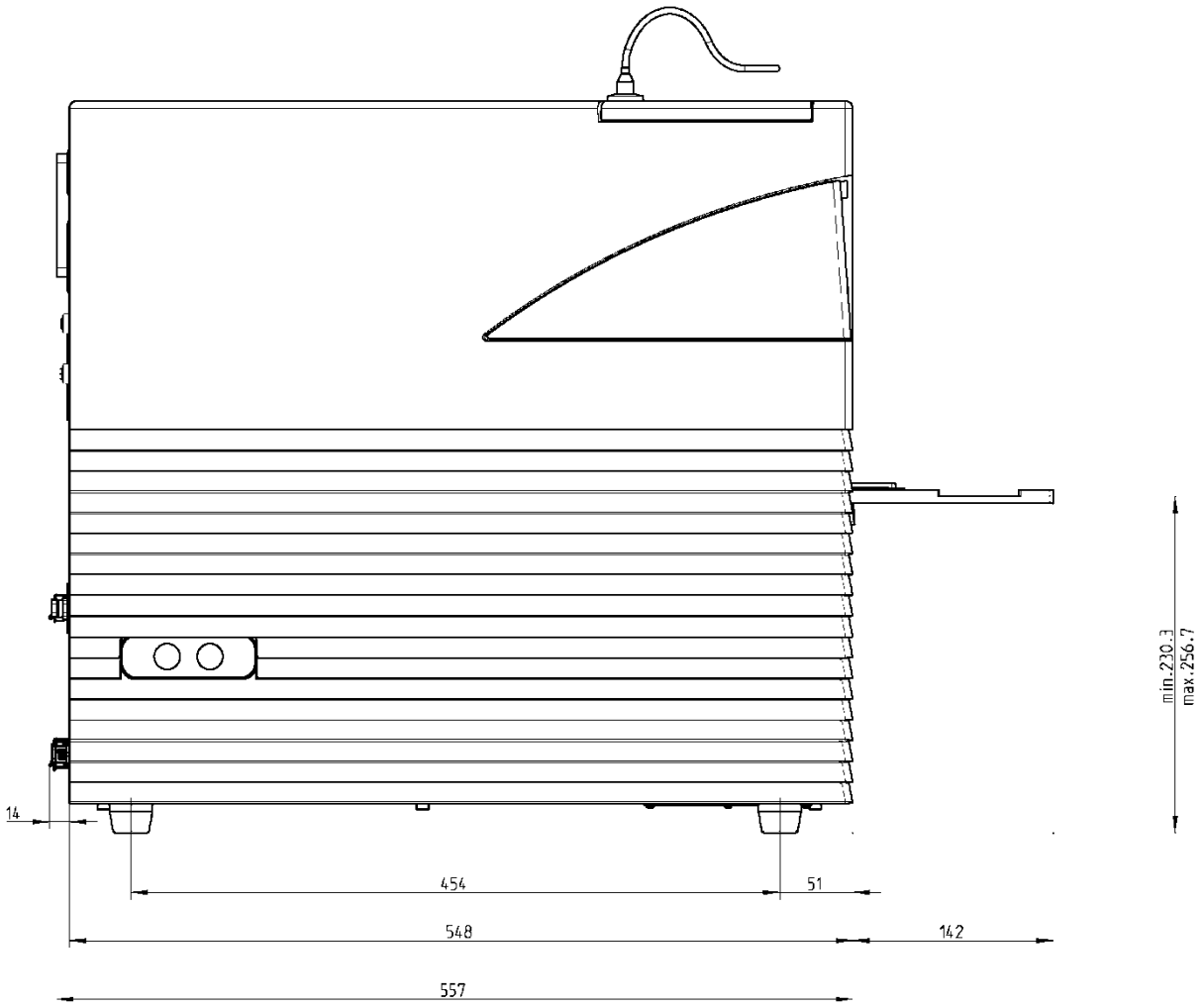
Heater/Stirrer - Top View



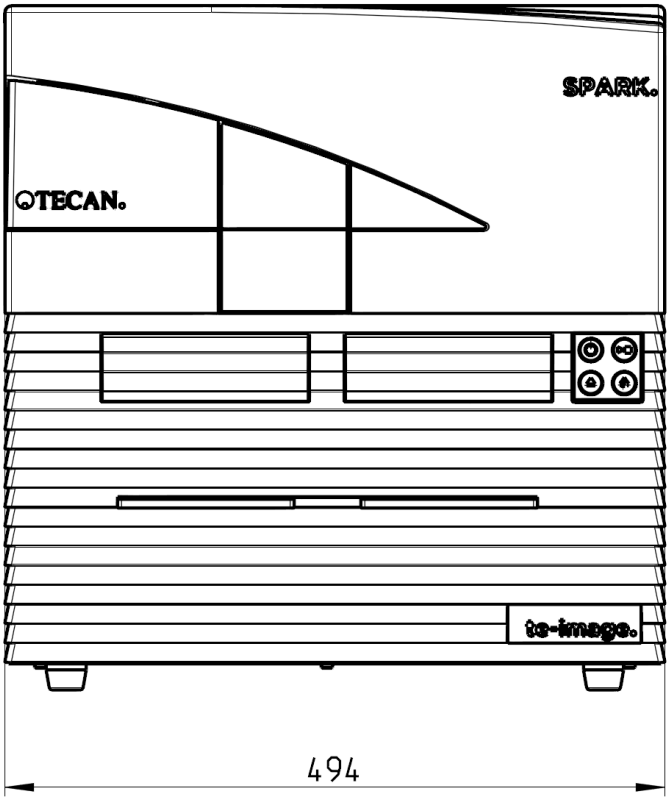
SPARK with Te-Cool - Front View



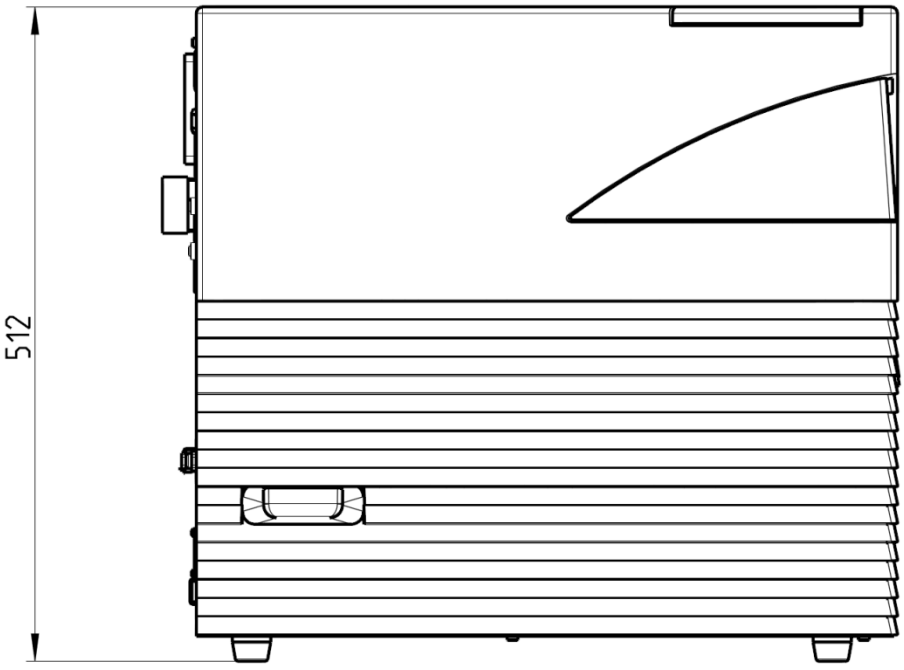
SPARK with Te-Cool - Side View



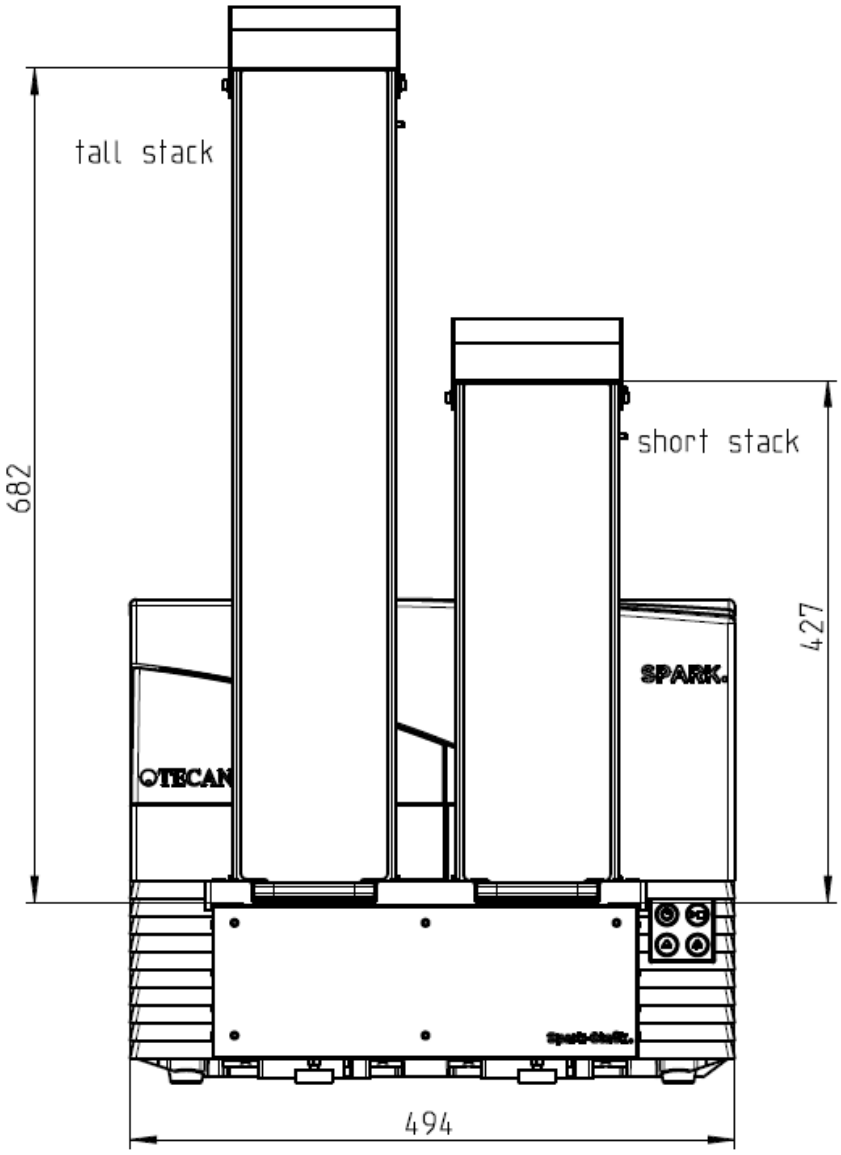
SPARK with Cell Imager Front View



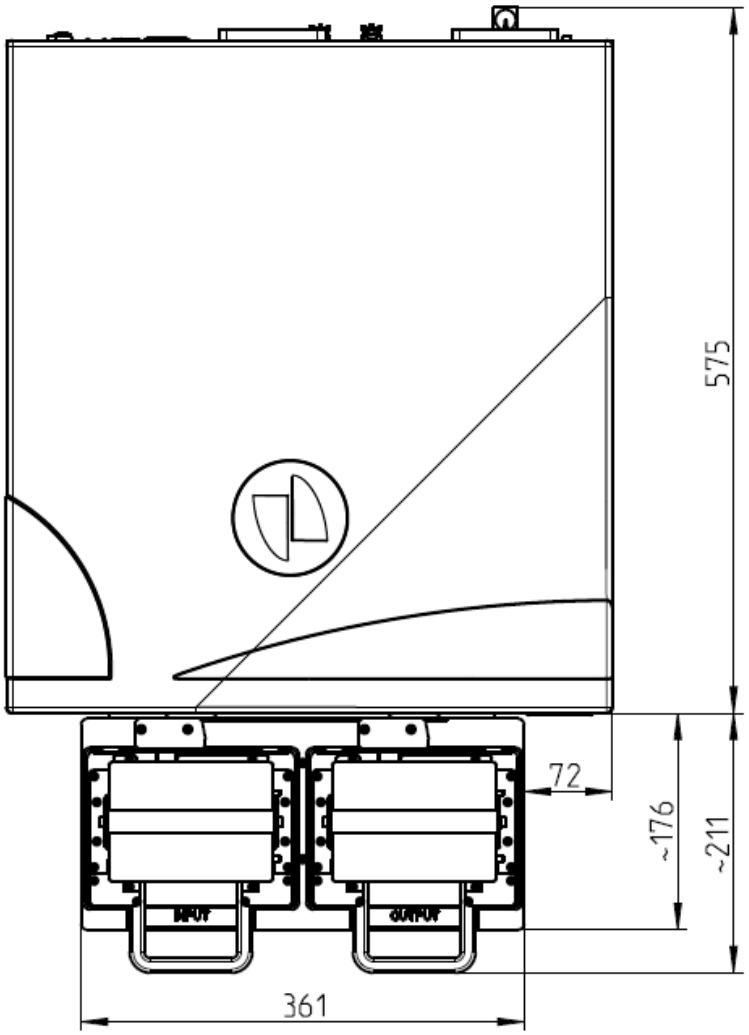
SPARK with Cell Imager Side View



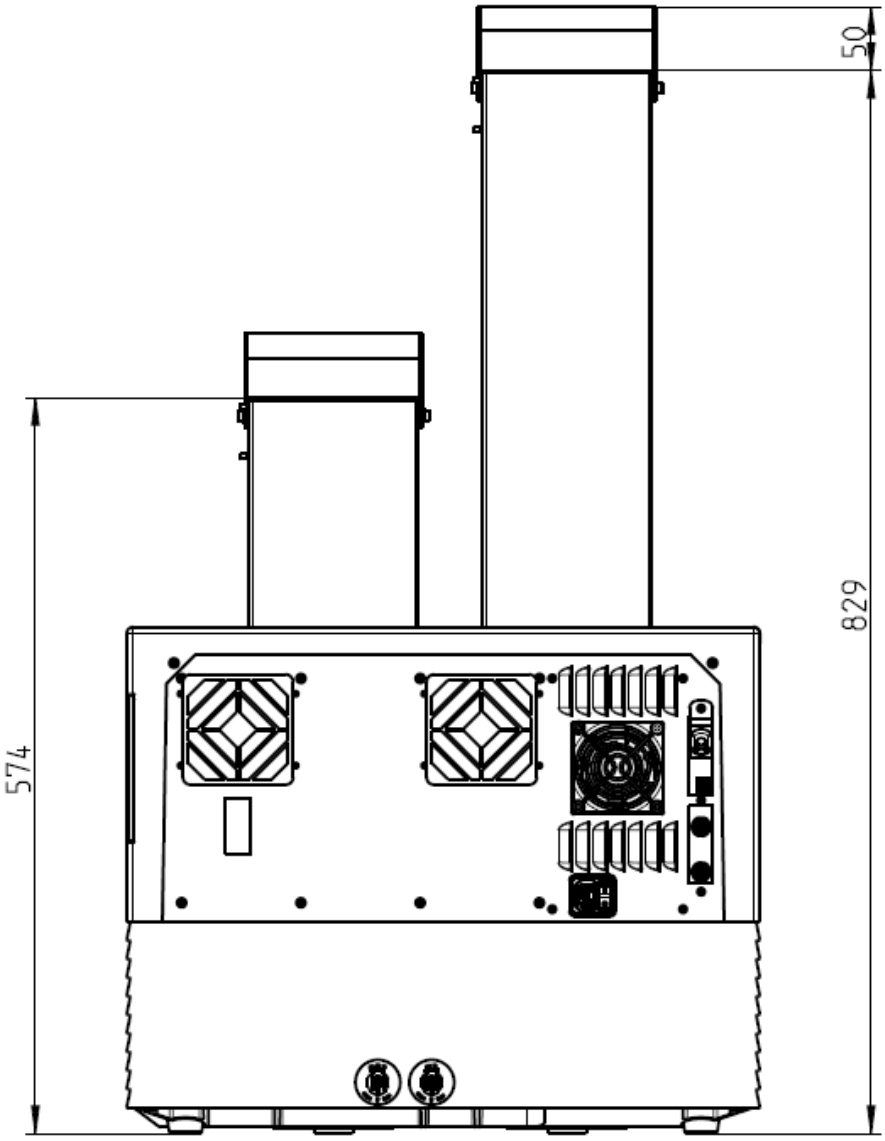
SPARK with Spark-Stack – Front View



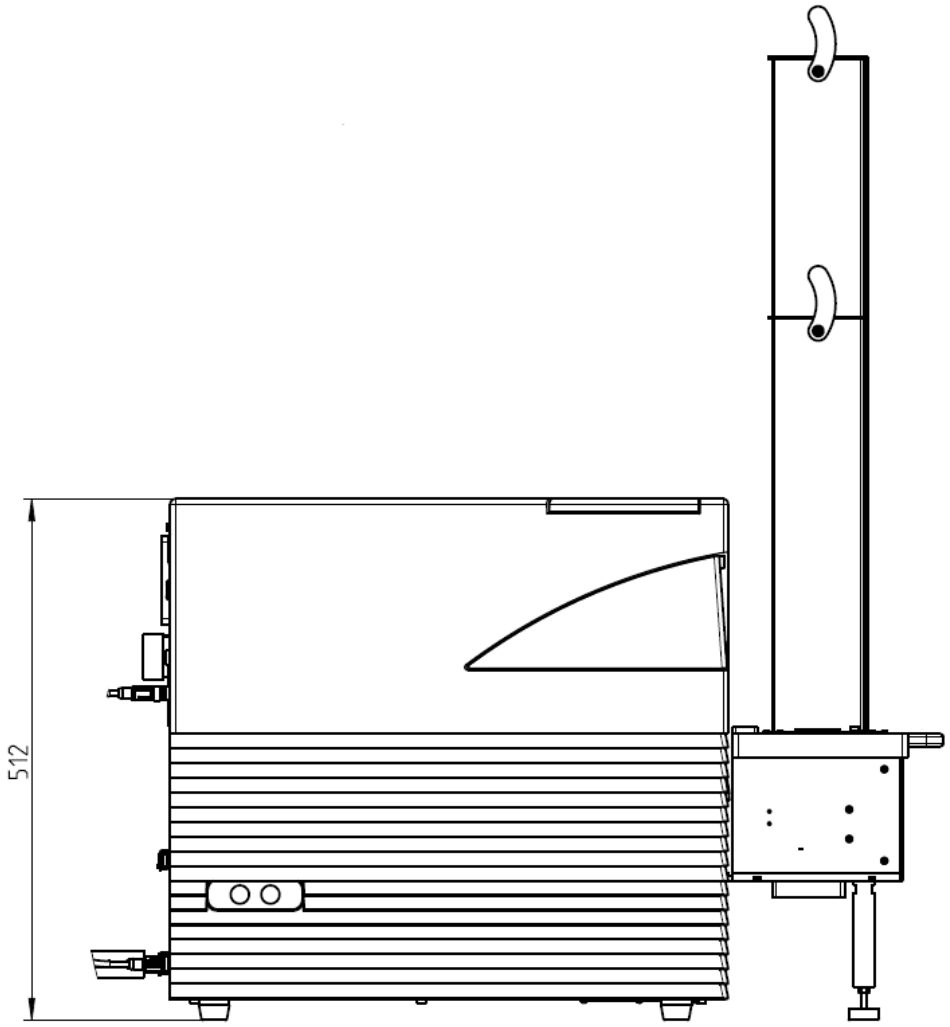
SPARK with Spark-Stack – Top View



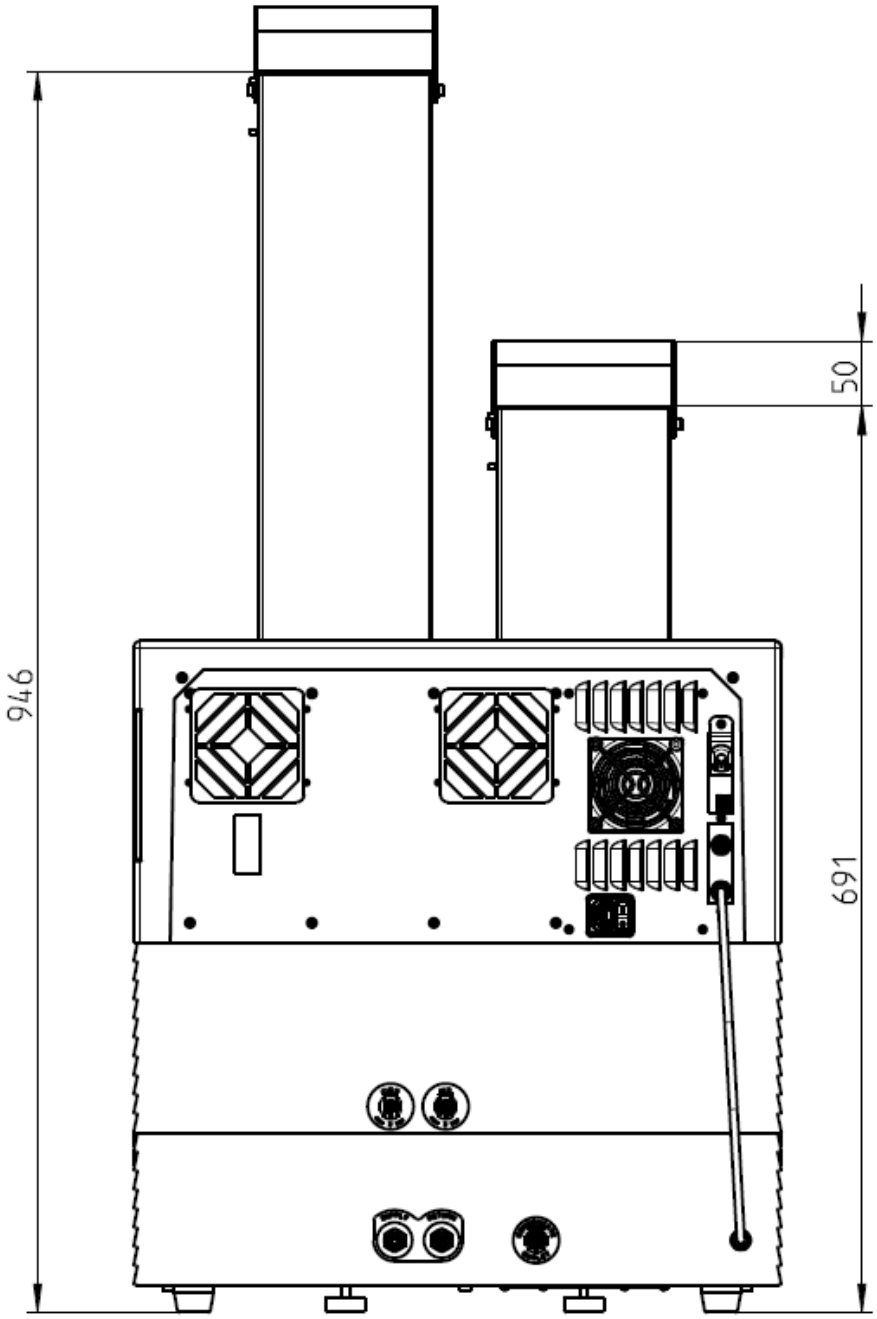
SPARK with Spark-Stack – Rear View



SPARK with Te-Cool and Spark-Stack - Side View



SPARK with Te-Cool and Spark-Stack - Rear View



3 Instrument Installation

3.1 Installing the SPARK

When installing, moving, or connecting the instrument, follow the instructions in this document. Tecan does not accept responsibility for injury suffered by anyone attempting these operations nor for damage incurred to the instrument.

Make sure the laboratory meets all the requirements and conditions described in this chapter.

3.2 Installation Requirements for SPARK

3.2.1 Required Working Area

Select a location to place the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors. Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment and 5 cm distance to any other equipment left and right to the instrument. See chapter 6 Instrument Specifications for further details regarding the environmental specifications.

The cell imaging performance of Spark's Cell Imager module is especially sensitive to external vibrations in the research laboratory, which can lead to blurred images and/or autofocus errors. Therefore, an appropriate location must be chosen to install the instrument, where external vibrations are kept to a minimum, or for best results use a vibration insulated laboratory table.

Ensure that the plate carrier and injector carrier cannot be accidentally hit when moved out. For the installation procedure for the Injector and the Heater/Stirrer, see 15 Injectors and 16.1 Heating Module.

For the installation procedure for the Cooling Module (Te-Cool), see chapter 16.2 Cooling System.

NOTE: A service engineer is required to install the Spark-Stack option.

Ensure that the main switch and the main cable can be reached at all times and are in no way obstructed.

For information regarding outer dimensions and weight of the instrument see chapter 2.9 Instrument Dimensions.

CAUTION: Install the instrument in a location that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors. Ensure that the plate carrier and injector carrier cannot be accidentally hit when moved out.

CAUTION: Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment and 5 cm distance to any other equipment left and right to the instrument. Do not cover the instrument while it is in operation.

CAUTION: Do not place heavy objects on the instrument cover. The maximum load for the SPARK cover is 20 kg. However, the load must be distributed evenly across the entire surface of the cover.

CAUTION: Only use the supplied USB cable. The instrument has been tested with the USB cable delivered with the instrument. If another USB cable is used, Tecan Austria cannot guarantee the correct performance of the instrument.

3.3 Unpacking & Inspection

1. Visually inspect the container for damage before it is opened.
   Report any damage immediately.
2. Select a location according to chapter 3.2.1 Required Working Area.
3. Place the carton in an upright position and open it.
4. Lift the instrument out of the carton and place it in the selected location. Take care when lifting the instrument and ensure that it is held on both sides.
5. Visually inspect the instrument for loose, bent or broken parts. Report any damage immediately.
6. Compare the serial number on the rear panel of the instrument with the serial number on the packing list. Report any discrepancy immediately.
7. Compare the contents of the subpackages to the packing list.
   Report any discrepancy immediately.
8. Save packaging materials and transport locks for further transportation purposes.

WARNING: The fully equipped SPARK is a precision instrument and weighs approximately 50 kg. At least two people must carefully lift the instrument from the box.

CAUTION: Do not overload the plate carrier. The maximum load for the plate transport is 275 g. Overloading the plate carrier can cause instrument damage which may require service.

3.4 Power Requirements

The instrument is auto-sensing and it is therefore unnecessary to make any changes to the voltage range. Check the voltage specifications on the rear panel of the instrument and ensure that the voltage supplied to the instrument is correct to this specification.

The voltage range is from 100-120 V and 220-240 V. If the voltage is not correct, please contact your distributor.

Connect the instrument only to an electricity supply system with a protective ground.

CAUTION: Do not use the instrument if the voltage setting is not correct. If the instrument is switched ON with the incorrect voltage setting it will become damaged.

CAUTION:. Do not replace detachable main power supply cords with inadequately rated cords.

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules and CISPR 11/EN 55011. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates uses and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

3.5 Subpackages

NOTE: Always compare the contents of the subpackages with the delivered packing list. Report any discrepancy immediately.

The instrument packaging includes the following items:

- Cables (USB 2.0 and main)
- Software (USB stick)
- Instruction for Use (optional)
- OOB Quality Report
- CE Declaration of Conformity
- Final Test Protocol (COC)
- RoHS Notice
- Cuvette adapter
- Transport Lock Install/Uninstall Procedure

Additional subpackages depend on the modules installed:

- Filter slide metal box (Fluorescence Filter/Fusion Optics Module)
- Magnetic pad (Lid Lifter)
- Hose kit (Gas Control)
- Tecan Adapter for Cell Chips (cardboard box including 15 cell chips (Cell Counter))
- Injector dummy (Injector/Injector Ready)
- RoboFlask metal box (Centering clamp with set screw and spare screw)
- Metal box with user dichroic mirror (including Allen key for installation)

3.6 Options Packages

NOTE: Always compare the contents of the packaging with the delivered packing list. Report any discrepancy immediately.

The injector module packaging for one injector (basic module) includes the following items:

- Injector cardboard box
- Injector carrier
- Bottle holder
- PVC clasps
- Carbon needle
- Beakers for priming (2 x 1 ml; 1 x 50 ml)
- 125 ml bottle (light protective)
- 15 ml bottle (light protective)

The injector module packaging for the second injector (extension module) includes the following items:

- Injector cardboard box
- Bottle holder
- PVC clasps
- Carbon needle
- Beakers for priming (2 x 1 ml)
- 125 ml bottle (light protective)
- 15 ml bottle (light protective)

The Heater/Stirrer option includes the following items:

- Heater/Stirrer module
- Main cable (basic module)
- Power supply (basic module)
- Beaker glass 100 ml (basic and extension module)
- Magnetic stirring bar (basic and extension module)
- Allen key

The NanoQuant option includes the following items:

- NanoQuant storage box (aluminum case)
- NanoQuant Plate
- Pipetting Aid
- Safety Certificate

The Humidity Cassette standard option includes the following items:

- Humidity Cassette (cassette plus lid)
- Magnetic pad

The Humidity Cassette Cell Imager option includes the following items:

- Humidity Cassette Cell Imager (cassette plus lid)
- Magnetic pad

The Te-Cool option includes the following items:

- External liquid cooling device
- Tubing set
- Condensate tubing
- CAN-cable
- Stoppers
- Coolant concentrate

The Spark-Stack microplate stacker consists of the following items (according to the order):

- Stacker module option
- Short stack option
  - Set of 2 plate magazines for 30 plates per run
  - Dark covers and lids
- Long stack option
  - Set of 2 plate magazines for 50 plates per run
  - Dark covers and lids

The Cell Imager option includes a dedicated computer.

NOTE: A service engineer is required to install the Spark-Stack option.

CAUTION: All items delivered with the instrument and also all spare parts or supplemental parts for the instrument are intended for use with the instrument only and are not for general use.

3.7 Upgrades

The instrument consists of various modules and can be ungraded if required. Contact your local Tecan representative for more information.

3.8 Removal of the Transport Locks

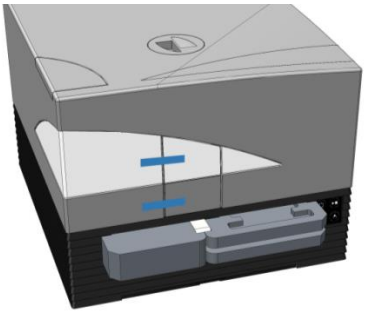
3.8.1 Plate Carrier Transport Lock

CAUTION: Remove the transport lock before operating the instrument.

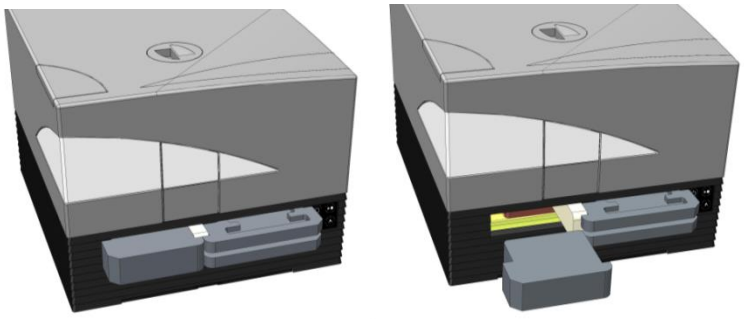
The instrument is delivered with the plate carrier locked into place, so that it cannot become damaged.

Before the instrument can be used, the transport locks (foam pieces) must be removed using the following procedure:

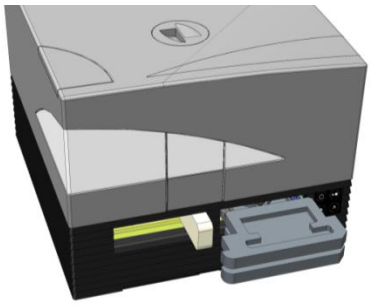
1. Ensure that the instrument is disconnected from the main power supply.
2. Remove the tape from the filter compartment doors.



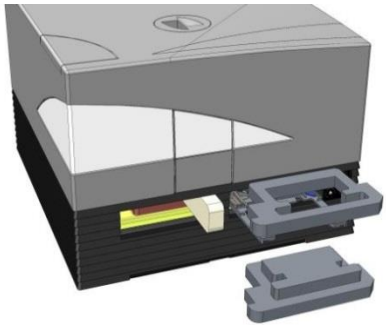
3. Remove the piece of foam from the left plate carrier compartment (see picture below).



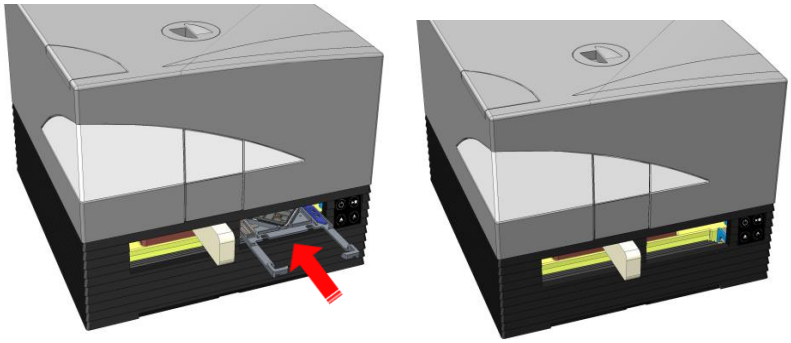
4. Move the plate carrier out manually by pulling on the pieces of foam in the right plate carrier compartment (see picture below).



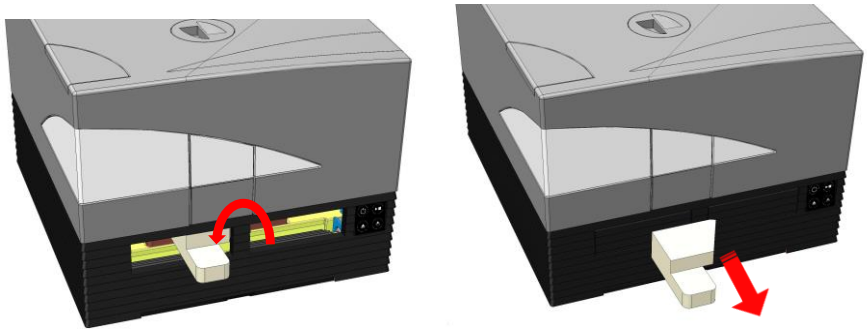
5. Remove the top piece of foam first and then the bottom piece (see picture below).



6. Move the plate carrier in carefully by hand. It must be pushed in far enough so that the plate carrier compartment door can close (see picture below).



7. Rotate the remaining piece of foam 90° counter-clockwise and pull it out of the instrument (see picture below).



CAUTION: Save packaging materials and transport locks (foam pieces) for further transportation purposes. The instrument must be shipped only with the original packaging and installed transport locks.

3.9 Switching the Instrument On

CAUTION: Before the instrument is switched on for the first time, it should be left to stand for at least 3 hours, so there is no possibility of condensation causing a short circuit.

1. Make sure that the main power switch on the rear panel of the instrument is in the OFF position.
2. Connect the computer to the instrument only with the delivered USB interface cable.
3. Insert the power cable into the main power socket (with protective ground connection) on the rear panel of the instrument.
4. Connect the USB cable of the camera of the Cell Module (guided through the rear panel of the instrument) to the USB 3.0 port of the computer.

CAUTION: The Cell Module camera or the Cell Imager module camera, respectively, must be connected to the USB 3.0 port of the computer to avoid performance loss.

5. All connected devices must be approved and listed as per IEC 60950-1 Information Technology Equipment – Safety or equivalent local standards.
6. Connect the injector, if required.
7. Plug in the heater/stirrer, if required.

CAUTION: Switch off the instrument before plugging in or unplugging the injector module.

CAUTION: Switch off the instrument before plugging in or unplugging the cooling module.

8. Switch ON the instrument using the main power switch on the rear panel of the instrument.
9. Start the software to work with the instrument. For instrument control via software, see the SparkContro software manual.

WARNING: Do not reach into the instrument while it is in operation!

3.10 Switching the Instrument Off

1. Ensure that the plate transport is empty.
2. In the SparkControl software, disconnect from the instrument by selecting Exit in the File menu in the Method Editor (see chapter Menu Bar in the SparkControl software manual) or Shut Down via the expandable Navigation bar on the left side of the Dashboard.
3. Switch OFF the instrument by either using the onboard control button or the main power switch on the rear panel of the instrument.

CAUTION: When switched off wait at least 5 seconds until switching the instrument on again. Instrument errors can occur.

3.11 Preparing the Instrument for Shipping

Before shipping an instrument with integrated cooling module (Te-Cool) the cooling liquid has to be removed from the cooling system. This procedure must be done by a service technician.

CAUTION: Do not ship an instrument with integrated cooling module! Only Tecan authorized service technicians are allowed to prepare instrument for transportation. Residual cooling fluid might damage the instrument.

Before shipping an instrument with the microplate stacker module (Spark-Stack), the stacker has to be removed from the instrument. This procedure must be done by a service technician.

CAUTION: Do not ship an instrument with integrated stacker module! Only Tecan authorized service technicians are allowed to remove the stacker module for transportation of instrument or stacker module.

Before shipping the instrument, perform the parking procedure to avoid any damage to the optics and plate transport (see 3.11.1 Parking Procedure).

After the parking procedure has been performed, the plate carrier transport locks must be installed (see 3.11.2 Installing the Plate Carrier Transport Locks).

Before shipping, the instrument (including the injector(s), heater/stirrer, humidity cassette, NanoQuant Plate and any other external optional components) must be thoroughly disinfected (see 7.3 Instrument Decontamination/Disinfection). For injector maintenance, see 15.3 Injector Cleaning and Maintenance).

CAUTION: Switch off the instrument before plugging in or unplugging the injector module.

CAUTION: Switch off the instrument before plugging in or unplugging the cooling module.

The instrument (including the injector(s), heater/stirrer, humidity cassette, NanoQuant Plate and any other external optional components) must be shipped in the original packaging.

WARNING: Always move the injector and the heater/stirrer separately, as the two units are not attached to each other. When carried together, one of the units can easily fall and become damaged.

3.11.1 Parking Procedure

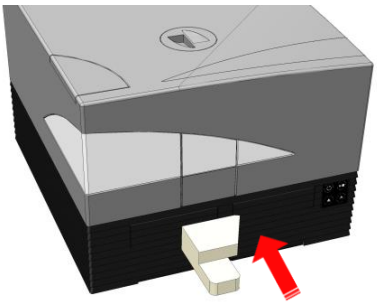
1. Ensure that the plate transport is empty.
2. Ensure that the injector (dummy) is removed from the injector port.
3. In the SparkControl software, disconnect from the instrument by selecting **Exit** in the File menu in the Method Editor (see chapter Menu Bar in the SparkControl software manual) or Shut Down via the expandable Navigation bar on the left side of the Dashboard.
4. Remove filter slides by using the onboard control button in the front of the instrument.
5. Move out the plate transport by using the onboard control button in the front of the instrument.
6. Switch OFF the instrument by using the onboard control button in the front of the instrument to start parking procedure. Starting the parking procedure may take a few seconds.
7. Switch OFF the instrument by using the main power switch on the rear panel of the instrument.
8. Install the plate carrier transport lock (see 3.11.2 Installing the Plate Carrier Transport Locks).

CAUTION: The parking procedure has to be performed and the transport lock must be mounted before shipping. If the instrument is shipped without these safety measures, the instrument guarantee is rendered null and void. Use original packaging for shipping.

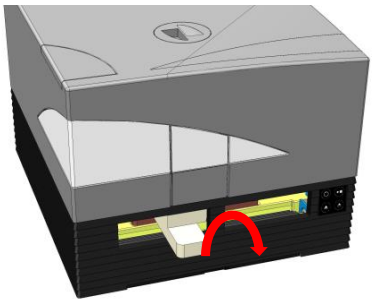
3.11.2 Installing the Plate Carrier Transport Locks

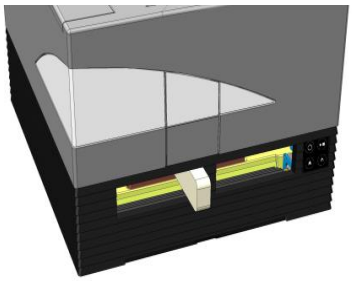
The instrument must be shipped with the plate carrier locked into place, so that it cannot become damaged. Before the instrument can be shipped, the transport locks (foam pieces) must be inserted using the following procedure:

1. Ensure that the instrument is disconnected from the main power supply.
2. Hold the plate carrier compartment door down and insert the white piece of foam (shown below) into the left compartment.

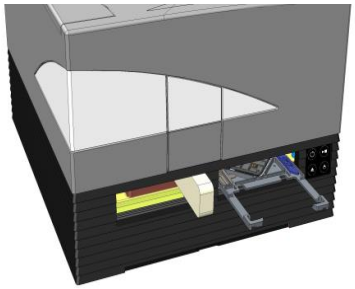


3. With the foam piece inserted, turn it 90° clockwise, so that the pointed end sticks down into the space between the two compartment openings. This piece of foam holds the compartment doors open.



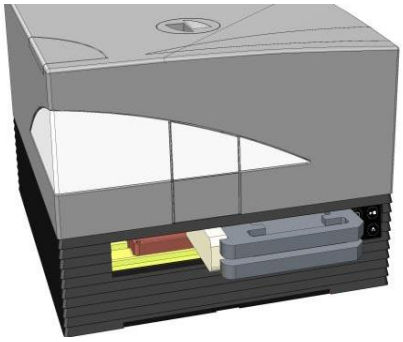


4. Move the plate carrier out carefully by hand until it lightly presses against the inserted white piece of foam from behind and cannot be moved out further.

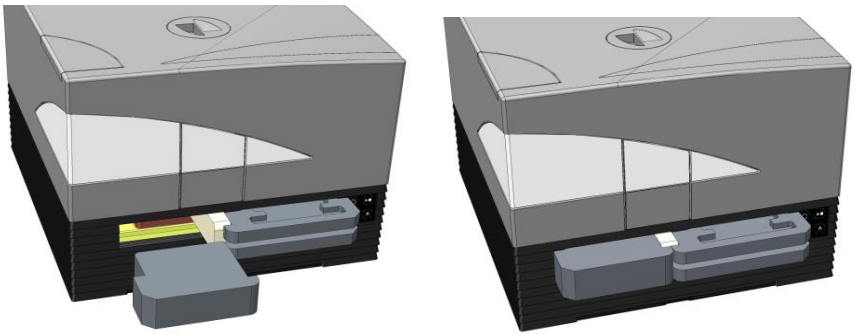


5. Insert the bottom piece of foam first and then interlock the top piece into place (see picture below).

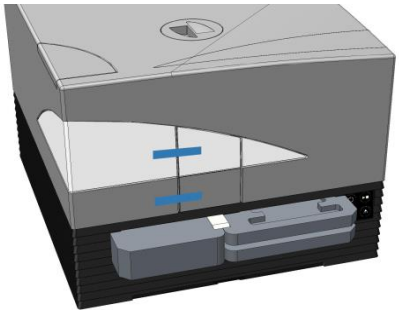
6. Move the plate carrier into the right compartment manually as far as it can go by pushing on the pieces of foam on the plate carrier.



7. Insert the piece of foam into the left plate carrier compartment (see picture below).



8. Tape the filter compartment doors shut (see picture below).



4 Plate Control

The plate transport is capable of moving horizontally (in the x- and y-directions) as well as vertically (in the z-direction), so that for each measurement mode, top or bottom, the optimal measurement position can be reached regardless of which plate type or filling volume is used. The movement speed is optimized according to the plate type and detection mode.

NOTE: For additional requirements when operating the instrument with the microplate stacker module, see chapter 14 Spark-Stack Microplate Stacker Module.

CAUTION: Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 has to be on the upper left side.

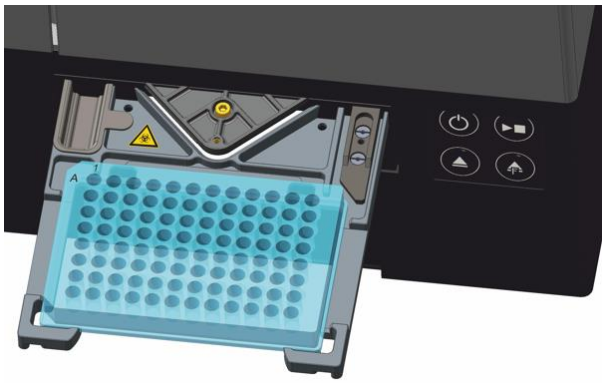


Figure 2: Microplate on the plate carrier with the A1 well in the upper left-hand corner

CAUTION: Do not leave microplates inside the instrument overnight when working with aggressive solutions. Acids, bases, or cleaning solutions (bleach) will evaporate inside the reader and cause corrosion. This may lead to severe damage of the instrument and can impair its proper functioning. Tecan cannot take any responsibility nor be held liable if the reader is damaged due to improper plate handling.

CAUTION: Users should also take care that no potential fluorescent or luminescent contamination lies on top of the plate as droplets, and also be aware that some plate sealers leave a sticky residue that should be removed before measurement.

CAUTION: Tecan Austria GmbH has taken great care when creating the Plate Definition Files (.pdfx) that are delivered with the instrument.

We take every precaution to ensure that the plate heights and well depths are correct according to the defined plate type. These parameters are used to determine the minimum distance between the top of the plate and the ceiling of the measurement chamber. Additionally, Tecan Austria adds a very small safety gap to prevent any damage that may occur to the measurement chamber as a result of small changes in plate height. This does not affect the performance of the instrument.

Make sure that the selected plate definition file corresponds to the currently used microplate, so that the safety gap is correctly calculated, otherwise the instrument could become damaged.

4.1 Z-Position

The height of the objective above the sample can be adjusted using the Z-position function. As excitation light is reflected by the sample fluid, the Z-adjustment helps to maximize the signal-to-noise ratio. For further details about Z-positioning, see chapter Optimizing Fluorescence and Fluorescence Polarization Measurement in the SparkControl software manual.

4.2 Shaking

The SPARK is capable of plate shaking before start of a measurement or in between kinetic cycles. Three shaking modes are available: linear, orbital and double orbital. The shaking amplitude can be selected from 1 to 6 mm in steps of 0.5 mm. The frequency is a function of the amplitude. The shaking duration is selectable from 3-3600 seconds.

4.3 Incubation/Cooling Position

The SPARK has a predefined incubation/cooling position with an optimum temperature distribution. These positions can be used for shaking or waiting steps within a measurement run.

4.4 Lid Lifter

The lid lifter option consists of a permanent magnet and a magnetic pad. The magnetic pad can be mounted on the lids of all commonly used microplate types with a maximum lid height of 11.5 mm. The magnetic mechanism is regulated by the software.

To attach the pad, peel the paper backing off of the metal disk and stick the pad onto the center of the lid.

CAUTION: The lid height must not exceed 11.5 mm.

CAUTION: Clean the lid with 70% ethanol before attaching the magnetic pad.

Figure 3: Lid with magnetic pad mounted in the middle

CAUTION: Make sure that the magnetic pad is mounted on the plate lid if **Removable Lid** or **Humidity Cassette** is activated in the method.

CAUTION: Mount the magnetic pad in the middle of the appropriate plate lid to guarantee optimal performance.

The lid lifter option is used to temporarily remove the lid of the microplate to execute, e.g. injection steps or measurement steps within the workflow of a long term experiment thus avoiding sample evaporation.

The lid lifter in combination with the Gas module option can also be used to improve the gas exchange between the medium and the surrounding environment in the case of cell-based studies. Ventilation steps can be inserted simply into the workflow and timed accordingly.

The lid lifter option can also be used in combination with Tecan's humidity cassette (see chapter 16 Environmental Control).

4.5 Securing the RoboFlask Cell Culture Vessels

A centering clamp is necessary to secure the RoboFlask Cell Culture Vessels (Corning, Inc.) onto the plate carrier. This centering clamp must be installed by the user before starting measurements using RoboFlask Cell Culture Vessels. Follow the given instructions.

- Move the plate transport out
- Put the centering clamp on the plate fixing mechanism as indicated in the figure below.
- Fasten the screw, taking care to avoid putting pressure on the plate carrier.

CAUTION: Do not put pressure on the plate carrier when attaching the centering clamp. A bent plate carrier can negatively influence the performance of the instrument and may require service.

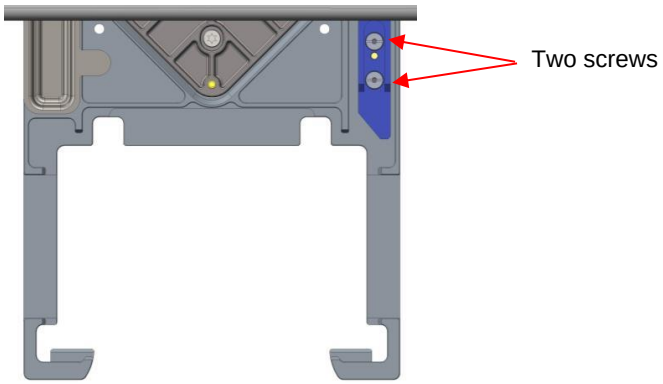


Figure 4: Centering clamp for the RoboFlask Cell Culture Vessels

CAUTION: Do not use the RoboFlask Cell Culture Vessels without the centering clamp. This can result in damage to the instrument.

NOTE: By using a higher number of flashes and/or a settle time for measurements with the RoboFlask, the result will be more accurate.

5 SPARK Platform

The SPARK is a multimode reader platform. Each instrument variant can be equipped with a large number of modules and functions. The following chapter provides an overview.

Module/Function	Characteristics
Absorbance	Absorbance (Fast absorbance scan included) or Absorbance Enhanced (Up to 1536-well)
NanoQuant Plate	For low volume nucleic acid samples; Ready to use apps available for nucleic acid quantitation and labeling efficiency.
Cuvette module	For Absorbance measurements. Ready to use app available.
Luminescence Standard	Attenuation function (OD1 and OD2). Up to 384-well.
Luminescence Enhanced	Attenuation function (OD1, OD2 and OD3). Wavelength discrimination. Luminescence scan included. Up to 1536-well
Alpha Technology	AlphaScreen, AlphaLISA and AlphaPlex. Alpha Enhanced (Up to 1536-well)
Fluorescence Standard Top	Filter-only-, Monochromator-only- or Fusion Optics-System available. Up to 384-well.
Fluorescence Standard Bottom	Filter-only-, Monochromator-only- or Fusion Optics-System available. VIS or UV-VIS fiber. Up to 384-well.
Fluorescence Standard Bottom Area Scan	Up to 100x100 data points/well
Fluorescence Standard Polarization	Filter-only-, Monochromator-only- or Fusion Optics-System available. >300 nm or >390 nm fiber. Up to 384-well.
Fluorescence Enhanced Top	Filter-only-, Monochromator-only- or Fusion Optics-System available. More sensitive than Standard option. Up to 1536-well.
Fluorescence Enhanced Bottom	Filter-only-, Monochromator-only- or Fusion Optics-System available. Equipped with UV-VIS fiber. More sensitive than Standard option. 1536-well optional.
Fluorescence Enhanced Bottom Area Scan	up to 100x100 data points/well
Fluorescence Enhanced Polarization	Filter-only-, Monochromator-only- or Fusion Optics-System available. Equipped with >300 nm fiber. More sensitive than Standard option. Up to 1536-well
Cell Module: Cell Counting and Confluence	Cell counting and viability in Tecan Cell Chips (Ready to use apps). Cell confluence in microplates.

Module/Function	Characteristics
Cell Imager	Bright field Imaging and Fluorescence Imaging in microplates.
Spark-Stack	Built-in microplate stacker designed for automated loading, unloading and restacking of plates.
Injector (one or two injectors)	One or two injector options with different syringe sizes.
Heater/Stirrer	Both injector options can be equipped with Heater/Stirrer module.
Heating	3 °C above ambient up to 42 °C
Cooling (Te-Cool)	18 °C up to 42 °C.
Gas control	CO_2 only or CO_2 and O_2.
Humidity Control	Evaporation protection for different plate formats for long-term studies (with cells).
Lid lifter	Interactions during long-term studies (gas exchange, injection).
Barcode reader	Reads barcodes automatically.

Fluorescence Standard and Fluorescence Enhanced options cannot be installed together in one instrument.

6 Instrument Specifications

NOTE: All specifications are subject to change without prior notification.

The table below lists the technical specifications of the basic instrument:

General

Parameters	Characteristics	
Measurement	Software controlled	
Interface	USB 2.0 or 3.0 (SPARK); 3.0 (SPARK CYTO)	
Fusion Optics system	Monochromator and Filter (external filter exchange) based	
Microplates	From 1-well to 1536-well SBS plates	
Temperature control	From 18 °C up to 42 °C (depending on installed modules)	
Plate shaking	Linear, orbital and double orbital shaking	
Light source	High energy Xenon flash lamp	
Optics	Fused Silica Lenses	
Fluorescence detector	Low dark current photomultiplier tube	
Luminescence detector	Low dark count photomultiplier tube	
Absorbance detector	Silicon photodiode	
Power supply	100-120 V and 220-240 V, auto-sensing	
Power consumption	Operation: 350 VA	Standby: 25 VA

Physical

Parameters	Characteristics		
Outer dimensions	Width:	494 mm	(19.5 in.)
	Height:	395 mm	(15.5 in.)
	Height (with Te-Cool):	512 mm	(20.2 in.)
	Height (with Cell Imager):	512 mm	(20.2 in.)
	Height (with injector carrier):	455 mm	(17.9 in.)
	Depth:	557 mm	(21.9 in.)
	Depth (carrier moved out):	699 mm	(27.5 in.)
	Depth (with Spark-Stack):	786 mm	(30.9 in.)

Weight

Parameters	Characteristics	
Instrument	40 kg	(88 lb.)
Instrument with Te-Cool	50 kg	(110 lb.)
Instrument with Cell Imager (for CYTO600, the heaviest configuration)	max. 50 kg	(max. 110 lb.)
Injector (2 channel)	4.0 kg	(8.8 lb.)
Heater/Stirrer	2.7 kg	(6 lb.)
Spark-Stack module		
Stacker	8.5 kg	(18.7 lb.)
Short Stack (2 plate magazines, including dark covers and dark lids)	4.5 kg	(9.9 lb.)
Long Stack (2 plate magazines, including dark covers and dark lids)	5 kg	(11 lb.)

Environmental

Parameters	Characteristics	
Operating temperature	+15 °C to +35 °C	59 °F to 95 °F
Operating temperature with active cooling	+15 °C to +30 °C	59 °F to 86 °F
Transportation temperature	-30 °C to +60 °C	-22 °F to +140 °F
Operating humidity	20 % to 90 % (non-condensing)	
Operating humidity with active cooling	20 % to 80 % (non-condensing)	
Transportation humidity	20 % to 95 % (non-condensing)	
Operating pressure	700-1050 hPa	
Transportation pressure	500-1100 hPa	
Overvoltage category	II	
Pollution degree	2	
Usage	Commercial	
Noise level	< 60 dBA	
Method of disposal	Electronic waste (infectious waste)	

7 Cleaning and Maintenance

7.1 Introduction

- For maintenance of the NanoQuant, see chapter NanoQuant Maintenance in the SparkControl software manual.
- For injector maintenance, see 15.3 Injector Cleaning and Maintenance.
- For maintenance of the cell chip adapter, see 12.3.3 Maintenance and Cleaning of the Cell Chip Adapter.
- For maintenance of the Cooling Module, see 16.2.6 Maintenance.
- For maintenance of the Spark-Stack, see 14.2.7 Cleaning and Maintenance of the Spark-Stack.

The cleaning and maintenance procedures are important in order to prolong the instrument's life and to reduce the need for service.

This section contains the following information:

- Liquid Spills
- Instrument Disinfection
- Disinfection Procedure
- Safety Certificate
- Disposal

CAUTION: Keep the plate transport clean! Take special care of the clip mechanism that secures the microplates. Insufficient plate fixation can lead to instrument damage. Excessive soiling requires service.

7.2 Liquid Spills

1. Wipe up the spill immediately with absorbent material.
2. Dispose of contaminated material appropriately.
3. Clean the instrument surfaces with a mild detergent.
4. For biohazard spills, clean with B33 (Orochemie, Germany).
5. Wipe cleaned areas dry.

WARNING: Always switch off the instrument before removing any kind of spills on the instrument. All spills must be treated as potentially infectious. Therefore, always adhere to applicable safety precautions (including the wearing of powder-free gloves, safety glasses and protective clothing) to avoid potential infectious disease contamination.

Additionally, all resulting waste from the clean-up procedure must be treated as potentially infectious and the disposal must be performed according to the information given in chapter 7.4 Disposal.

7.3 Instrument Decontamination/Disinfection

WARNING: The disinfection procedure should be performed according to national, regional, and local regulations.

WARNING: All parts of the instrument that come into contact with potentially infectious or any hazardous material must be treated as potentially infectious areas.

It is advisable to adhere to applicable safety precautions (including the wearing of powder-free gloves, safety glasses and protective clothing) to avoid potential infectious disease contamination when performing the disinfection procedure.

WARNING: It is very important that the instrument is thoroughly disinfected before it is removed from the laboratory or before any service is performed on it.

WARNING: The disinfection procedure for the injector described in this chapter is valid only for the cover of the injector box. For cleaning and maintenance of the syringes, tubes and pumps, see chapter 15.3 Injector Cleaning and Maintenance.

CAUTION: Ensure that the microplate is removed from the instrument before it is prepared for shipment. If a microplate is left in the instrument, fluorescent solutions may spill onto the optical parts and damage the instrument.

Before the instrument is returned to the distributor or service center, all outer surfaces and the plate transport must be disinfected and a safety certificate must be completed by the operating authority. If a safety certificate is not supplied, the instrument may not be accepted by the distributor or service center or custom authorities may hold it.

7.3.1 Disinfection Solutions

The instrument (Front, Cover, Plate transport) should be disinfected using the following solution:

- B33 (Orochemie, Germany)

CAUTION: The disinfection procedure should be performed by authorized trained personnel in a well-ventilated room wearing disposable gloves and protective glasses and clothing.

WARNING: The disinfection procedure for the injector is valid only for the cover of the injector box. For cleaning and maintenance of the syringes, see 15.3 Injector Cleaning and Maintenance.

7.3.2 Disinfection Procedure

CAUTION: The surface disinfectant can negatively influence the performance of the instrument, if it is applied or accidentally gets inside the instrument.

CAUTION: Make sure that the microplate has been removed from the instrument before starting disinfection procedure.

If the laboratory has no specific disinfection procedure the following procedure should be used to disinfect the outside surfaces of the instrument:

1. Wear protective gloves, protective glasses and protective clothing.
2. Prepare a suitable container for all disposables used during the disinfection procedure.
3. Disconnect the instrument from the main power supply.
4. Disconnect the instrument from any external components that are used.
5. Carefully wipe all outside surfaces of the instrument with a lint-free paper towel soaked in the disinfection solution.
6. Perform the same disinfection procedure on the plate carrier.
7. Perform the disinfection procedure on any external components that are used with the instrument.
8. Complete the Safety Certificate and attach it to the outside of the box so that it is clearly visible.

See below for information about the Safety Certificate, which must be completed before the instrument is returned to the distributor/ service center.

CAUTION: The plate transport should only be moved manually when the instrument is disconnected from the main power supply.

7.3.3 Safety Certificate

The Safety Certificate must be requested from your local Tecan Customer Support (see http://www.tecan.com/ for contact information).

To ensure the safety and health of personnel, our customers are kindly asked to complete two copies of the **Safety Certificate** and attach one copy to the top of the container in which the instrument is returned (visible from the outside of the shipping container!) and attach the other copy to the shipping documents before shipping it to the service center for service or repair.

The instrument must be decontaminated and disinfected at the operating authority's site before shipping (see chapter 7.3.2 Disinfection Procedure). The decontamination and disinfection procedure must be performed in a well-ventilated room by authorized and trained personnel wearing disposable powder-free gloves, safety glasses and protective clothing. The decontamination and disinfection procedure must be performed according to national, regional, and local regulations.

If a Safety Certificate is not supplied, the instrument may not be accepted by the service center.

7.4 Disposal

Follow laboratory procedures for bio-hazardous waste disposal, according to national, regional and local regulations.

This section provides instructions on how to lawfully dispose of waste material accumulated in connection with the instrument.

CAUTION: Observe all federal, state and local environmental regulations.

WARNING: Directive 2012/19/EU on waste electrical and electronic equipment (WEEE)

Negative environmental impacts associated with the treatment of waste:

- Do not treat electrical and electronic equipment as unsorted municipal waste
- Collect waste electrical and electronic equipment separately

7.4.1 Disposal of Packaging Material

According to Directive 94/62/EC on packaging and packaging waste, the manufacturer is responsible for the disposal of packaging material.

Returning Packaging Material

If you do not intend to keep the packaging material for future use, e.g. for transport and storage purposes, return the packaging of the product, spare parts and modules via the field service engineer to the manufacturer.

7.4.2 Disposal of Operating Material

WARNING: Biological hazards can be associated with the waste material (i.e. microplate) of the processes run on the SPARK.

Treat the used microplate, cell chips, other disposables, and all substances used, in accordance with good laboratory practice guidelines.

Inquire about appropriate collecting points and approved methods of disposal in your country, state or region.

7.4.3 Disposal of the Instrument

Please contact your local Tecan service representative before disposing of the instrument.

CAUTION: Always disinfect the instrument before disposing.

Pollution Degree	2 (IEC/EN 61010-1)
Method of Disposal	Contaminated waste

WARNING: Depending on the applications, parts of the instrument may have been in contact with biohazardous material. Make sure to treat this material according to the applicable safety standards and regulations.

Always decontaminate all parts before disposal.

8 Luminescence

8.1 Basic Principles

Luminescence is the emission of light caused by processes other than heat. Chemiluminescence is the emission of light due to a chemical reaction. If a living organism is the light emitting source (e.g. fireflies, some deep-sea organisms), this process is called bioluminescence.

Luminescence-based assays are often used to measure the activity of enzyme-labeled compounds. Light emission results from a luminescence substrate being decomposed by enzymes, such as peroxidase, phosphatase, or luciferase. The luminescence signal is only considered to be proportional to the abundance of the enzyme-labeled compound when sufficient substrate is provided.

NOTE: Luminescence is often used as umbrella term for all non-thermal emission types, such as fluorescence, phosphorescence, bio- and chemi-luminescence, etc.

At Tecan, however, the term luminescence is only used for emission types occurring without excitation.

8.2 Measurement Techniques

8.2.1 Glow Luminescence

Glow type luminescence assays provide a stable luminescence signal for a long time. There are several luminescence substrates that ensure a stable light output even over hours.

8.2.2 Flash Luminescence

Flash luminescence assays provide a very short-lived luminescence signal that has to be measured during the dispensing of activating agent or after a short time delay. Due to the short-lived signal, use of injectors may be advantageous for these measurements.

8.2.3 Multicolor Luminescence

Certain assays use two or more emitting species, giving light of two or more different wavelength ranges at the same time. For theses assays, discrimination of wavelength ranges during luminescence detection may be required. The enhanced luminescence module from Tecan is perfectly suitable for commercial application such as Chroma-Glo Luciferase assay system and BRET based applications.

For example, Chroma-Glo assays generate red and green luminescence from two types of luciferase within a single well upon the addition of a single substrate. This homogeneous dual-reporter gene assay permits each reporter to be measured independently by detecting two luminescence signals per well at two different wavelengths ranges, i.e. red and green.

8.2.4 Luminescence Scan

A luminescence scan measures emission spectra of luminescent substances (luminophores). For example, emission spectra of new/mutated types of luciferase (e.g. new recombinants of renilla- and firefly-luciferase) can be recorded in order to determine their emission maxima. Furthermore, luminescence scans can be applied to study environmental influences such as pH, solvent, or buffer on the spectral behavior of luminescent systems.

Luminescence intensity scan data can be displayed either as corrected spectra or as technical (uncorrected) spectra. The luminescence intensity values depend on instrument measurement characteristics (spectral sensitivity, filter transmission) as well as the wavelength and therefore may distort the measured spectra. Calibration data is saved on the instrument and intensity value corrections of the raw data are performed by default (however, they can also be switched off in the software).

8.3 Standard Luminescence Module

The standard luminescence module enables the integral measurement of a luminescence signal, without distinguishing between emission wavelengths.

The standard luminescence module can be used with all microplate formats up to 384 wells.

8.3.1 Luminescence Optics

The standard luminescence module consists of the luminescence fiber [1], the filter wheel [2], and the detection unit [3] (see Figure 5).

The luminescence fiber guides the luminescence light from the sample to the detection unit, where it passes the filter wheel on its way to the detector. A single luminescence fiber covers all plate formats up to 384 wells.

The sensitivity of the detection system requires attenuation of high luminescence light levels. The attenuators, two neutral density filters (OD1 and OD2) installed on the filter wheel, can be moved into the light path via the software.

The z-drive of the plate transport [4] ensures the optimal distance between microplate and luminescence optics, in order to maximize the signal and to minimize cross-talk. The z-adjustment is performed automatically after the appropriate plate type is selected in the software (see chapter 4.1 Z-Position).

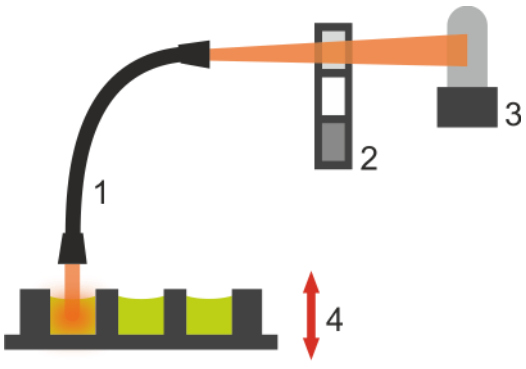


Figure 5: Optical system in standard luminescence module:
[1] luminescence fiber, [2] filter wheel,
[3] detection unit, [4] z-drive of plate transport

8.3.2 Standard Luminescence Detection

The standard luminescence module is equipped with a photon multiplier tube (PMT) which is operated in single photon counting mode. This technique is well-suited for the detection of small signals due to its low noise level. The photon-counting PMT provides a high dynamic range, which is ideal for luminescence measurements with a strong variation in luminescence intensity levels.

To achieve optimal performance, white plates are recommended for luminescence measurements.

CAUTION: Switch on the instrument at least 15 minutes before starting a luminescence measurement to ensure stable conditions for the measurement.

8.4 Enhanced Luminescence Module

The enhanced luminescence module has the ability of performing all available multicolor applications, as well as fast and high-sensitive luminescence scans. Additionally, it is able to measure luminescence signals without wavelength discrimination and to attenuate strong signals like the standard luminescence module.

The luminescence enhanced module can be used with all microplate formats supported by the instrument.

8.4.1 Luminescence Filters

The essential parts of the enhanced luminescence module are two sets of 19 spectral filters, built into two filter wheels. One filter wheel contains all the long pass filters; the other one contains the short pass filters.

A short pass filter transmits light of all wavelengths below the filter's cutoff wavelength. In contrast, a long pass filter transmits light of all wavelengths above the filter's cutoff wavelength. By combining short and long pass filters, arbitrary band pass filters can be realized allowing the spectral selection of the luminescence wavelengths to be detected.

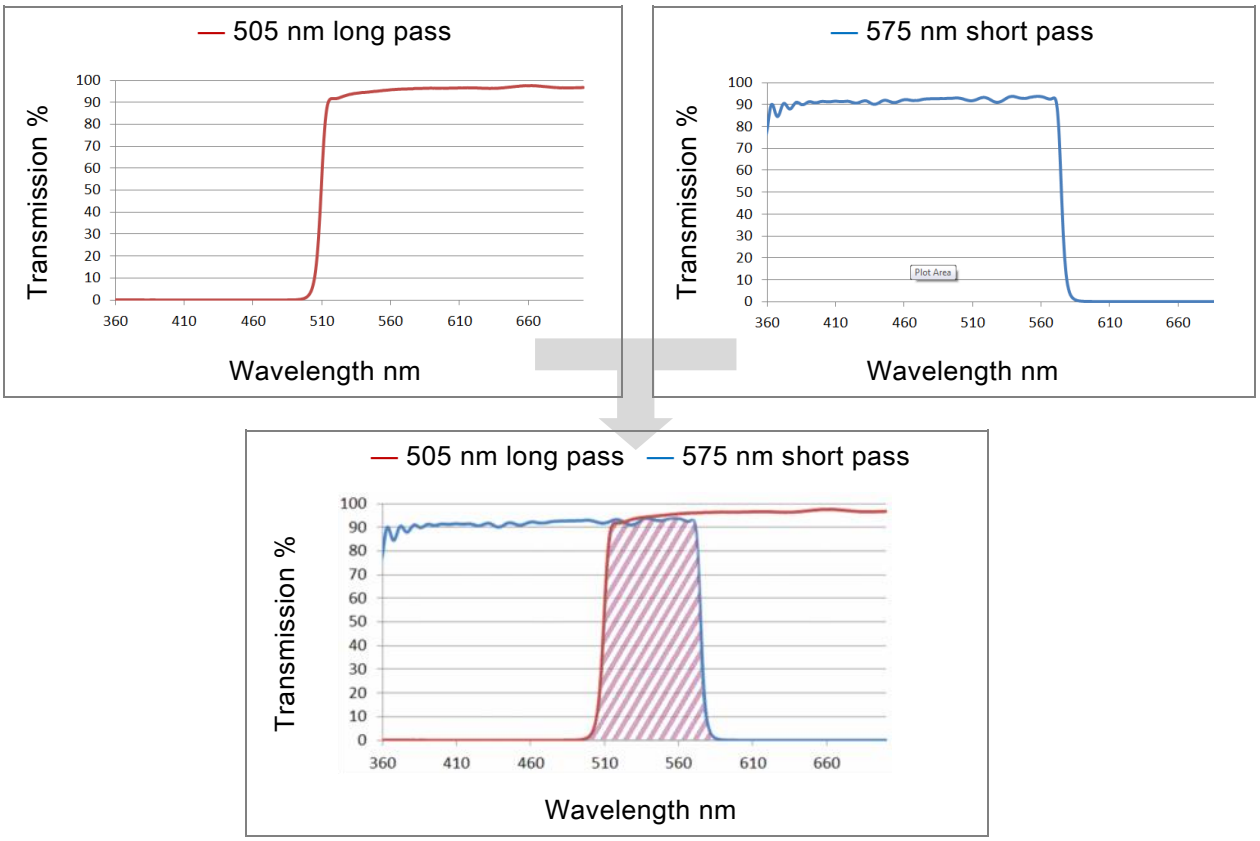


Figure 6: The combination of a 505 nm long pass filter with a 575 nm short pass filter yields a spectral measurement window for the luminescence emission from 505 to 575 nm.

Cutoff values of the short pass filters:

395, 410, 425, 440, 455, 470, 485, 500, 515, 530, 545, 560, 575, 590, 605, 620, 635, 650 and 665 nm.

Cutoff values of the long pass filters:

385, 400, 415, 430, 445, 460, 475, 490, 505, 520, 535, 550, 565, 580, 595, 610, 625, 640 and 655 nm.

The band pass filters formed by the combination of long and short pass filters are also used to perform luminescence scans. A complete wavelength scan (390 – 660 nm) consists of 18 measurement points, carried out by 18 dedicated filter pairs. The spectral bandwidth is fixed to 25 nm and the step size is fixed to 15 nm.

8.4.2 Enhanced Luminescence Optics

The enhanced luminescence module consists of the luminescence fiber [1], the aperture wheel [2], two filter wheels [3], and the detection unit [4] (see Figure 7).

The luminescence fiber guides the luminescence light from the sample to the detection unit, where it passes the long and short pass filter wheels on its way to the detector. The filter wheels are used for spectral discrimination of luminophores and suppression of unwanted luminescence wavelengths. The aperture wheel matches the light beam diameter to the microplate wells in order to prevent cross-talk.

The enhanced luminescence module can be used for all plate formats up to 384 wells.

The sensitivity of the detection system requires attenuation of high luminescence light levels. The attenuators, two neutral density filters (OD1 and OD2) installed on the filter wheel, are moved into the light path via the software. Combining OD1 and OD2 neutral density filters allows a light level attenuation of 1000x (corresponding to an OD3 filter).

The z-drive of the plate transport [5] ensures the optimal distance between microplate and luminescence optics, in order to maximize the signal and to minimize cross-talk. The z-adjustment is performed automatically after selecting the appropriate plate type in the software.

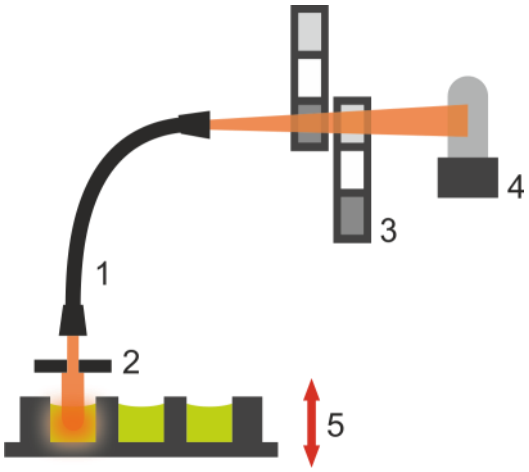


Figure 7: Optical system in luminescence enhanced module:
[1] luminescence fiber, [2] aperture wheel,
[3] two filter wheels, [4] detection unit, [5] z-drive of plate transport

8.4.3 Luminescence Detection

See chapter 8.3 Standard Luminescence Module.

8.5 Luminescence Specifications

NOTE: All specifications are subject to change without prior notification.

8.5.1 General Specifications

Parameters	Standard Luminescence Module	Enhanced Luminescence Module
Wavelength range	370-700 nm	370-700 nm
Wavelength range Luminescence scan	n.a.	390-660 nm
Wavelength discrimination and multicolor luminescence	n.a.	via filter sets
Integration time/well	10 - 60000 ms	10 - 60000 ms
Attenuation	1 OD, 2 OD	1 OD, 2 OD, 3 OD
Dynamic range	107-109	107-1010

8.5.2 Performance Specifications

Glow Luminescence detection limit (Standard and Enhanced Module)		
Plate type/Filling Volume	**Parameter**	**Criteria**
96-well plate, white, 200 µl	Integration time/well: 1000 ms	ATP: < 50 pM (< 10 fmol/well)
384-well plate, white, 100 µl	Integration time/well: 1000 ms	ATP: < 10 pM (< 1 fmol/well)
1536-well plate, white, 10 µl	Integration time/well: 1000 ms	ATP: < 1 nM (< 10 fmol/well)

Flash Luminescence detection limit (Standard and Enhanced Module)		
Plate type/Filling Volume	**Parameter**	**Criteria**
96-well plate, white, 200 µl	Integration time/well: 10000 ms	ATP: < 0.4 pM (< 80 amol/well)
384-well plate, white, 100 µl	Integration time/well: 10000 ms	ATP: < 0.8 pM (< 80 amol/well)

8.6 Quality Control of the Luminescence Module

8.6.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on site at Tecan.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

CAUTION: Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 has to be on the upper left side.

WARNING: The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

8.6.2 Detection Limit ATP 384-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

CAUTION: Switch on the instrument at least 15 minutes before starting a luminescence measurement to ensure stable conditions for the measurement.

Material:

- ATP Kit SL (BioThema AB, article no. 144-041)
- Greiner 384-well plate, flat bottom, white
- Pipettes + tips

Procedure:

Prepare reagents according to the manufacturer's instructions. Adjust ATP Standard to 10^{-7} M.

Pipette 100 µl of the Blank into the wells A4 – D10.

Pipette 20 µl of ATP standard 10^{-7} M into the wells A2 – D2, add 80 µl of ATP reagent and mix in well **(use fresh tip for each well); ATP reagent must NOT be contaminated with ATP standard!**

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	...	24
A		ATP		B	B	B	B	B	B	B			
B		ATP		B	B	B	B	B	B	B			
C		ATP		B	B	B	B	B	B	B			
D		ATP		B	B	B	B	B	B	B			
E													
...													
P													

ATP: 100µl, 2*10-8 M ATP (final concentration in well)
B: 100 µl Blank

Measurement Parameters:

Measurement mode: Luminescence

Integration time: 1000 ms

Plate definition file: GRE384fw

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(\text{fmol / well}) = \frac{2 \cdot 10^{-8} * 3 * SD_B}{\text{mean}_{ATP} - \text{mean}_B} * 0.0001 * \frac{1}{1e^{-15}}$$

$2*10^{-8}$	Concentration of ATP standard [M]
SD_B	Standard deviation of Blank (B: A4 – D10)
mean_{ATP}	Mean of wells filled with ATP standard
mean_B	Mean of Blank wells (B: A4 – D10)
0.0001	Conversion into mol/well
$1/1e^{-15}$	Conversion into fmol/well

8.6.3 Detection Limit ATP 1536-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

CAUTION: Switch on the instrument at least 15 minutes before starting a luminescence measurement to ensure stable conditions for the measurement.

Material:

- ATP Kit SL (BioThema AB, article no. 144-041)
- Greiner 1536-well plate, flat bottom, white
- Pipettes + tips

Procedure:

Prepare reagents according to the manufacturer's instructions. Adjust ATP Standard to 10^{-7} M.

Pipette 10 µl of the Blank into the wells A4 – D10.

Pipette 2 µl of ATP standard 10^{-7} M into the wells A2 – D2, add 8 µl of ATP reagent and mix in well (use fresh tip for each well); ATP reagent must NOT be contaminated with ATP standard!

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	...	24
A		ATP		B	B	B	B	B	B	B			
B		ATP		B	B	B	B	B	B	B			
C		ATP		B	B	B	B	B	B	B			
D		ATP		B	B	B	B	B	B	B			
E													
...													
P													

ATP: 10µl, 2*10-8 M ATP (final concentration in well)
B: 10 µl Blank

Measurement Parameters:

Measurement mode:	Luminescence
Integration time:	1000 ms
Plate definition file:	GRE1536fw

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(\text{fmol / well}) = \frac{2 \cdot 10^{-8} * 3 * SD_B}{\text{mean}_{ATP} - \text{mean}_B} * 0.00001 * \frac{1}{1e^{-15}}$$

$2*10^{-8}$	Concentration of ATP standard [M]
SD_B	Standard deviation of Blank (B: A4 – D10)
mean_{ATP}	Mean of wells filled with ATP standard
mean_B	Mean of Blank wells (B: A4 – D10)
0.0001	Conversion into mol/well
$1/1e^{-15}$	Conversion into fmol/well

9 Alpha Technology

9.1 Basic Principles

Amplified Luminescent Proximity Homogeneous Assays (AlphaScreen and AlphaLISA) are bead-based nonradioactive, homogeneous and sensitive assays perfectly suited for the study of biochemical interactions. The interaction between an acceptor and donor bead leads to the light output: Upon illumination with a high-energy light source, the photosensitive molecules contained in the donor beads produce high level of oxyradicals. These oxyradicals travel to the acceptor beads and trigger a cascade of reactions that ultimately lead to the generation of a strong chemiluminescent signal.

By using multiple acceptor beads which emit at different wavelengths, multiple analytes can be detected in one well (AlphaPlex).

9.2 Alpha Module

The Alpha module is used for detection of assays based on the Alpha technology (AlphaScreen, AlphaLISA and AlphaPlex). The Alpha module consists mainly of luminescence enhanced and laser module coupled with a contactless IR temperature sensor.

9.2.1 Filter

Predefined filters for Alpha based applications are available. Each band pass filter is generated by combination of a long pass and short pass filter built into the filter wheels of the luminescence enhanced module (see chapter 8.4.1 Luminescence Filters). The following table shows the wavelength characteristic of the predefined band pass filter:

Alpha Technology	Filter Choice	Central Wavelength/ Bandwidth
AlphaScreen	Long pass filter: 520 nm, Short pass filter: 620 nm	570 nm/100 nm
AlphaLISA	Long pass filter: 610 nm, Short pass filter: 635 nm	622.5 nm/25 nm
AlphaPlex	Long pass filter: 610 nm, Short pass filter: 635 nm Long pass filter: 535 nm, Short pass filter: 560 nm	622.5 nm/25 nm 547.5 nm/25 nm

9.2.2 Optics

As excitation light source for Alpha based assays a high-power laser [1] is used. The luminescence fiber [2] guides the light from the sample to the detector passing the filter wheels [4]. Long and short pass filters are installed on the filter wheels. Appropriate filter combinations result in dedicated band pass filters. The aperture wheel [3] adapts the light beam diameter to the used well size.

The Alpha module can be used with all microplate formats supported by the instrument.

Low light levels benefit from the single photon counting detector [5].

The Alpha module is combined with an IR-temperature sensor [6] to compensate for temperature-caused signal differences in every microplate well.

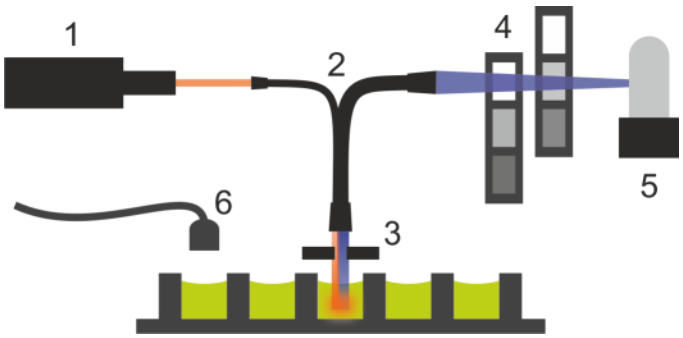
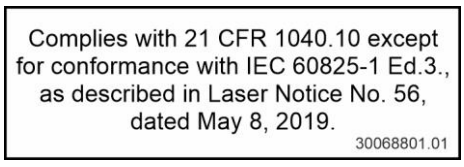


Figure 8: Optical system in Alpha module: [1] laser module; [2] luminescence fiber; [3] aperture wheel; [4] filter wheels; [5] detection unit; [6] IR-temperature sensor

9.2.3 Laser

The laser module uses a high power laser (680 nm/750 mW) as the excitation light source. A SPARK instrument equipped with an Alpha module is a LASER CLASS 1 product. The instrument complies with FDA radiation performance standards 21 CFR 1040.10 except for conformance with IEC 60825-1 Ed.3, as described in Laser Notice No. 56, dated May 8, 2019.

The following labels are attached to the rear of the instrument:



WARNING: Laser radiation Class IV inside the instrument - Keep the instrument lid closed during measurement.

9.2.4 Detection

CAUTION: Switch on the instrument at least 15 minutes before starting a measurement to ensure stable conditions for the measurement.

The luminescence and Alpha module detection system utilizes the single photon counting measurement technique. This is based on a dedicated luminescence detector with appropriate measurement circuitry. This technique is very robust against noise, and is, therefore, the preferred method for performing measurements at very low light levels.

CAUTION: Use white or light grey plates for Alpha Technology based measurements. Never use black plates and don't measure empty wells to avoid damages caused by laser radiation.

9.2.5 Temperature Correction

To compensate for the temperature sensitive nature of Alpha based assays, the Alpha module offers a temperature correction system.

A contactless temperature sensor measures the temperature inside each well and the measured count rates are automatically normalized to a temperature of 22.5 °C. Temperature and signal detection is performed in parallel. Due to the position of the temperature sensor the reading direction is from right to left (A12 to A1, B12 to B1 in case of a 96-well plate) if using the temperature correction function.

NOTE: To ensure the best performance for Alpha Technology based assays, the SPARK should be operated in a temperature-regulated environment (±1 °C in the range of 20–25 °C).

9.3 Alpha Specifications

NOTE: All specifications are subject to change without prior notification.

9.3.1 General and Performance Specifications

Parameters	Specification
Excitation time/well	10 - 1000 ms
Integration Time/well	10 - 60000 ms
Predefined Filter	AlphaScreen, AlphaLISA, AlphaPlex
Temperature correction	available
Detection Limit 384-well plate low volume (Omnibeads)	< 12.5 ng/ml
Uniformity 384-well plate low volume (Omnibeads)	< 8 CV%

9.4 Quality Control of the Alpha Module

9.4.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on Tecan site.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

We recommend adapting these tests and the acceptance criteria to the laboratory's primary application. Ideally these tests must be performed with the laboratory's own plates, fluorophore, buffers, volumes and all the appropriate settings (filters, flashes, delays, etc.).

WARNING: Before starting measurements, make sure that the microplate position A1 is inserted correctly. The position of well A1 has to be on the upper left side.

WARNING: This gives instructions on how to check the specifications of the instrument. If the results of these control tests do not lie within the official specifications of the instrument, please contact your local service center for further advice.

9.4.2 Detection Limit AlphaScreen Omnibeads 384-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare instrument for measurement and start measurement immediately after pipetting.

CAUTION: Switch on the instrument at least 15 minutes before starting a measurement to ensure stable conditions for the measurement.

Material:

- AlphaScreen Omnibeads
- Greiner 384-well plate, flat bottom, white
- Phosphate-buffered saline (PBS)
- Pipettes + tips

Procedure:

Dilute the Omnibeads stock solution 1:500 in PBS by adding 3 µl of the stock solution (5 mg/ml) to 1497 µl PBS yielding a solution of 10 µg/ml. Prepare 12 further dilutions in 1:2 steps by pipetting 750 µl of the previous dilution step to 750 µl PBS. Use a new tip for each dilution step.

Pipette 100 µl of each dilution into 5 replicate wells of the microplate according to the plate layout. Use 100 µl PBS for the blank wells.

CAUTION: Use a fresh tip for each concentration and take care NOT to contaminate the blank with any Omnibeads dilution!

Plate Layout:

	2	3	4	5	6	7	8	9	10	11	12	...	24
A		10.00 µg/ml											
B		5.00 µg/ml											
C		2.50 µg/ml											
D		1.25 µg/ml											
E		0.62 µg/ml											
F		0.31 µg/ml											
G		0.15 µg/ml											
H		0.08 µg/ml											
I		0.04 µg/ml											
J		0.02 µg/ml											
K		0.01 µg/ml											
L		0.005 µg/ml											
M		0.0025 µg/ml											
N		PBS											
O													
P													

100 µl of each Omnibeads concentration (5 replicate wells each)
100 µl PBS = Blank

Measurement Parameters:

- Measurement mode: AlphaScreen
- Excitation time: 100 ms
- Integration time: 300 ms
- Temperature correction: Activated
- Plate definition file: GRE384fw

Evaluation:

Calculate the average and standard deviation for each Omnibeads concentration. Perform a blank reduction by subtracting the average signal of the blank wells from the average signal of each Omnibeads concentration.

Plot the average blank-corrected values against the final Omnibeads concentration in a XY scatter diagram. Add a linear trend line with intercept set to 0 and solve the trend line equation (y=kx) using the 3-fold standard deviation of the blank as y.

$$x = \frac{y}{k}$$

y = 3*standard deviation of the blank

Extrapolate the detection limit [ng/ml] by using the 3-fold standard deviation of the blank as y.

9.4.3 Uniformity AlphaScreen Omnibeads 384-Well Plate

The uniformity defines the well-to-well variations when measuring a multi-well plate. The uniformity is calculated as percentage deviation from the mean value.

Before pipetting the plate, prepare instrument for measurement and start measurement immediately after pipetting.

CAUTION: Switch on the instrument at least 15 minutes before starting a measurement to ensure stable conditions for the measurement.

Material:

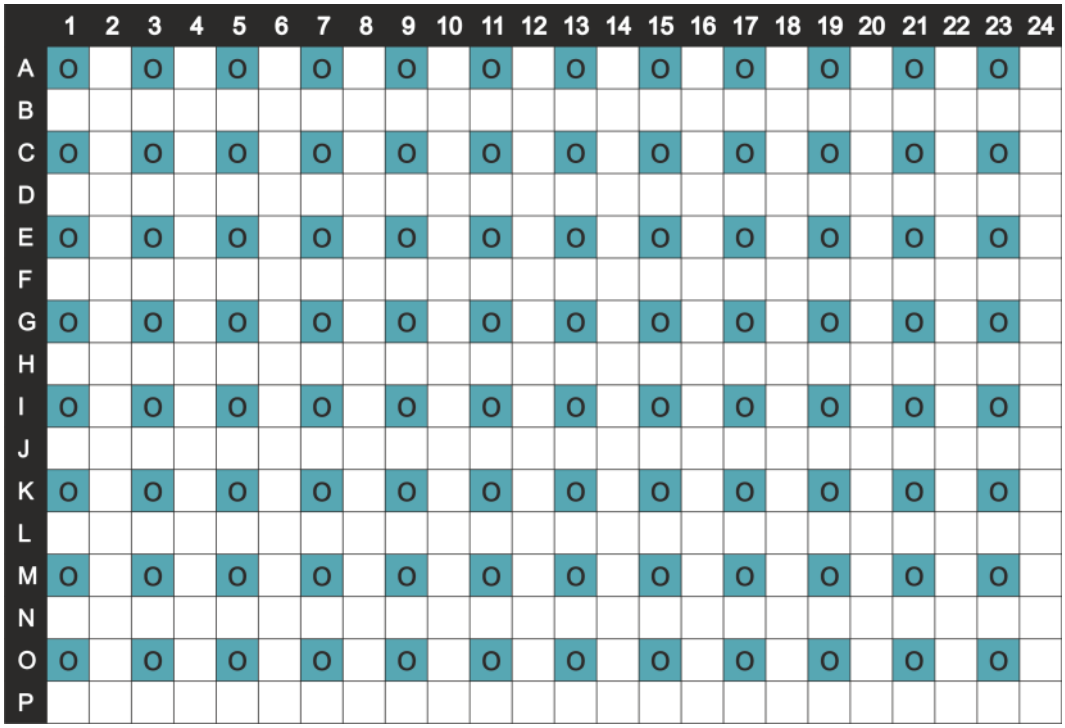
- AlphaScreen Omnibeads
- Greiner 384-well plate, flat bottom, white
- Phosphate-buffered saline (PBS)
- Pipettes + tips

Procedure:

Dilute the Omnibeads stock solution 1:2000 in PBS by adding 3 µl of the stock solution (5 mg/ml) to 5997 µl PBS yielding a solution of 2.5 µg/ml.

Pipette 100 µl of the Omnibeads dilution into the wells of the microplate according to the plate layout.

Plate Layout:



O: 100 µl/well Omnibeads dilution (2.5 µg/ml)

Measurement Parameters:

Measurement mode:	AlphaScreen
Excitation time:	100 ms
Integration time:	300 ms
Temperature correction:	Activated
Plate definition file:	GRE384fw

Evaluation:

Calculate the Uniformity as follows:

$$\text{Uniformity (CV\%)} = \frac{SD_O * 100}{mean_O}$$

SD_O	Standard deviation of wells filled with 2.5 µg/ml Omnibeads solution
$mean_O$	Mean of wells filled with 2.5 µg/ml Omnibeads

10 Absorbance

10.1 Basic Principles

Absorbance assays provide a fast and convenient method for qualitative and quantitative characterization of biological compounds. They are based on measuring the amount of light absorbed by the molecules included in a liquid sample when light passes through the sample. The reduced light intensity due to the light absorption by a compound is proportional to the corresponding concentration of that species (Beer-Lambert law) and can be applied for its quantitative analysis (ELISA, protein, nucleic acid quantitation). On the other hand, color changes due to a biochemical reaction in the sample are used to detect a compound of interest in the solution (e.g. qualitative ELISA).

Absorbance signal is a measure for the attenuation of monochromatic light when transmitted through a sample. Absorbance is defined by Beer-Lambert law as:

$$A = LOG10(I_0/I_{SAMPLE})$$

Where I_{SAMPLE} is the intensity of the transmitted light, I_0 the initial light intensity not attenuated by the sample.

Furthermore, the absorbance depends on the concentration of absorbing compound (c), its extinction coefficient (ε) and the path length (d) which the light passes through:

$$A = \varepsilon * c * d$$

The unit is assigned as OD (Optical Density):

1 OD means 10-fold light attenuation, i.e. 10 % transmission

2 OD means 100-fold light attenuation, i.e. 1 % transmission

10.2 Absorbance Measurement Techniques

10.2.1 Absorbance Scan

Absorbance scans measure the absorbance behavior of compounds under investigation within a selected wavelength range. The absorbance spectrum of a compound is often affected by environmental influences such as pH, temperature or protein folding. Therefore it is an essential detection mode for every research group.

10.3 Absorbance Module

Absorbance applications can be performed at any wavelength from 200 to 1000 nm. For absorbance measurements, a fiber bundle guides the light from the monochromator to the absorbance optics, which focuses the light into the wells of a microplate. The transmitted light is detected by a silicon photodiode.

Before the measurement of the microplate is performed, a reference measurement is made with the plate carrier moved away from the light beam.

10.3.1 Absorbance Optics

The standard absorbance module consists of the flash lamp, the monochromator, the absorbance fiber, and the photodiode (see picture below).

The light of the Xenon-flash lamp [1] (light source) passes an order sorting filter [2] and is focused onto the entrance slit of a single grating monochromator by a condenser mirror. By moving the optical grating [3] the measurement wavelength is selected and focused onto the exit slit of the monochromator. There the light enters the absorbance fiber [4], which guides the light onto the sample by an elliptical mirror [5]. A part of this light is reflected onto a reference photodiode. Afterwards the light is collected by a lens and focused onto the measurement photodiode [6].

The Absorbance module can be used with all microplate formats supported by the instrument.

At the focal point the spot diameter of the absorbance light beam is about 1 mm.

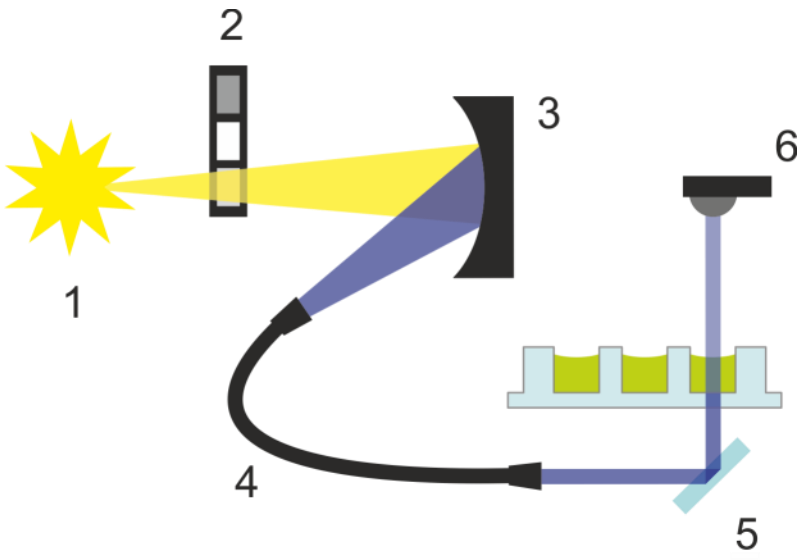


Figure 9: Optical system in absorbance module
Xenon-flash lamp [1] (light source), order sorting filter [2], optical grating [3], absorbance fiber [4], elliptical mirror [5], measurement photodiode [6]

Detection

A silicon photodiode is used for the measurement of the transmitted light. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels of absorbance measurements below 4 OD.

10.4 Cuvette Module

Cuvette based applications can be performed at any wavelength from 200 to 1000 nm. The optical path of the cuvette module is similar to the optical path of the absorbance standard module. A fiber bundle guides the light from the monochromator to the absorbance optics, which focuses the light into the cuvette. The transmitted light is detected by a photodiode.

10.4.1 Cuvette Optics

The absorbance cuvette module consists of the flash lamp, the monochromator, the absorbance fiber, and the photodiode (see figure below).

The light of the Xenon-flash lamp [1] (light source) passes an order sorting filter [2] and is focused onto the entrance slit of a single grating monochromator [3] by a condenser mirror. By moving the optical grating the measurement wavelength is selected and focused onto the exit slit of the monochromator. There the light enters the absorbance fiber [4] which guides the light onto the sample in the cuvette [5]. A part of the light is reflected onto to the reference photodiode. The transmitted light is detected by the measurement photodiode [6]. At the focal point the spot diameter of the absorbance cuvette light beam is about 1 mm.

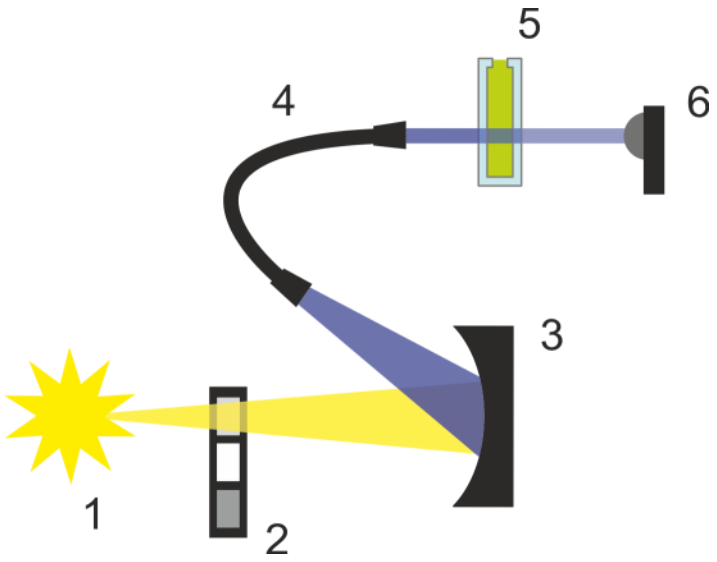


Figure 10: Optical system absorbance cuvette module
Xenon-flash lamp [1] (light source), order sorting filter [2], optical grating [3], absorbance fiber [4], cuvette [5], measurement photodiode [6]

Detection

A silicon photodiode is used for the measurement of the transmitted light. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels of absorbance measurements below 4 OD.

10.5 Measurement Equipment

10.5.1 Microplates

Generally, for absorbance measurements, transparent or UV-transparent microplates are used. For high OD values, black microplates with transparent bottoms are superior. In general, to obtain accurate OD values measurements above OD3 are not recommended, especially when using 1536-well plates. Dilutions of the measurement samples will result in more accurate data.

CAUTION: Use UV compatible microplates for absorbance measurements in UV wavelength range.

NOTE: For absorbance measurements of nucleic acids in small volumes (2 µl) use Tecan's NanoQuant Plate. With this device it is possible to measure 16 different samples in one measurement.

NOTE: To obtain more accurate measurement data avoid OD values above 3.

10.5.2 Cuvette Adapter

The Tecan cuvette adapter can be used to measure four cuvettes in one measurement. For suitable cuvette dimensions, see the table below. When using the cuvette adapter, the cuvette has to be inserted horizontally and closed tightly to avoid any liquid leakage. Additionally, the cuvette has to be filled with the maximum filling volume to prevent the formation of air bubbles at the measurement window.

The cuvette adapter was created to perform measurements with cuvettes that comply with the following dimensions (table):

Dimension	Parameters
Absolute height (including lid)	35 - 55 mm
Foot print (outer dimension)	12.5 x 12.5 mm
Optical path	10 mm

*If using a cuvette with different optical path measurement results have to be corrected accordingly.

CAUTION: When performing measurements with the cuvette adapter, always use the maximum filling volume of the cuvette to prevent the formation of air bubbles at the measurement window. Close the cuvette tightly to avoid any liquid leakage.

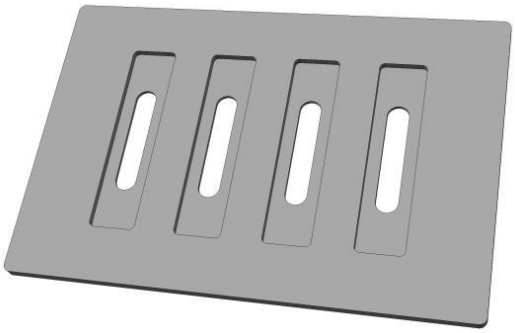


Figure 11: Cuvette adapter

Figure 12: Example of a suitable cuvette type

10.5.3 Cuvette Port

Instead of in a microplate, absorbance measurement can be performed in a cuvette that is inserted into the cuvette port of the instrument. The cuvette port was created to perform measurements with cuvettes that comply with the following dimensions (table):

Dimension	Parameters
Absolute height (including lid)	35 - 55 mm
Foot print (outer dimension)	12.5 x 12.5 mm
Optical path	10 mm*
Central height	15 mm
Measurement window	> 2 x 2 mm

*If using a cuvette with different optical path measurement results have to be corrected accordingly.

CAUTION: Always use a valid filling volume. Make sure that the liquid level in the cuvette exceeds 20 mm (height). Too low liquid levels lead to wrong measurement results.

CAUTION: The cuvette port has a measurement window of 2 x 2 mm and a central height of 15 mm.

CAUTION: The cuvette has to be inserted into the carrier so that the measurement window of the cuvette lines up with the measurement window of the cuvette carrier. For correct insertion please follow the arrow on the cuvette port (see figure below).

Figure 13: Cuvette Port.
The direction of the arrow on the panel represents the direction of light.

CAUTION: Close the cuvette port properly when it is not used. Contaminations lead to wrong measurement results.

CAUTION: Ensure that the cuvette port is closed properly before starting a cuvette measurement. Open cuvette port lids lead to wrong measurement results.

CAUTION: Ensure that the cuvette is inserted properly into the cuvette port before starting a cuvette measurement. Misalignments lead to wrong measurement results.

10.6 NanoQuant Application

Tecan provides a ready-to-use NanoQuant app for:

- Quantifying nucleic acids
- Labeling efficiency of nucleic acids
- Quantifying proteins

When using the app, the calculations of the nucleic acid, protein, and dye contents as well as the purity checks are performed automatically.

For details, see chapter NanoQuant App in the SparkControl software manual.

10.6.1 NanoQuant Plate

Tecan's NanoQuant Plate is a powerful measurement tool for the quantification of small volumes (2 µl) of nucleic acids in absorbance mode. The NanoQuant Plate can also be used for determining the nucleic acid labeling efficiency. In that case, absorbance is measured at 230 nm, 260 nm and 280 nm as well as at the wavelength of the labeled dyes, e.g. 550 and 649 nm for Cy3 and Cy5, respectively.

Special quartz optics ensures outstanding performance and a high rate of reproducibility. It is possible to measure 16 different samples in a single measurement procedure. The NanoQuant Plate is compatible with an eight-channel pipette for quick and easy sample application.

Figure 14: NanoQuant Plate

10.7 Absorbance Specifications

NOTE: All specifications are subject to change without prior notification.

10.7.1 General Specifications

Parameters	Characteristics
Wavelength range	200 - 1000 nm, selectable in 1 nm steps
Wavelength accuracy	≤ 0.8 nm
Wavelength reproducibility	≤ 0.5 nm
Bandwidth fixed wavelength	3.5 nm
Measurement range	0 - 4 OD

10.7.2 Performance Specifications in Microplates

Plate Type/ Filling Volume	Parameters	Specifications	Criteria
96-well plate, transparent, 200 µl	Flashes/well: 25	Accuracy 0–0.8 OD	+/- 0.008 OD
96-well plate, transparent, 200 µl	Flashes/well: 25	Accuracy 0.8–2.5 OD	< +/- 1.0%
96-well plate, transparent, 200 µl	Flashes/well: 25	Accuracy 2.5–3.0 OD	< +/- 1.5%
96-well plate, transparent, 200 µl	Flashes/well: 25	Precision 0–1.2 OD	< +/- 0.006 OD
96-well plate, transparent, 200 µl	Flashes/well: 25	Precision 1.2–3.0 OD	< +/- 0.5%
96-well plate, UV transparent, 200 µl	Flashes/well: 25	Linearity 0–3 OD at 260 nm	R2 > 0.999
96-well plate, transparent, 200 µl	Flashes/well: 25	Uniformity at 1 OD	< 3 %

10.7.3 Measurement Times

Parameters	Measurement time
Measurement time 96-well, 1 flash	< 14 seconds
Measurement time 384-well, 1 flash	< 30 seconds
Fast Scan (200-1000 nm, 1 nm steps)	< 5 seconds

Fast reading times are determined by using one flash only, plate-in and plate-out movements are not included in the measurement time.

10.7.4 Performance Specifications in Cuvettes (Cuvette port)

Cuvette Type	Parameters	Specifications	Criteria
Quartz cuvette, 1 cm light path	Flashes: 25 Wavelength: 260 nm	Detection limit (DNA)	< 0.2 ng/µl dsDNA
Quartz cuvette, 1 cm light path	Flashes: 25 Wavelength: 280 nm	Detection limit (Protein: BSA, IgG, Lysozyme)	< 0.1 mg/ml
Quartz cuvette, 1 cm light path	Flashes: 1	Fast Scan (200-1000 nm, 1 nm steps)	< 5 seconds

10.7.5 Specifications for the NanoQuant Plate

General Specifications for the NanoQuant Plate:

Parameters	Characteristics
Optics	16 Quartz lenses (= 16 sample positions)
Quartz lenses	Diameter: 2.2 mm
Optical path	0.5 mm
Sample volume	2 µl

Performance Specifications for the NanoQuant Plate:

Absorbance	Performance
Detection limit (DNA)	< 2 ng/µl dsDNA
260/280 nm OD ratio accuracy	< 0.07
260/230 nm OD ratio accuracy	< 0.08
Measurement time for DNA quantification (consisting of a full wavelength scan plus fixed wavelength measurements at 230, 260, 280 and 310 nm)	< 8 s / sample
Detection limit (Cy3, Cy5, Alexa Fluor 555, Alexa Fluor 647, Alexa Fluor 660)	0.1 µM
Detection limit (Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 594)	0.2 µM

10.8 Quality Control of the Absorbance Module

10.8.1 Periodic Quality Control Tests

Depending on usage and application, we recommend a periodic evaluation of the instrument on Tecan site.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

CAUTION: Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 has to be on the upper left side.

WARNING: If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

10.8.2 Uniformity 96-Well Plate

Uniformity is a measure for the well-to-well variations when measuring a multi-well-plate. The uniformity is calculated as percentage deviation from the mean value.

Material:

- Orange G [60 mg/l] diluted in distilled water (Sigma-Aldrich, O3756)
- Greiner 96-well plate, flat bottom, transparent
- Pipette + tips

Procedure:

Pipette 200µl of the reagent into the wells of a Greiner 96-well plate (flat bottom, transparent) according to the plate layout.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG
B												
C	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG
D												
E	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG
F												
G	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG	OG
H												

OG: Orange G [60 mg/l]

Measurement Parameters:

Measurement mode:	Absorbance
Measurement wavelength:	492 nm
Number of flashes:	25
Settle time	300 ms
Plate definition file:	GRE96ft

Evaluation:

Calculate the Uniformity (CV %) as follows:

$$\text{Uniformity} \quad (\text{CV}\%) \quad = \frac{SD_{OG} * 100}{mean_{OG}}$$

SD_{OG}	Standard deviation of wells filled with OG
$mean_{OG}$	Mean of wells filled with OG

10.8.3 Quality Control of the NanoQuant Plate

Material:

- Tris-EDTA buffer (BioThema, no. 21-103)
- Tecan NanoQuant Plate
- Pipette + tips

Procedure:

Pipette 2 µl of the reagent onto all positions of the NanoQuant Plate.

Measurement Parameters:

Start the NanoQuant application and perform average blanking procedure over all wells (16 positions).

Evaluation:

The test is passed if the average blanking results at OD 260 are in the range of 10 % (CV). If average blanking is out of range, the failed wells are highlighted indicating that these wells are dirty due to lint, fingerprints, etc.

11 Fluorescence

11.1 General Description

Fluorescence is the emission of light by a molecule when struck by light of a specific wavelength. A single fluorescent molecule can release one fluorescence photon (quantum of light). This is a part of the energy, which has been absorbed by electronic excitation, but could not be released fast enough into thermal energy. Due to this loss of energy by thermal processes, the fluorescence always results at a wavelength higher than the excitation wavelength (Stokes Shift).

The average time it takes between excitation and emission is called the fluorescence lifetime. For many fluorescent molecular species, fluorescence lifetime is on the order of nanoseconds (prompt fluorescence). After excitation, fluorescence emission occurs with a certain probability (quantum yield), which depends on the fluorescent species and its environmental conditions.

11.2 Fluorescence Intensity (FI)

In many fluorescence intensity based applications, the intensity of fluorescence emission is measured to determine the abundance of fluorescent labeled compounds. In these assays, all factors having an influence on fluorescence emission need to be controlled experimentally. Temperature, pH-value, dissolved oxygen, type of solvent, etc. may significantly affect the fluorescence quantum yield and therefore the measurement results.

11.3 Fluorescence Resonance Energy Transfer (FRET)

The Fluorescence Resonance Energy Transfer is the transfer of excitation energy from a donor molecule to an acceptor molecule without emitting a photon. The acceptor may receive excitation energy from the donor, if both are in close proximity and the emission spectrum of the donor overlaps the excitation spectrum of the acceptor (resonance condition). Therefore, FRET is a process in which the binding events between two fluorescent-labeled compounds can be detected.

11.4 Time–Resolved Fluorescence (TRF)

Time-resolved fluorescence applications apply fluorescent acceptors with a long-lived fluorescence signal (e.g. lanthanides like Europium and Terbium). Consequently, the short-lived unspecific background fluorescence signal can be excluded by using a time delay between the excitation and signal integration thus maximizing the signal to background ratio.

11.5 Fluorescence Polarization (FP)

Fluorescence polarization measures rotational immobility of a fluorescently labeled compound due to its environment.

Fluorescence polarization is defined by the following equation:

$$P = \frac{(I_{||} - I_{\perp})}{(I_{||} + I_{\perp})}$$

Where ***P*** equals polarization, $I_{||}$ equals the emission intensity of the polarized light parallel to the plane of excitation and $I_{\perp}$ equals the emission intensity of the polarized light perpendicular to the plane of excitation.

FP is suitable for binding studies, because tumbling of molecules may be dramatically reduced after binding to a much larger site, resulting in high polarization values.

For a simplified picture of FP, fluorescent molecules may be visualized as antennae, which need suitable orientation to pick up light waves of excitation successfully. Using planar polarized light, only a specifically oriented subset of the randomly oriented molecules is susceptible to excitation.

The FP measurement result will be calculated from two successive fluorescence intensity measurements. They differ in the mutual orientation of polarizing filters, one being inserted into the excitation light path, another in the emission light path.

11.6 Fluorescence Intensity Module

The fluorescence module is designed as a Fusion Optics system. The wavelength selection for excitation and emission can be performed by either the monochromator or the filter option. The monochromator and the filter mode are independently combinable for the excitation and the emission and therefore provide a detection system with the maximum flexibility and maximum signal output. Furthermore, the fluorescence signals can be read from top and bottom.

11.6.1 How a Monochromator Works

A monochromator is an optical instrument that enables any wavelength to be selected from a defined optical spectrum. Its method of operation can be compared to a tunable optical filter, which allows both the wavelength and bandwidth to be adjusted.

A monochromator consists of an entrance slit, a dispersive element (optical grating) and an exit slit. The dispersive element diffracts the light into the optical spectrum and projects it onto the exit slit.

Rotating the optical grating around its vertical axis moves the spectrum across the exit slit and only a small part of the spectrum (band pass) passes through the exit slit. This means that when the monochromator entrance slit is illuminated with white light, only light with a specific wavelength (monochromatic light) passes through the exit slit. The wavelength of this light is set by the rotation angle of the optical grating. The bandwidth is set by the width of the exit slit. The bandwidth is defined as full width at half maximum intensity (FWHM).

Monochromators block undesired wavelengths, typically amounting to 10^3. This means when the monochromator is set for light with a wavelength of 500 nm and the PMT detects a signal of 10.000 counts, light with different wavelengths creates a signal of only 10 counts. For applications in the fluorescence range, this blocking is often not sufficient because the fluorescence light to be detected is usually much weaker than the excitation light. To achieve a higher level of blocking, two monochromators are connected in series, i.e. the exit slit of the first monochromator acts as the entrance slit of the second monochromator simultaneously. This is known as a double monochromator. In this case, the blocking count reaches a factor of 10^6, a value typically achieved by Interference filters.

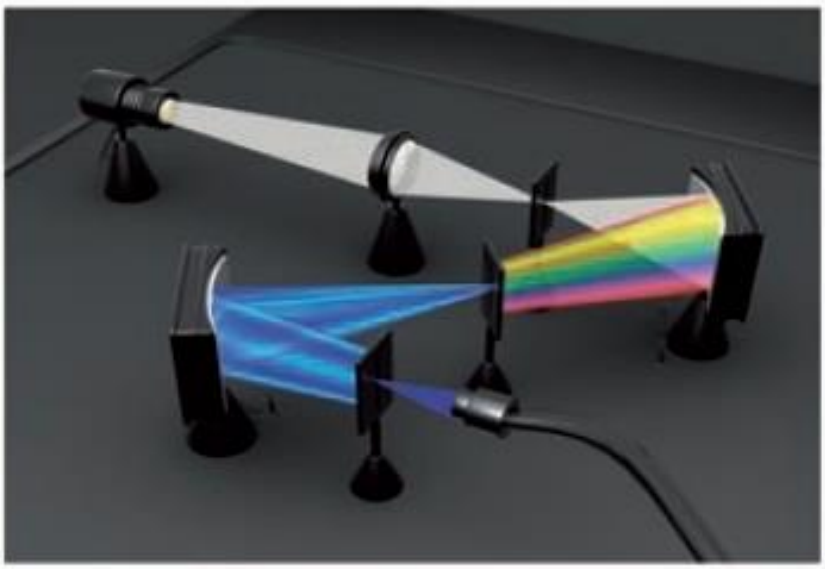


Figure 15: Double monochromator concept

11.7 Fluorescence Top Module

11.7.1 Fluorescence Top Module Optics

The fluorescence top module consists of a flash lamp as light source, monochromators and/or filters for wavelength selection, a measurement head for top reading, and a photomultiplier tube as detector.

The light source is a Xenon-flash lamp [1]. The measurement head is connected to the excitation and emission modules by fiber bundles. The excitation and the emission lights are guided directly to the fiber by rotating the mirror [2] and the fiber end [3]. The wavelength selection is done either by filters [4] in the measurement head or by double monochromators [5]. The mirror slide [6] in the measurement head can switch between different mirror options. A photomultiplier tube [7] serves as detector.

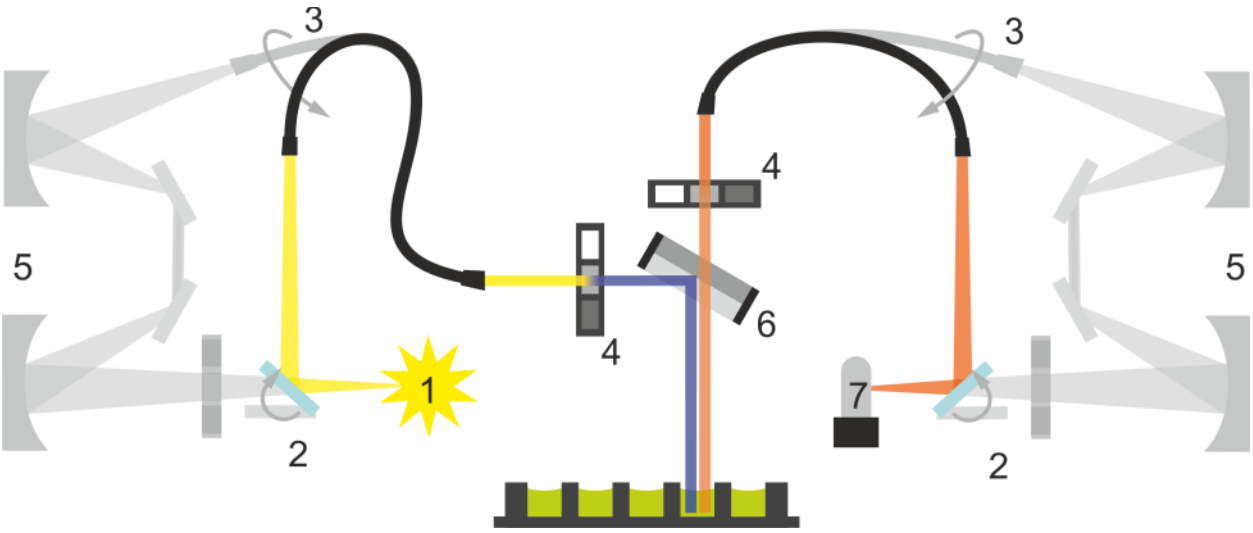


Figure 16: Fluorescence module (Fusion Optics concept) – filter option is highlighted

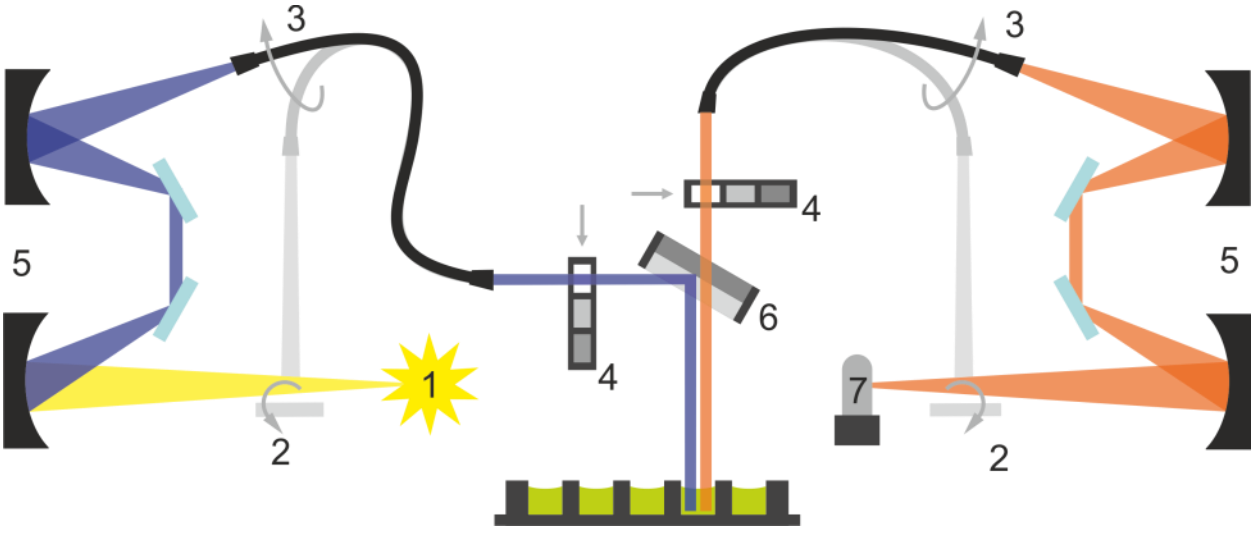


Figure 17: Fluorescence module (Fusion Optics concept) – monochromator option is highlighted

11.7.2 Fluorescence Top Module Options

The SPARK can either be equipped with the Fluorescence Standard Module or the Fluorescence Enhanced Module. In general, the Enhanced Module is more sensitive than the Standard Module.

The following table shows the main differences between Fluorescence Standard and Fluorescence Enhanced module:

Description	Standard Module	Enhanced Module
Monochromator bandwidth	Fixed: 20 nm	Variable: 5, 7.5, 10, 15, 20, 25, 30, 50 nm
Mirrors	2 mirror positions: 50% mirror, 510 Dichroic	5 mirror positions: 50% mirror, 510 Dichroic, 560 Dichroic, 625 Dichroic, user-definable Dichroic
Flash lamp	5 Watt, 50 Hz	20 Watt, 100 Hz
Plate compatibility	Up to 384-well plates	Up to 1536-well plates

11.8 Fluorescence Bottom Module

11.8.1 Fluorescence Bottom Module Optics

Based on the fluorescence Fusion Optics system the fluorescence bottom module can be combined with all of its options to select the appropriate wavelengths. The bottom module additionally consists of a fiber bundle, the bottom measurement head, and the bottom cube.

For the description of the Fusion Optics system, see 11.6 Fluorescence Intensity Module.

The bottom mirror unit is installed inside the mirror slide [1] and guides the excitation light via bottom fiber [2] to the bottom measurement head [3]. The bottom mirror [4] reflects the light onto the sample. The collected emission light is again guided to the bottom cube by the bottom fiber and focused to the emission fiber [5].

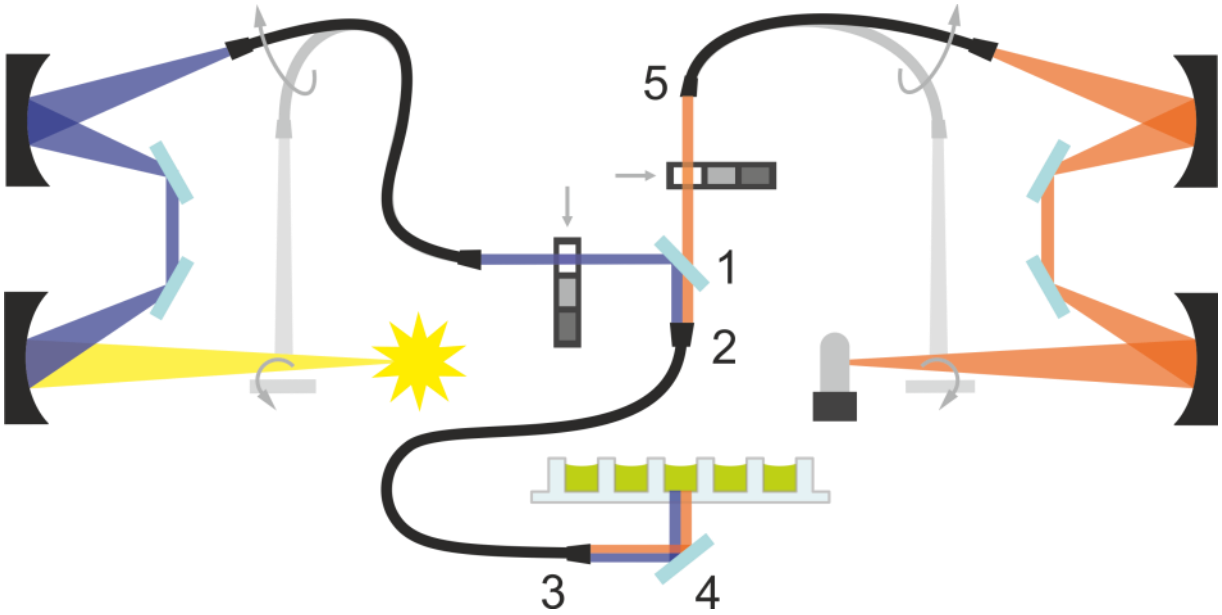


Figure 18: Fluorescence bottom module – monochromator option highlighted

11.8.2 Fluorescence Bottom Module Options

The SPARK can either be equipped with the Fluorescence Standard Module or the Fluorescence Enhanced Module. In general, the Enhanced Module is more sensitive than the Standard Module.

The Fluorescence Bottom Standard Module can either be equipped with a VIS- or a UV-VIS fiber. The Fluorescence Bottom Enhanced Module is equipped with a UV-VIS fiber by default.

For further differences between Fluorescence Standard and Fluorescence Enhanced Module, see chapter 11.7 Fluorescence Top Module.

11.9 Fluorescence Functions

11.9.1 Z-Positioning

The Z-position defines the distance between the measurement head and the surface of the microplate. It can be adjusted by moving the plate transport up and down. As light is reflected onto the sample liquid surface, a Z-adjustment helps to maximize signal to noise ratio.

The Z-position option is available for fluorescence intensity top as well as bottom measurements.

11.9.2 Fluorescence Detection

A photo-multiplier tube (PMT) is used for the detection of the low light levels associated with fluorescence. The fluorescence PMT is sensitive up to the near infrared (NIR) while still having low dark current. Electronic circuitry uses analog to digital conversion of PMT input current. The gain is an amplification factor for the PMT. Adjusting the PMT gain enables measurement of a wide range of concentrations in lower or higher concentration domains.

11.9.3 Fluorescence Scans

By holding the excitation light at a constant wavelength, the different wavelengths of fluorescent light emitted by a sample are detected via the monochromator. This process defines the recording of an **emission spectrum.** An **excitation spectrum** is the opposite, whereby the emission light is held at a constant wavelength, and the excitation light is scanned through many different wavelengths.

A **3D spectrum** is generated by recording the emission spectra resulting from a range of excitation wavelengths and combining them all together. A three dimensional surface data set is thus obtained: fluorescence emission intensity as a function of excitation and emission wavelengths. A 3D scan over the full wavelength range consists of more than $4x10^5$ single measurement points.

11.9.4 Spectral Intensity Calibration

The fluorescence intensity values are influenced by instrument components as well as by the selected wavelengths and therefore may distort the measured spectra. Calibration data is saved on the instrument and intensity value corrections are performed automatically.

11.10 Measurement Equipment

11.10.1 Filters

The optical filters (band pass filters) are mounted in the filter slides. The spectral transmission and the bandwidth of the fluorescence filters are optimized for achieving excellent sensitivity.

Contact Tecan for filters other than those supplied on the delivered filter slides.

11.10.2 Filter Slides

Two separate filter slides, an excitation and an emission filter slide, enable the user to work with six independent filter pairs for fluorescence measurements. The information about the inserted filters is saved on the microchip integrated into each filter slide.

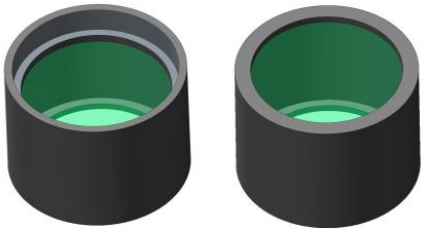
CAUTION: There are two types of filters available. It is important that light travels through the filter in the correct direction. Before inserting a new filter carefully consider the orientation of the filter and the direction of light through the filter slide.

For filters with an arrow on the side, the light must travel in the same direction as the arrow.

For filters without an arrow, the end with the lip must face away from the light source:

Filters have two different ends – one has a lip and one doesn't have a lip.

End with lip	End without lip



Direction of light through the filter:

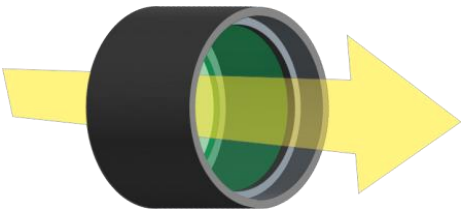


Figure 19: The light travels from the end without the lip towards the end with the lip.

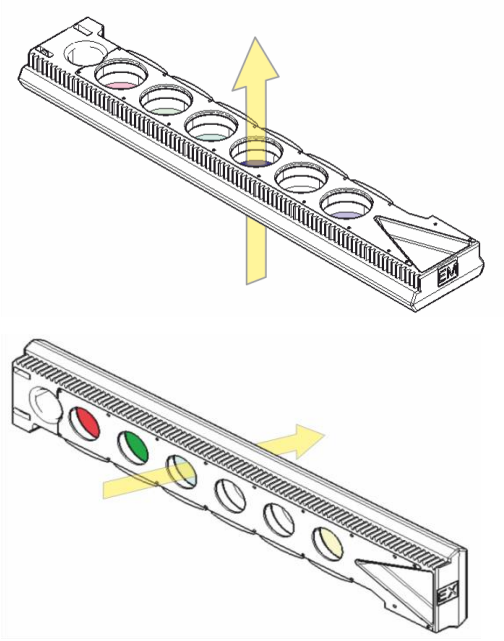


Figure 20: Direction of light through the filter slides

11.10.3 Installing and Removing Filters

No special tool is necessary to install or remove the filters of the excitation or emission filter slide.

To install a filter simply push the button next to the appropriate filter slot, insert the filter and release the button to secure the filter in the slot. Check that the filter is positioned firmly on the bottom of the filter slot.

NOTE: Make sure that the filters are inserted in the correct direction (see chapter 11.10.2 Filter Slides).

CAUTION: The filters are precision optical components, which should be handled by the edges and not scratched or stored face down in a drawer. Once the filters are installed in the slide, they are relatively well protected, but care should be exercised when handling or storing them.

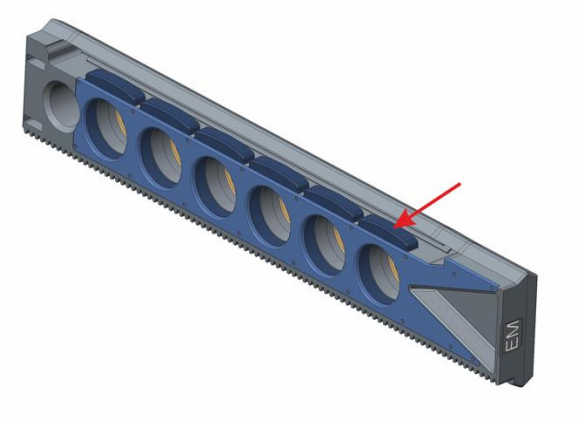


Figure 21: Remove the filter by pushing the button next to the appropriate filter slot (see picture above), turn the filter slide over and the filter will slide out of the slot.

11.10.4 Inserting Filter Slides

To insert the filter slides open the door flap manually. For the ease of identification the excitation and emission filter slides are labeled differently. Move the filter slides gently into the respective slots as indicated (chip side first) and push until the drive retract them automatically.

CAUTION: Do not push a filter slide further into the instrument when the drive has started to retract it.

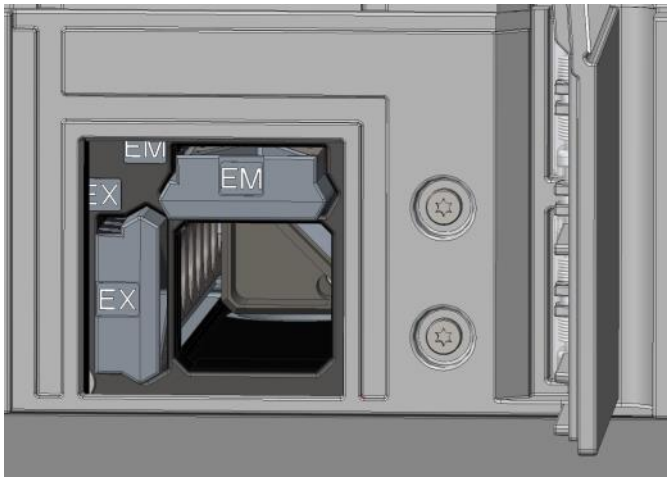


Figure 22: Inserting filter slides

Eject the filter slides via the software or by using the Onboard control button on the front of the instrument (see 2.6 Onboard Control Buttons).

11.10.5 Defining the Filters

CAUTION: Any changes to the filters in the filter slide are to be carried out by trained personnel! The instrument is able to recognize predefined filter slides and you should not attempt to change the filter values.

However, if the filters in the filter slide have been changed or if a new undefined customized filter slide is to be used, the filter slides need to be defined.

A custom filter can be defined via the Filter Definition window in the Dashboard or Method Editor:

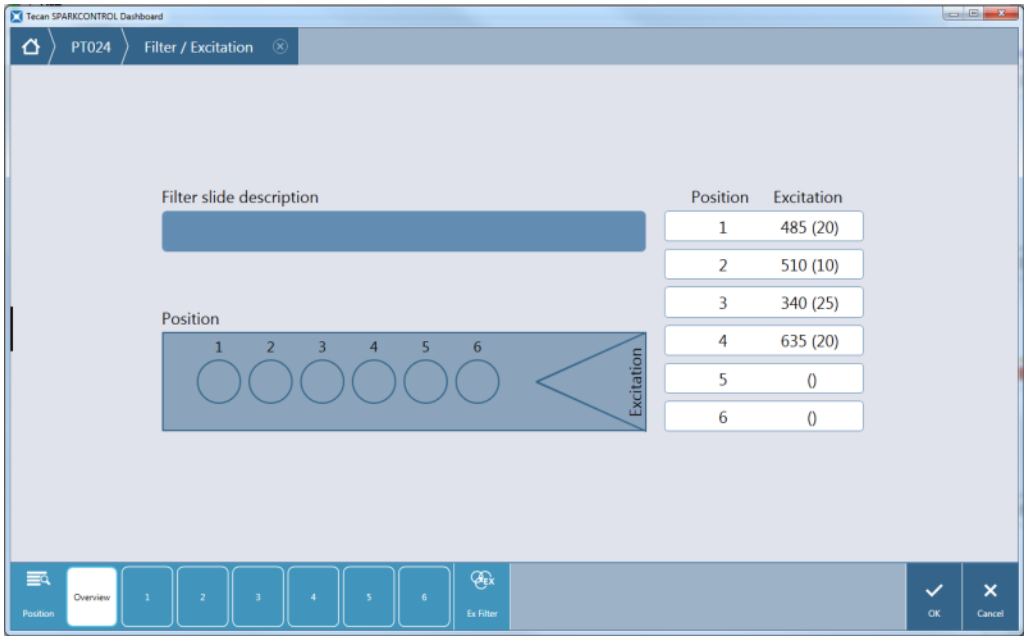


Figure 23: Filter Definition window

Position/Overview: The overview provides the user with the current filter slide definition. Optionally, a **Filter slide description** can be entered.

NOTE: Alphanumeric Latin characters are allowed as well as defined special characters, including blank, ?, $, %, ., /.

Position 1 – 6: Positions 1 to 6 correspond to the position of a filter on the filter slide. Select the appropriate filter position and enter the **Wavelength** and the **Bandwidth** for each new filter:

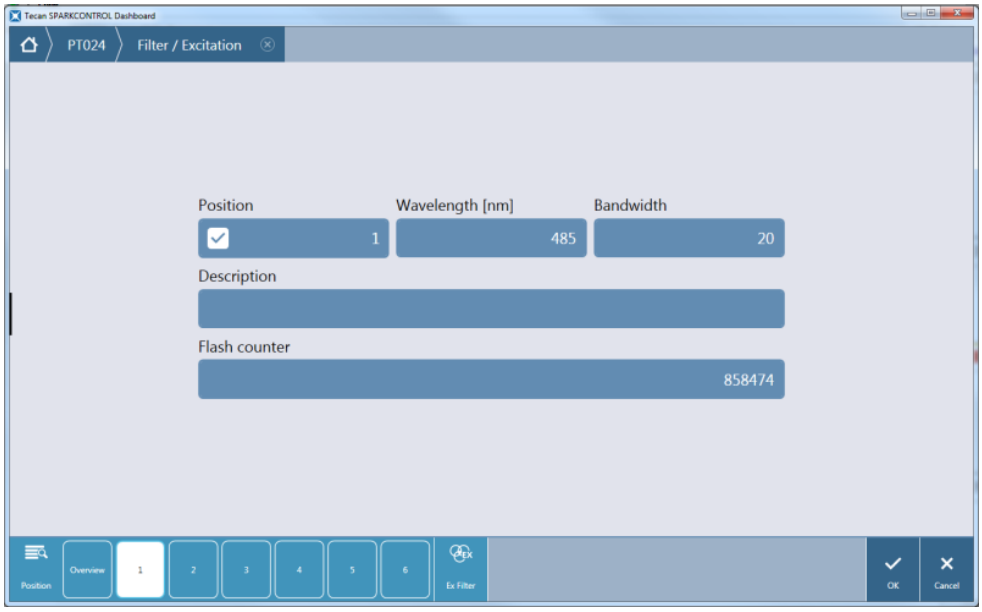


Figure 24: Defining a filter for position 1

Deselect the current **Position** if the selected position is empty, i.e. no filter inserted. Use the **Description** field to add remarks about the filter, e.g. filter name, application, etc.

NOTE: Alphanumeric Latin characters are allowed as well as defined special characters, including blank, ?, $, %, ., /.

Flash Counter: The flash counter monitors the number of flashes the filter is exposed to. The number of flashes is saved together with other information about the filter on the microchip. If the filter is replaced this information will be lost.

CAUTION: It is recommended to manually document the last flash counter number before replacing a filter. Otherwise this information will be lost.

11.10.6 Mirror Slides

Mirrors are used for all Fluorescence Top measurements to reflect the excitation light onto the samples. In case of the Fluorescence Top Standard module the mirror slide is equipped with two different mirror types, in case of the Fluorescence Top Enhanced module five mirror positions are available (one custom dichroic option).

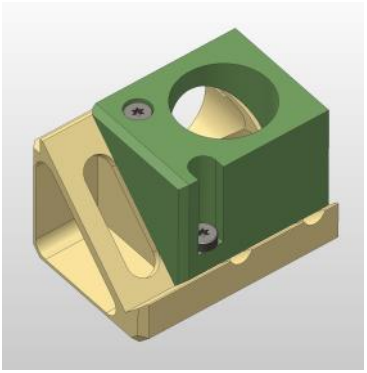
For the performance characteristics of the different mirrors and their availability for the Standard or Enhanced module, see the following table: The 50 % mirror can be used for all fluorescence measurements independent of the selected wavelength.

Mirror	Reflection (Excitation)	Transmission (Emission)	Availability
50% Mirror	230-900 nm	230-900 nm	FI Top Standard and Enhanced
510 Dichroic (e.g. Fluorescein, HTRF)	320-490 nm	515-750 nm	FI Top Standard and Enhanced
560 Dichroic (e.g. Cy3)	510-545 nm	575-620 nm	FI Top Enhanced
625 Dichroic (e.g. Cy5)	565-610 nm	640-700 nm	FI Top Enhanced
Custom Dichroic 410	360-395 nm	425-470 nm	FI Top Enhanced
Custom Dichroic 430	380-415 nm	445-490 nm	FI Top Enhanced
Custom Dichroic 458	350-450 nm	470-900 nm	FI Top Enhanced
Custom Dichroic 593	350-585 nm	605-900 nm	FI Top Enhanced
Custom Dichroic 660	350-650 nm	670-900 nm	FI Top Enhanced

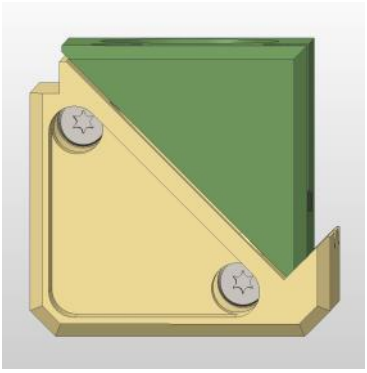
NOTE: A dichroic mirror needs to match the selected fluorescence excitation and emission wavelength.

11.10.7 Installing the Custom Dichroic Mirror

If desired, the mirror slide can be extended with a custom type dichroic mirror. The custom dichroic mirror is delivered separately with the subpackaging and needs to be installed and defined before usage.



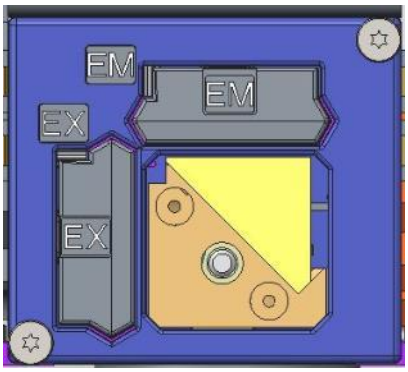
Inclined view



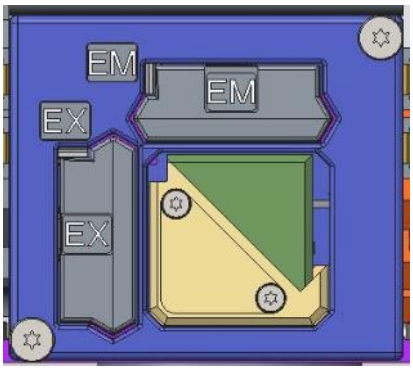
Front view

In order to install the custom dichroic mirror follow these instructions:

1. Open the Mirror Definition window in the Dashboard or Method Editor and select **Mirror Out**. The mirror slide moves to the load position.
2. To install the Custom Dichroic Mirror open the door flap manually. Slide the custom dichroic into the Mirror Carriage as indicated in the figure below. Apply and carefully tighten the mounting screws.



Load position



Installed custom dichroic

CAUTION: Do not apply too much torque to the mirror slide to avoid damages.

3. Carefully release the door flap and click **Mirror In**. The Mirror Slide moves back into the instrument.
4. The custom dichroic mirror is now ready to be defined (see chapter 11.10.8 Defining the Custom Dichroic Mirror).

11.10.8 Defining the Custom Dichroic Mirror

CAUTION: If a new dichroic is to be used, it needs to be defined in the software.

A custom dichroic can be defined via the Mirror Definition window in the Dashboard or Method Editor:

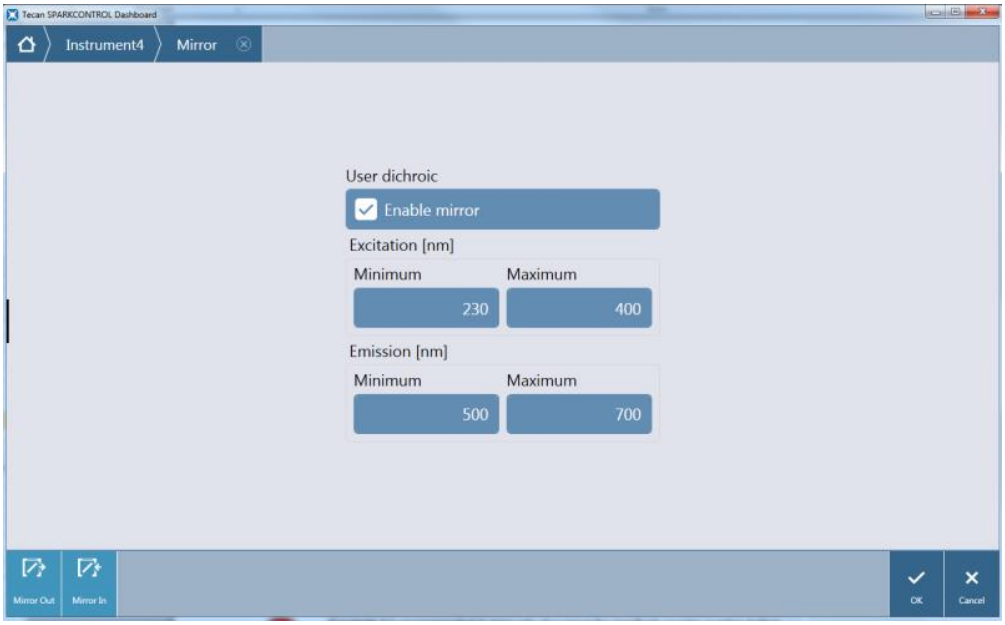


Figure 25: Mirror Definition window

Select **Enable mirror** and define the **Excitation** and the **Emission** range by entering the corresponding **Minimum** and **Maximum** wavelength.

11.11 Fluorescence Polarization Module

The fluorescence polarization module is designed as a Fusion Optics system. The wavelength selection for excitation and emission can be performed by either the monochromator or the filter option. The monochromator and the filter mode are independently combinable for the excitation and the emission side and therefore provide a detection system with the maximum flexibility and maximum signal output. The polarization option is available for top measurements only.

The fluorescence polarization Standard Module is available in two versions: a >300 nm or a >390 nm version. The fluorescence polarization Enhanced Module is equipped with the >300 nm version by default.

For further differences between Fluorescence Standard and Fluorescence Enhanced Module see chapter 11.7 Fluorescence Top Module.

11.11.1 Fluorescence Polarization Optics

Optics

The polarization module consists of a flash lamp as light source, monochromators and/or filters for wavelength selection, a measurement head, and a photomultiplier tube as detector (see chapter 11.7.1 Fluorescence Top Module Optics). Polarizers are installed on the measurement head and switched in automatically when performing polarization measurements.

Z-Positioning

The Z-position defines the distance between the measurement head and the surface of the microplate. The Z-position can be adjusted by moving the plate transport up and down. As light is reflected onto the sample liquid surface, a Z-adjustment helps to maximize signal to noise ratio.

11.11.2 Fluorescence Polarization Detection

See chapters 11.5 Fluorescence Polarization (FP) and 11.9.2 Fluorescence Detection.

G-Factor Calculation

The equation for calculation of fluorescence polarization assumes that the sensitivity of the detection system is equivalent for parallel and perpendicular polarized light. This is generally not the case. Therefore the so called G-factor calculation has to be performed. The G-factor compensates for differences in response of the optical components to the parallel and perpendicular polarized light. The G-factor calculation is an important requirement for each fluorescence polarization measurement.

The G-factor depends on the selected wavelength and bandwidth. Its calculation requires at least one well containing the fluorophore used in the assay as reference and at least one well for blanking containing the (assay) solution without the fluorophore.

In the plate layout, define the RF identifier as reference fluorophore or use an identifier other than BL (Blank) or BF (Blank fluorophore) for referencing the fluorophore. For blanking the assay system, the identifier BF (Blank Fluorophore) or BL (Blank) can be selected.

NOTE: By using more than one well filled for referencing the fluorophore and more than one well without fluorophore for blanking, mean values will be calculated and the G-factor calibration becomes more accurate.

Once the G-factor was calculated for a certain fluorophore (assay type) it can be set manually for all further measurements using the same fluorophore. Recalculation of the G-factor has to be performed under following conditions:

- The wavelengths/filters have changed
- New filters are installed on the filter slide
- New assay type with different fluorophore is used

NOTE: It is recommended to use a free fluorophore or a fluorophore with a low polarization value for the G-factor calibration.

11.12 Fluorescence Specifications

NOTE: All specifications are subject to change without prior notification.

11.12.1 General Specifications of Fluorescence Intensity (Standard and Enhanced Module)

If not otherwise stated the specifications are valid for Standard as well as Enhanced module.

Fluorescence Intensity Top:

Parameters	Monochromator	Filter
Wavelength range	Excitation: 230 – 900 nm Emission: 280 – 900 nm selectable in 1 nm steps	Excitation: 230 – 900 nm Emission: 230 – 900 nm
Bandwidth Standard module	20 nm	depends on the filter used
Bandwidth Enhanced module	5, 7.5, 10, 15, 20, 25, 30, 50 nm	depends on the filter used

Fluorescence Intensity Bottom (Monochromator and Filter Option):

Parameters	VIS Bottom Fiber	UV-VIS Bottom Fiber
Wavelength range	**Monochromator and Filter:** 390 – 900 nm, selectable in 1 nm steps (Monochromator only)	**Monochromator:** Excitation: 230 – 900 nm Emission: 280 – 900 nm, selectable in 1 nm steps **Filter:** Excitation: 230 – 900 nm Emission: 230 – 900 nm
Bandwidth Standard module - Monochromator	20 nm	
Bandwidth Enhanced module - Monochromator	5, 7.5, 10, 15, 20, 25, 30, 50 nm	
Bandwidth Standard and Enhanced module - Filter	depends on the filter used	

NOTE: The UV/VIS bottom fiber is more sensitive than the VIS bottom fiber. Running assays below 400 nm with the VIS fiber leads to results with lower sensitivity.

Gain Options

Gain setting	Values
Manual	1 – 255
Optimal	Automatic
Calculated from well	Automatic
Extended dynamic range	Automatic
Use gain regulation	Automatic

TRF Parameters

Parameters	Characteristics
Integration time	20 µs – 2000 µs
Lag time	0 µs – 2 ms

11.12.2 Performance Specifications of Fluorescence Intensity

Performance Specifications of Fluorescence Intensity Top Standard Module			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Monochromator	96-well plate, black, 200 µl	Flashes/well: 30	Detection Limit: < 20 pM (1 nM Fluorescein)
Monochromator	384-well plate, black, 100 µl	Flashes/well: 30	Detection Limit: < 20 pM (1 nM Fluorescein)
Filter	96-well plate, black, 200 µl	Flashes/well: 30	Detection Limit: < 10 pM (1 nM Fluorescein)
Filter	384-well plate, black, 100 µl	Flashes/well: 30	Detection Limit: < 10 pM (1 nM Fluorescein)
Monochromator and filter	96-well plate, black, 200 µl	Flashes/well: 30	Uniformity: < 3 CV% (25 nM Fluorescein)
Monochromator and filter	384-well plate, black, 100 µl	Flashes/well: 30	Uniformity: < 5 CV% (25 nM Fluorescein)

Performance Specifications of Fluorescence Intensity Top Enhanced Module			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Monochromator	384-well plate, black, 100 µl	Flashes/well: 30	Detection Limit: < 3 pM (1 nM Fluorescein)
Monochromator	1536-well plate, black, 10 µl	Flashes/well: 30	Detection Limit: < 10 pM (1 nM Fluorescein)
Filter	384-well plate, black, 100 µl	Flashes/well: 30	Detection Limit: < 2 pM (1 nM Fluorescein)
Filter	1536-well plate, black, 10 µl	Flashes/well: 30	Detection Limit: < 7 pM (1 nM Fluorescein)
Monochromator and filter	384-well plate, black, 100 µl	Flashes/well: 30	Uniformity: < 3 CV% (25 nM Fluorescein)
Monochromator and filter	1536-well plate, black, 10 µl	Flashes/well: 30	Uniformity: < 5 CV% (100 nM Fluorescein)

Performance Specifications of Fluorescence Intensity Bottom Standard Module			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Monochromator	96-well plate, black, transparent bottom, 350 µl	Flashes/well: 30	Detection Limit: < 45 pM (1 nM Fluorescein)
Monochromator	384-well plate, black, transparent bottom, 100 µl	Flashes/well: 30	Detection Limit: < 45 pM (1 nM Fluorescein)
Filter	96-well plate, black, transparent bottom, 350 µl	Flashes/well: 30	Detection Limit: < 35 pM (1 nM Fluorescein)
Filter	384-well plate, black, transparent bottom, 100 µl	Flashes/well: 30	Detection Limit: < 35 pM (1 nM Fluorescein)
Monochromator and filter	96-well plate, black, transparent bottom, 200 µl	Flashes/well: 30	Uniformity: < 3 CV% (25 nM Fluorescein)
Monochromator and filter	384-well plate, black, transparent bottom, 100 µl	Flashes/well: 30	Uniformity: < 5 CV% (25 nM Fluorescein)

Performance Specifications of Fluorescence Intensity Bottom Enhanced Module			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Monochromator	96-well plate, black, transparent bottom, 350 µl	Flashes/well: 30	Detection Limit: < 30 pM (1 nM Fluorescein)
Monochromator	384-well plate, black, transparent bottom, 100 µl	Flashes/well: 30	Detection Limit: < 30 pM (1 nM Fluorescein)
Monochromator	1536-well plate, black, transparent bottom, 10 µl	Flashes/well: 30	Detection Limit: < 40 pM (1 nM Fluorescein)
Filter	96-well plate, black, transparent bottom, 350 µl	Flashes/well: 30	Detection Limit: < 15 pM (1 nM Fluorescein)
Filter	384-well plate, black, transparent bottom, 100 µl	Flashes/well: 30	Detection Limit: < 17 pM (1 nM Fluorescein)
Filter	1536-well plate, black, transparent bottom, 10 µl	Flashes/well: 30	Detection Limit: < 40 pM (1 nM Fluorescein)
Monochromator and filter	384-well plate, black, transparent bottom, 100 µl	Flashes/well: 30	Uniformity: < 3 CV% (25 nM Fluorescein)
Monochromator and filter	1536-well plate, black, transparent bottom, 10 µl	Flashes/well: 30	Uniformity: < 5 CV% (100 nM Fluorescein)

Performance Specifications of Time Resolved Fluorescence (TRF) Standard Module			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Monochromator	96-well plate, white, 200 µl	Flashes/well: 30	Detection Limit: < 5 pM (1 nM Europium)
Monochromator	384-well plate, white, 100 µl	Flashes/well: 30	Detection Limit: < 5 pM (1 nM Europium)
Filter	96-well plate, white, 200 µl	Flashes/well: 30	Detection Limit: < 150 fM (1 nM Europium)
Filter	384-well plate, white, 100 µl	Flashes/well: 30	Detection Limit: < 150 fM (1 nM Europium)

Performance Specifications of Time Resolved Fluorescence (TRF) Enhanced Module			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Monochromator	96-well plate, white, 200 µl	Flashes/well: 30	Detection Limit: < 750 fM (1 nM Europium)
Monochromator	384-well plate, white, 100 µl	Flashes/well: 30	Detection Limit: < 750 fM (1 nM Europium)
Monochromator	1536-well plate, white, 10 µl	Flashes/well: 30	Detection Limit: < 900 fM (1 nM Europium)
Filter	96-well plate, white, 200 µl	Flashes/well: 30	Detection Limit: < 75 fM (0.1 nM Europium)
Filter	384-well plate, white, 100 µl	Flashes/well: 30	Detection Limit: < 75 fM (0.1 nM Europium)
Filter	1536-well plate, white, 10 µl	Flashes/well: 30	Detection Limit: < 100 fM (0.1 nM Europium)

11.12.3 General Specifications of Fluorescence Polarization (Standard and Enhanced Polarization Module)

If not otherwise stated the specifications are valid for Standard as well as Enhanced module.

Parameters	**>390 nm Fiber**	**>300 nm Polarization Fiber**
Wavelength range	**Monochromator and Filter:** 400 – 850 nm, selectable in 1 nm steps (Monochromator only)	**Monochromator and Filter:** 300 – 850 nm, selectable in 1 nm steps (Monochromator only)
Bandwidth Standard Polarization module - Monochromator	20 nm	
Bandwidth Enhanced Polarization module - Monochromator	5, 7.5, 10, 15, 20, 25, 30, 50 nm	
Bandwidth Standard and Enhanced Polarization module - Filter	depends on the filter used	

11.12.4 Performance Specifications of Fluorescence Polarization

Performance Specifications of Fluorescence Polarization Standard Module (>300 nm and >390 nm)			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Filter	96-well plate, black, 200 µl	Flashes/well: 30	Precision: < 5 mP (1 nM Fluorescein)
Filter	384-well plate, black, 100 µl	Flashes/well: 30	Precision: < 5 mP (1 nM Fluorescein)

Performance Specifications of Fluorescence Polarization Enhanced Module (>300 nm and >390 nm)			
Module	**Plate type/Filling Volume**	**Parameter**	**Criteria**
Filter	96-well plate, black, 200 µl	Flashes/well: 30	Precision: < 3 mP (1 nM Fluorescein)
Filter	384-well plate, black, 100 µl	Flashes/well: 30	Precision: < 3 mP (1 nM Fluorescein)
Filter	1536-well plate, black, 10 µl	Flashes/well: 30	Precision: < 5 mP (1 nM Fluorescein)

11.12.5 Fastest Measurement Times

Fastest measurement times are determined by using one flash only, manual gain, and manual Z-position. Plate-in and plate-out movements are not included in the measurement time.

Standard module		
Measurement Technique	**Measurement Time**	
Plate type	96-well	384-well
Fluorescence intensity top filter	≤ 13 seconds	≤ 30 seconds
Fluorescence intensity top monochromator	≤ 14 seconds	≤ 32 seconds
Fluorescence intensity bottom monochromator	≤ 21 seconds	≤ 35 seconds

Enhanced module			
Measurement Technique	**Measurement Time**		
Plate type	96-well	384-well	1536-well
Fluorescence intensity top filter	≤ 13 seconds	≤ 22 seconds	≤ 34 seconds
Fluorescence intensity top monochromator	≤ 14 seconds	≤ 23 seconds	≤ 36 seconds
Fluorescence intensity bottom monochromator	≤ 19 seconds	≤ 24 seconds	≤ 42 seconds

11.13 Quality Control of the Fluorescence Module

11.13.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on site at Tecan.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

CAUTION: Before starting measurements, make sure that the microplate is inserted correctly. The position of well A1 has to be on the upper left side.

WARNING: The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

11.13.2 Detection Limit Top/Bottom 96-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 96-well plate, flat bottom, black (for top measurement)
- Greiner 96-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

Procedure:

Pipette 200 µl for top and 350 µl for bottom measurements of a 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	F	B	F	B	F	B	F	B	F	B	F	B
B	F	B	F	B	F	B	F	B	F	B	F	B
C	F	B	F	B	F	B	F	B	F	B	F	B
D	F	B	F	B	F	B	F	B	F	B	F	B
E	F	B	F	B	F	B	F	B	F	B	F	B
F	F	B	F	B	F	B	F	B	F	B	F	B
G	F	B	F	B	F	B	F	B	F	B	F	B
H	F	B	F	B	F	B	F	B	F	B	F	B

F: 200/350 µl 1 nM Fluorescein
B: 200/350 µl Blank (10 mM NaOH)

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Fluorescence Top/Bottom**	**Fluorescence Top/Bottom**
Excitation	485 nm	485 nm
Bandwidth excitation	20 nm	20 nm
Emission	535 nm	535 nm
Bandwidth emission	20 nm	25 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Plate definition file	GRE96fb	GRE96fb

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(pM) = \frac{(3 * SD_B * 1000)}{(mean_F - mean_B)}$$

SD_B	Standard deviation of wells filled with Blank (10 mM NaOH)
1000	Concentration of Fluorescein in pM
$mean_F$	Mean of wells filled with 1 nM Fluorescein
$mean_B$	Mean of wells filled with Blank (10 mM NaOH)

11.13.3 Uniformity Top/Bottom 96-Well Plate

The uniformity defines the well-to-well variations when measuring a multi-well plate. The uniformity is calculated as percentage deviation from the mean value.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Fluorescence Standard Module: Fluorescein, 25 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- Fluorescence Enhanced Module: Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- Greiner 96-well plate, flat bottom, black (for top measurement)
- Greiner 96-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

Procedure:

Pipette 200 µl of Fluorescein solution into the appropriate wells according to the plate layout.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	F		F		F		F		F		F	
B	F		F		F		F		F		F	
C	F		F		F		F		F		F	
D	F		F		F		F		F		F	
E	F		F		F		F		F		F	
F	F		F		F		F		F		F	
G	F		F		F		F		F		F	
H	F		F		F		F		F		F	

F: 200 µl Fluorescein

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Fluorescence Top/Bottom**	**Fluorescence Top/Bottom**
Excitation	485 nm	485 nm
Bandwidth excitation	20 nm	20 nm
Emission	535 nm	535 nm
Bandwidth emission	20 nm	25 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Plate definition file	GRE96fb	GRE96fb

Evaluation:

Calculate the Uniformity as follows:

$$\text{Uniformity (CV\%)} = \frac{SD_F * 100}{mean_F}$$

SD_F	Standard deviation of wells filled with Fluorescein
$mean_F$	Mean of wells filled with Fluorescein

11.13.4 Detection Limit Top/Bottom 384-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 384-well plate, flat bottom, black (for top measurement)
- Greiner 384-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

Procedure:

Pipette 100 µl of 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	F		B		F		B		F		B		F		B		F		B		F		B	
B																								
C	F		B		F		B		F		B		F		B		F		B		F		B	
D																								
E	F		B		F		B		F		B		F		B		F		B		F		B	
F																								
G	F		B		F		B		F		B		F		B		F		B		F		B	
H																								
I	F		B		F		B		F		B		F		B		F		B		F		B	
J																								
K	F		B		F		B		F		B		F		B		F		B		F		B	
L																								
M	F		B		F		B		F		B		F		B		F		B		F		B	
N																								
O	F		B		F		B		F		B		F		B		F		B		F		B	
P																								

F: 100 µl 1 nM Fluorescein
B: 100 µl Blank (10 mM NaOH)

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Fluorescence Top/Bottom**	**Fluorescence Top/Bottom**
Excitation	485 nm	485 nm
Bandwidth excitation	20 nm	20 nm
Emission	535 nm	535 nm
Bandwidth emission	20 nm	25 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Plate definition file	GRE384fb	GRE384fb

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(pM) = \frac{(3 * SD_B * 1000)}{(mean_F - mean_B)}$$

SD_B	Standard deviation of wells filled with Blank (10 mM NaOH)
1000	Concentration of Fluorescein in pM
$mean_F$	Mean of wells filled with 1 nM Fluorescein
$mean_B$	Mean of wells filled with Blank (10 mM NaOH)

11.13.5 Uniformity Top/Bottom 384-Well Plate

The uniformity defines the well-to-well variations when measuring a multi-well plate. The uniformity is calculated as percentage deviation from the mean value.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

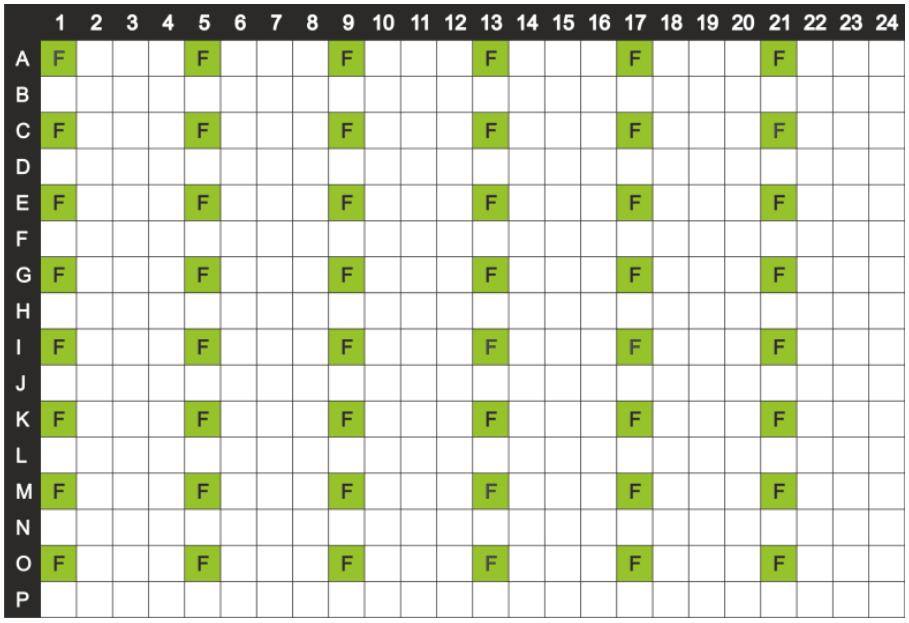
Material:

- Fluorescence: Fluorescein, 25 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- Greiner 384-well plate, flat bottom, black (for top measurement)
- Greiner 384-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

Procedure:

Pipette 100 µl of Fluorescein solution into the appropriate wells according to the plate layout.

Plate Layout:



F: 100 µl Fluorescein

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Fluorescence Top/Bottom**	**Fluorescence Top/Bottom**
Excitation	485 nm	485 nm
Bandwidth excitation	20 nm	20 nm
Emission	535 nm	535 nm
Bandwidth emission	20 nm	25 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Plate definition file	GRE384fb	GRE384fb

Evaluation:

Calculate the Uniformity as follows:

$$\text{Uniformity (CV\%)} = \frac{SD_F * 100}{mean_F}$$

SD_F	Standard deviation of wells filled with Fluorescein
$mean_F$	Mean of wells filled with Fluorescein

11.13.6 Detection Limit Top/Bottom 1536-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

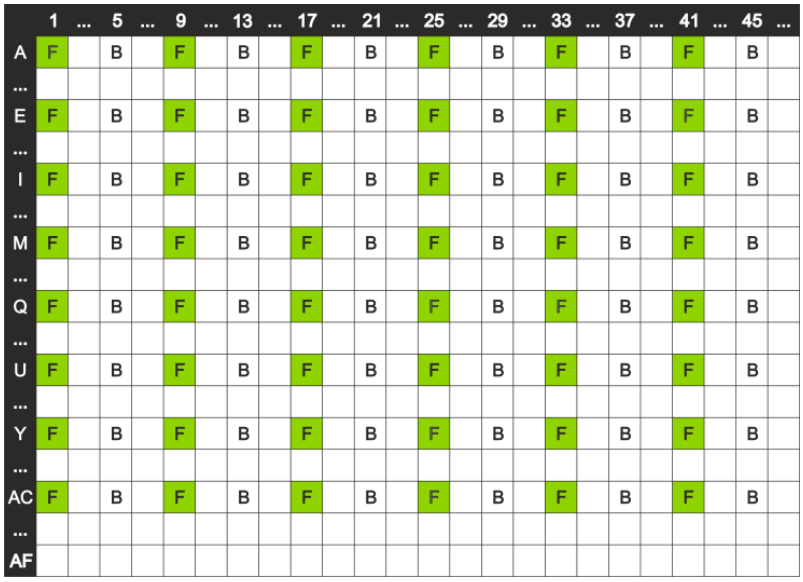
Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 1536-well plate, flat bottom, black (for top measurement)
- Greiner 1536-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

Procedure:

Pipette 10 µl of 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

Plate Layout:



F: 10 µl 1 nM Fluorescein
B: 10 µl Blank (10 mM NaOH)

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Fluorescence Top/Bottom**	**Fluorescence Top/Bottom**
Excitation	485 nm	485 nm
Bandwidth excitation	20 nm	20 nm
Emission	535 nm	535 nm
Bandwidth emission	20 nm	25 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Plate definition file	GRE1536fb	GRE1536fb

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(pM) = \frac{(3 * SD_B * 1000)}{(mean_F - mean_B)}$$

SD_B	Standard deviation of wells filled with Blank (10 mM NaOH)
1000	Concentration of Fluorescein in pM
$mean_F$	Mean of wells filled with 1 nM Fluorescein
$mean_B$	Mean of wells filled with Blank (10 mM NaOH)

11.13.7 Uniformity Top/Bottom 1536-Well Plate

The uniformity defines the well-to-well variations when measuring a multi-well plate. The uniformity is calculated as percentage deviation from the mean value.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

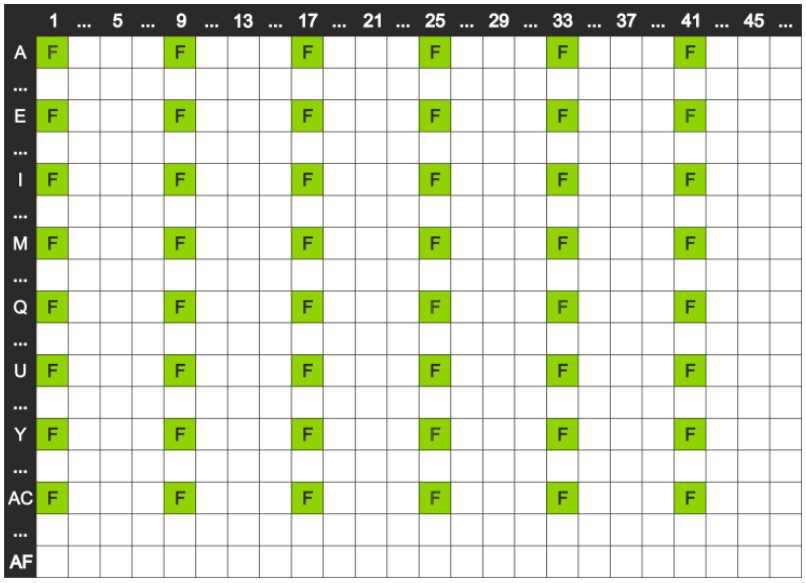
Material:

- Fluorescein, 100 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- Greiner 1536-well plate, flat bottom, black (for top measurement)
- Greiner 1536-well plate, flat transparent bottom, black (for bottom measurement)
- Pipettes + tips

Procedure:

Pipette 10 µl of 100 nM Fluorescein solution into the appropriate wells according to the plate layout.

Plate Layout:



F: 10 µl 100 nM Fluorescein

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Fluorescence Top/Bottom**	**Fluorescence Top/Bottom**
Excitation	485 nm	485 nm
Bandwidth excitation	20 nm	20 nm
Emission	535 nm	535 nm
Bandwidth emission	20 nm	25 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Plate definition file	GRE1536fb	GRE1536fb

Evaluation:

Calculate the Uniformity as follows:

$$\text{Uniformity (CV\%)} = \frac{SD_F * 100}{mean_F}$$

SD_F	Standard deviation of wells filled with Fluorescein
$mean_F$	Mean of wells filled with Fluorescein

11.13.8 Detection Limit TRF 96-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Europium, 1 nM (HVD Life Sciences)
- Enhancement Solution = Blank (HVD Life Sciences)
- Greiner 96-well plate, flat bottom, white
- Pipettes + tips

Procedure:

Pipette 200 µl of 1 nM Europium solution or the blank solution (Enhancement Solution) into the appropriate wells according to the plate layout. Dilute the Europium solution 10 times by using the Enhancement Solution when testing the filter system (0.1 nM Europium solution).

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Eu	B	B	B	B	B						
B	Eu	B	B	B	B	B						
C	Eu	B	B	B	B	B						
D	Eu	B	B	B	B	B						
E	Eu	B	B	B	B	B						
F	Eu	B	B	B	B	B						
G	Eu	B	B	B	B	B						
H	Eu	B	B	B	B	B						

Eu: 200 µl 1 nM Europium (Monochromator) / 0.1 nM Europium (Filter)
B: 200 µl Blank (Enhancement Solution)

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Time Resolved Fluorescence**	**Time Resolved Fluorescence**
Excitation	340 nm	340 nm
Bandwidth excitation	Standard Module: 20 nm Enhanced Module: 30 nm	35 nm
Emission	617 nm	612 nm
Bandwidth emission	20 nm	10 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Integration time	400 µs	400 µs
Lag time	100 µs	100 µs
Plate definition file	GRE96fw	GRE96fw

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(fM) = \frac{(3 * SD_B * 10^6)}{(mean_F - mean_B)}$$

SDB	Standard deviation of wells filled with Blank (Enhancement Solution)
10^6	Concentration of Europium in fM (change to 10^5 in case of 0.1 nM Europium)
meanF	Mean of wells filled with Europium
meanB	Mean of wells filled with Blank (Enhancement Solution)

11.13.9 Detection Limit TRF 384-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Europium, 1 nM (HVD Life Sciences)
- Enhancement Solution = Blank (HVD Life Sciences)
- Greiner 384-well plate, flat bottom, white
- Pipettes + tips

Procedure:

Pipette 100 µl of 1 nM Europium solution or the blank solution (Enhancement Solution) into the appropriate wells according to the plate layout. Dilute the Europium solution 10 times by using the Enhancement Solution when testing the filter system (0.1 nM Europium solution).

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	...	24
A	Eu	B	B	B	B	B						
B	Eu	B	B	B	B	B						
C	Eu	B	B	B	B	B						
D	Eu	B	B	B	B	B						
E	Eu	B	B	B	B	B						
F	Eu	B	B	B	B	B						
...												
P												

Eu: 100 µl 1 nM Europium (Monochromator) / 0.1 nM Europium (Filter)
B: 100 µl Blank (Enhancement Solution)

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Time Resolved Fluorescence**	**Time Resolved Fluorescence**
Excitation	340 nm	340 nm
Bandwidth excitation	Standard Module: 20 nm Enhanced Module: 30 nm	35 nm
Emission	617 nm	612 nm
Bandwidth emission	20 nm	10 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Integration time	400 µs	400 µs
Lag time	100 µs	100 µs
Plate definition file	GRE384fw	GRE384fw

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(fM) = \frac{(3 * SD_B * 10^6)}{(mean_F - mean_B)}$$

SD_B	Standard deviation of wells filled with Blank (Enhancement Solution)
10^6	Concentration of Europium in fM (change to 10^5 in case of 0.1 nM Europium)
$mean_F$	Mean of wells filled with Europium
$mean_B$	Mean of wells filled with Blank (Enhancement Solution)

11.13.10 Detection Limit TRF 1536-Well Plate

The detection limit is the lowest quantity of a substance that can be distinguished from the blank within a stated confidence limit.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Europium, 1 nM (HVD Life Sciences)
- Enhancement Solution = Blank (HVD Life Sciences)
- Greiner 1536-well plate, flat bottom, white
- Pipettes + tips

Procedure:

Pipette 10 µl of 1 nM Europium solution or the blank solution (Enhancement Solution) into the appropriate wells according to the plate layout. Dilute the Europium solution 10 times by using the Enhancement Solution when testing the filter system (0.1 nM Europium solution).

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	···	48
A	Eu	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B							
B	Eu	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B							
C	Eu	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B							
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
...																								
AF																								

Eu: 10 µl 1 nM Europium (Monochromator) / 0.1 nM Europium (Filter)
B: 10 µl Blank (Enhancement Solution)

Measurement Parameters:

	Monochromator	Filter
Measurement mode	**Time Resolved Fluorescence**	**Time Resolved Fluorescence**
Excitation	340 nm	340 nm
Bandwidth excitation	Standard Module: 20 nm Enhanced Module: 30 nm	35 nm
Emission	617 nm	612 nm
Bandwidth emission	20 nm	10 nm
Flashes	30	30
Gain	Optimal	Optimal
Mirror	510 Dichroic	510 Dichroic
Z-position	Calculate from A1	Calculate from A1
Integration time	400 µs	400 µs
Lag time	100 µs	100 µs
Plate definition file	GRE1536fw	GRE1536fw

Evaluation:

Calculate the detection limit (DL) as follows:

$$DL(fM) = \frac{(3 * SD_B * 10^6)}{(mean_F - mean_B)}$$

SD_B	Standard deviation of wells filled with Blank (Enhancement Solution)
10^6	Concentration of Europium in fM (change to 10^5 in case of 0.1 nM Europium)
$mean_F$	Mean of wells filled with Europium
$mean_B$	Mean of wells filled with Blank (Enhancement Solution)

11.13.11 Fluorescence Polarization Precision 96-Well Plate

This procedure is for filter-based devices only. The fluorescence polarization precision corresponds to the standard deviation of the mP (milli-polarization) values of the wells filled with fluorescein.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 96-well plate, flat bottom, black
- Pipettes + tips

Procedure:

Pipette 200 µl of 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	F	B	F	B	F	B	F	B	F	B	F	B
B	F	B	F	B	F	B	F	B	F	B	F	B
C	F	B	F	B	F	B	F	B	F	B	F	B
D	F	B	F	B	F	B	F	B	F	B	F	B
E	F	B	F	B	F	B	F	B	F	B	F	B
F	F	B	F	B	F	B	F	B	F	B	F	B
G	F	B	F	B	F	B	F	B	F	B	F	B
H	F	B	F	B	F	B	F	B	F	B	F	B

F: 200 µl 1 nM Fluorescein
B: 200 µl Blank (10 mM NaOH)

Measurement Parameters:

	Filter
Measurement mode	Fluorescence Polarization
Excitation	485 nm
Bandwidth excitation	20 nm
Emission	535 nm
Bandwidth emission	25 nm
Flashes	30
Gain	Optimal
Mirror	510 Dichroic
Z-position	Calculate from A1
Settle time	300 ms
G-factor	Calculate
Reference G-factor from/to	A1-H1
Reference Blank G-factor from/to	A2-H2
Measurement blank	Remaining wells filled with Blank
Plate definition file	GRE96fb

Evaluation:

Calculate the precision:

$$\text{Precision (mP)} = 1 * SD_F$$

SD_F	Standard deviation of mP values of wells filled with Fluorescein

11.13.12 Fluorescence Polarization Precision 384-Well Plate

This procedure is for filter-based devices only. The fluorescence polarization precision corresponds to the standard deviation of the mP (milli-polarization) values of the wells filled with fluorescein.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 384-well plate, flat bottom, black
- Pipettes + tips

Procedure:

Pipette 100 µl of 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	F		B		F		B		F		B		F		B		F		B		F		B	
B																								
C	F		B		F		B		F		B		F		B		F		B		F		B	
D																								
E	F		B		F		B		F		B		F		B		F		B		F		B	
F																								
G	F		B		F		B		F		B		F		B		F		B		F		B	
H																								
I	F		B		F		B		F		B		F		B		F		B		F		B	
J																								
K	F		B		F		B		F		B		F		B		F		B		F		B	
L																								
M	F		B		F		B		F		B		F		B		F		B		F		B	
N																								
O	F		B		F		B		F		B		F		B		F		B		F		B	
P																								

F: 100 µl 1 nM Fluorescein
B: 100 µl Blank (10 mM NaOH)

Measurement Parameters:

	Filter
Measurement mode	Fluorescence Polarization
Excitation	485 nm
Bandwidth excitation	20 nm
Emission	535 nm
Bandwidth emission	25 nm
Flashes	30
Gain	Optimal
Mirror	510 Dichroic
Z-position	Calculate from A1
G-factor	Calculate
Reference G-factor from/to	A1, C1, E1, G1, I1, K1, M1, O1;
Reference Blank G-factor from/to	A3, C3, E3, G3, I3, K3, M3, O3;
Measurement blank	Remaining wells filled with Blank
Plate definition file	GRE384fb

Evaluation:

Calculate the precision:

$$\text{Precision (mP)} = 1 * SD_F$$

SD_F	Standard deviation of mP values of wells filled with Fluorescein

11.13.13 Fluorescence Polarization Precision 1536-Well Plate

This procedure is for filter-based devices only. The fluorescence polarization precision corresponds to the standard deviation of the mP (milli-polarization) values of the wells filled with fluorescein.

Before pipetting the plate, prepare the instrument for measurement and start the measurement immediately after pipetting.

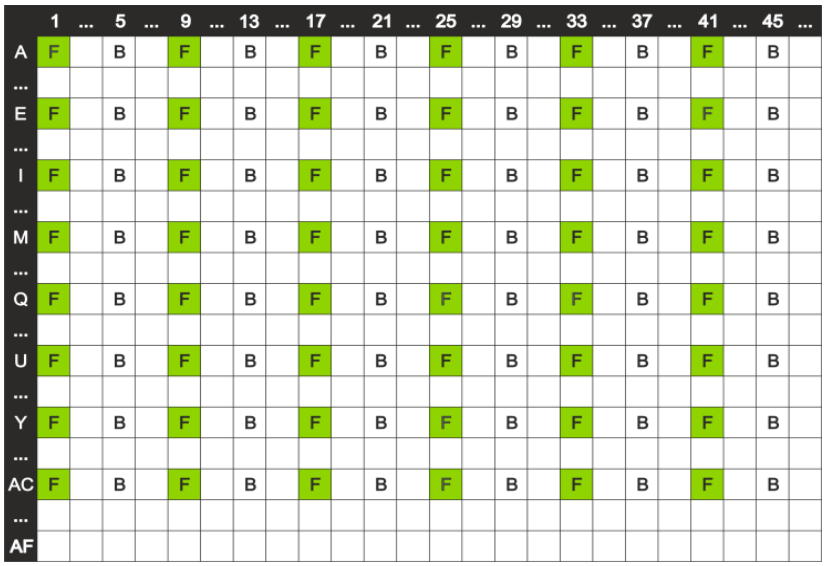
Material:

- Fluorescein, 1 nM in 10 mM NaOH (Fluorescein sodium salt, Sigma)
- 10 mM NaOH = Blank (NaOH pellets)
- Greiner 1536-well plate, flat bottom, black
- Pipettes + tips

Procedure:

Pipette 10 µl of 1 nM Fluorescein solution or the blank solution (10 mM NaOH) into the appropriate wells according to the plate layout.

Plate Layout:



F: 10 µl 1 nM Fluorescein
B: 10 µl Blank (10 mM NaOH)

Measurement Parameters:

	Filter
Measurement mode	Fluorescence Polarization
Excitation	485 nm
Bandwidth excitation	20 nm
Emission	535 nm
Bandwidth emission	25 nm
Flashes	30
Gain	Optimal
Mirror	510 Dichroic
Z-position	Calculate from A1
G-factor	Calculate
Reference G-factor from/to	A1;
Reference Blank G-factor from/to	A5;
Measurement blank	Remaining wells filled with Blank
Plate definition file	GRE1536fb

Evaluation:

Calculate the precision:

$$\text{Precision (mP)} = 1 * SD_F$$

SD_F	Standard deviation of mP values of wells filled with Fluorescein

12 Cell Module

SPARK can be equipped with either the Cell Module described in this chapter, or with the enhanced fluorescence imaging module, the Cell Imager. Only one of these imaging modules can be installed in the SPARK instrument.

Tecan's Cell Module is based upon the bright field illumination technique which is a commonly used tool for visualization of cells. Bright field cellular visualization requires a difference in contrast between cells and their surrounding medium. This contrast is caused by the different levels of light absorption of cells and their media. Counting of cells with low or no contrast can be improved by using various staining procedures.

12.1 Measurement Techniques

12.1.1 Cell Counting/Cell Viability

Tecan provides two fully-automated apps for counting cells and determining cell viability in single-use cell chips. Both apps are optimized to perform a routine quality check of cell cultures on a daily basis.

12.1.2 Cell Confluence

Confluence indicates the size of surface covered by adherent cells. Cell confluence is displayed in percentage of the measured area. Confluence measurements can be performed in cell culture plates from 6- to 96-well format.

12.2 Bright field Imaging

The Cell Module consists of an illumination module and a camera module. Samples are illuminated from the top and image acquisition is done from the bottom.

12.2.1 Optics

The illumination module consists of an LED (light emitting diode), an aperture wheel, and a lens system. An LED serves as the light source [1]. The light beam is shaped by apertures of the aperture wheel and the lens system [2] guides the bundled light to the sample.

The camera module consists of an objective, a camera and a laser diode. The light is collected by an objective and reflected to the camera [3] by a mirror [4]. The laser diode [5] is used for autofocusing.

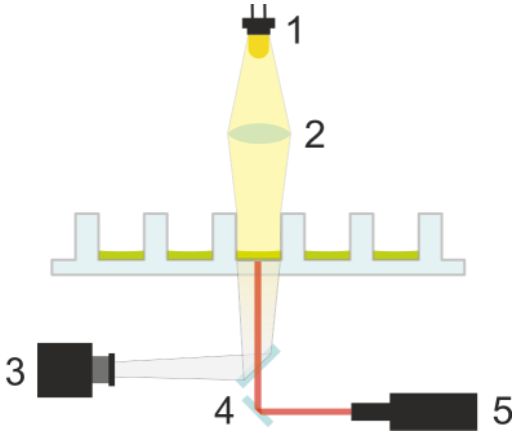
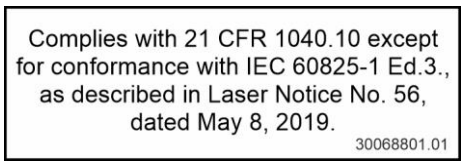


Figure 26: Cell counting optics
LED [1], lens system [2], camera [3], mirror [4], laser diode [5]

The Cell Module uses a laser diode for auto-focusing. A SPARK instrument equipped with a Cell Module is a LASER CLASS 1 product. The instrument complies with FDA radiation performance standards 21 CFR 1040.10 except for conformance with IEC 60825-1 Ed.3, as described in Laser Notice No. 56, dated May 8, 2019.

The following labels are attached to the rear of the instrument:



12.2.2 Detection

Basically, the counting and confluence procedure involves focusing, light exposure timing and image acquisition. An automatic laser-based focusing system enables precise autofocusing.

12.3 Measurement Equipment

12.3.1 Cell Chips

Tecan provides appropriate single-use cell chips consisting of two sample chambers each (see picture below). The filling volume for one sample chamber is 10 µl and can be filled using an appropriate standard pipette. For optimal performance, avoid the formation of air bubbles in the sample chamber during the filling procedure.

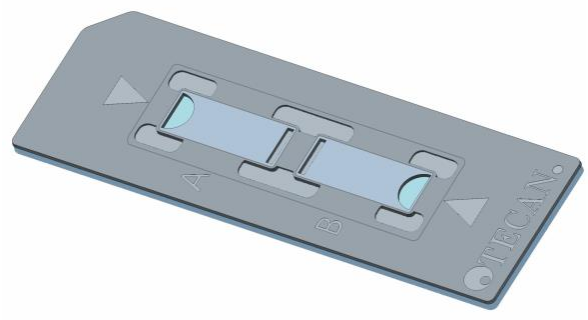


Figure 27: Cell chip

CAUTION: Proper performance can only be guaranteed if Tecan's cell chips are used for cell counting and cell viability analysis. Avoid the formation of air bubbles when filling the sample chambers of the cell chip.

CAUTION: Before using the cell chips control their date of expiry. Optimal performance is not guaranteed if expiry date is exceeded.

12.3.2 Adapter for Cell Chips

Tecan's cell chip adapter is designed to hold up to four cell chips. The cell chips have cropped corners to prevent incorrect insertion and avoid wrong data acquisition. The slides must be inserted correctly for the adapter to close properly. The lid is automatically secured by a magnetic mechanism. The denotation (e.g. A1, A2) of the sample positions on the adapter corresponds to the those presented in the software. Before starting measurements, make sure that the cell chip adapter is inserted correctly. The opening must be in front and chamber A1 must be on the upper left side.

The adapter can be cleaned by using 70 % ethanol.

Figure 28: Cell chip adapter

NOTE: A cell chip adapter as well as a package of 50 cell chips are included with the SPARK multimode reader.

CAUTION: Before starting measurements, make sure that the cell chip adapter is inserted correctly. The opening must be in front and chamber A1 must be on the upper left side.

12.3.3 Maintenance and Cleaning of the Cell Chip Adapter

The cell chip adapter can be cleaned by applying the following procedure:

1. Wear protective gloves, protective glasses and protective clothing.
2. Empty the cell chip adapter and carefully remove the springs installed on the inside of the adapter lid (see Figure 26: Cell counting optics).
3. Carefully wipe all outside surfaces of the adapter and the springs with a lint-free paper towel soaked with 70 % ethanol.
4. Allow to dry.
5. Reinstall springs before using the adapter.

CAUTION: Don't use the cell chip adapter without springs! Measurement errors may result.

12.4 Cell Counting Application

Tecan provides two ready-to-use cell counting apps for

- Cell counting
- Cell viability

When using these apps, the calculation of the cell concentration, cell size, and viability values are performed automatically. For more information, see the corresponding chapters in the SparkControl software manual.

12.5 Optimizing Cell Counting Measurements

12.5.1 Increase Number of Images

In general, cell counting and cell viability is performed in very small volumes. Cell concentrations below $1x10^5$ cells/ml result in a low number of counted objects per image and often an irregular distribution of the cells. To improve counting rates and therefore absolute numbers of cells/ml, more than one image per sample can be taken and analyzed using the cell counting and cell viability apps. Choose between 4 and 8 images/sample.

12.5.2 Live Viewer

See the corresponding chapters in the SparkControl software manual.

12.6 Cell Module Specifications

NOTE: All specifications are subject to change without prior notification.

12.6.1 General Specifications

Illumination	LED
Image	Bright field
Objective	4 x
Optical resolution	> 3 µm
Area/image	2.2 mm^2

12.6.2 Specifications Cell Counting/Viability

Disposable	Cell chips (Tecan brand)
Cell chips	2 Sample chambers per cell chip
Adapter for cell chips	4 Cell chips per adapter
Multiple images per sample	1, 4, 8
Cell size	4-90 µm
Cell concentration	$1x10^4$-$1x10^7$ cells/ml
Reproducibility	< 10% (1 Sigma), HeLa and CHO cell lines
Accuracy	± 10%, at $1x10^6$ cells/ml, HeLa and CHO cell lines

12.6.3 Measurement Time

Plate-in and plate-out movements as well as initialization steps are not included in the measurement time.

Measurement Technique	Measurement Time
Cell counting/viability check	< 30 seconds/sample
Confluence, 96-well, whole well imaging	< 45 minutes

12.7 Quality Control of the Cell Counting Module

12.7.1 Periodic Quality Control Tests

Depending on usage and application we recommend a periodic evaluation of the instrument on site at Tecan.

The tests described in the following chapter do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

CAUTION: Before starting measurements, make sure that the Tecan Adapter for Cell Chips is inserted correctly. The position of chamber A1 has to be on the upper left side.

WARNING: The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

12.7.2 Cell Counting Accuracy

Accuracy is the ability of a system to give responses close to a true value. The accuracy is calculated as percentage deviation from the true value.

Material:

- Cell suspension, approx. $1x10^6$ cells/ml
- Tecan Cell Chip
- Tecan Adapter for Cell Chips
- Cell counting chamber for manual counting (e.g. Neubauer chamber)
- Pipette and tips (10 µl)

Procedure:

Adjust the cell suspension to a concentration of approximately $1x10^6$ cells/ml. Perform manual counting of cell suspension with, for example, a Neubauer chamber. Pipette 10 µl of cell suspension into the counting chambers (chamber A and B) of a Tecan Cell Chip and load the cell chip into the adapter (position 1). Start the Cell counting application.

Measurement Parameters:

Measurement	Cell counting app
Position	A1, B1 (define as duplicates)
Cell size	Depends on cell line
Images	4

Evaluation:

Calculate the difference between cell concentration (cells/ml) obtained from manual counting and automated counting and calculate the accuracy as follows:

$$\text{Accuracy}\,(\%) = \frac{concentration_{manual} - concentration_{automated}}{(\text{concentration}_{\text{manual}}/100)}$$

Accuracy data were collected by using HeLa and CHO cell lines. Cell lines with varying characteristics may not result in the same accuracy data.

13 Fluorescence Imaging (Cell Imager)

13.1 General Description of Fluorescence Imaging with the Cell Imager Module

Depending on the user's needs and applications, SPARK can be equipped with Tecan's Cell Module, described in chapter 12 Cell Module, or with the enhanced fluorescence imaging module, Cell Imager, which incorporates high-end bright field and fluorescence systems. If equipped with the Cell Imager module, the SPARK is delivered with a dedicated stand-alone computer, which meets the required memory- and video-card demands. Furthermore, the Cell Imager module requires specific humidity cassettes as described in chapter 16.4 Humidity Control.

Cytometry is the method of choice for the evaluation/quantification of many cellular parameters, such as count, shape and texture. Image cytometers utilize optical microscopy to statically image a large number of cells, which can be stained with a variety of fluorophores to visualize specific biomolecules. The Cell Imager with a bright field channel, four different fluorescence channels, three levels of magnification, astigmatism-based autofocusing, whole-well imaging with well border correction and full environmental control, enable the unique combination of live-cell cytometry and cell incubation. A whole well or a specified area within a well can be illuminated and imaged. Imaging of adherent cells is possible in standard microplate formats (SBS/SLAS format) at single cell resolution.

More recently, cells grown in a 3D environment have shown a higher physiological relevance in terms of e.g. proliferation, differentiation, metabolism, and gene expression and thereby more accurately recapitulate the in vivo situation of the native tissue. The SPARK Cyto provides the suitable features and technologies for successful imaging of 3D objects in U-shaped bottom plates and flat bottom plates. Quantification of growth, number and investigation into morphological changes can be performed.

Image acquisition and initial image analysis is implemented in the easy-to-use reader control software, SparkControl. In addition, a dedicated image analysis software, SPARK's ImageAnalyzer, allows the re-opening of already acquired images in order to optimize the analysis results. Input-tuned adjustments of object segmentation and confluence algorithms can be performed, as well as object gating and object exclusion in histograms and heat maps.

13.2 Bright Field Imaging

The Cell Imager offers an improved bright field illumination system, which captures a whole well of a 96-well plate in a single acquired image.

The detection of non-labeled cells, which exhibit very low optical density and are therefore barely visible, can be a troublesome issue in bright field imaging. The Cell Imager provides digital phase imaging, which produces a very high level of contrast and detail as well as an optimized sharpness. If bright field images are requested during a method, phase images are automatically generated and the digital phase contrast is calculated by the software. Furthermore, the new astigmatism-based autofocus detection produces optimized results in less time. Samples are illuminated from the top and image acquisition is performed from the bottom.

13.2.1 Optics

The bright field illumination system consists of a light emitting diode (LED) (1) and two lenses (2). Homogenous illumination is achieved by acquiring the image at infinity, while high dynamic range imaging is performed simultaneously in order to compensate for any meniscus effects. The sample plane is imaged by a 2x, 4x or 10x microscope objective attached to a revolving objective turret (3) and is further guided via a tube lens (4) to the camera (5).

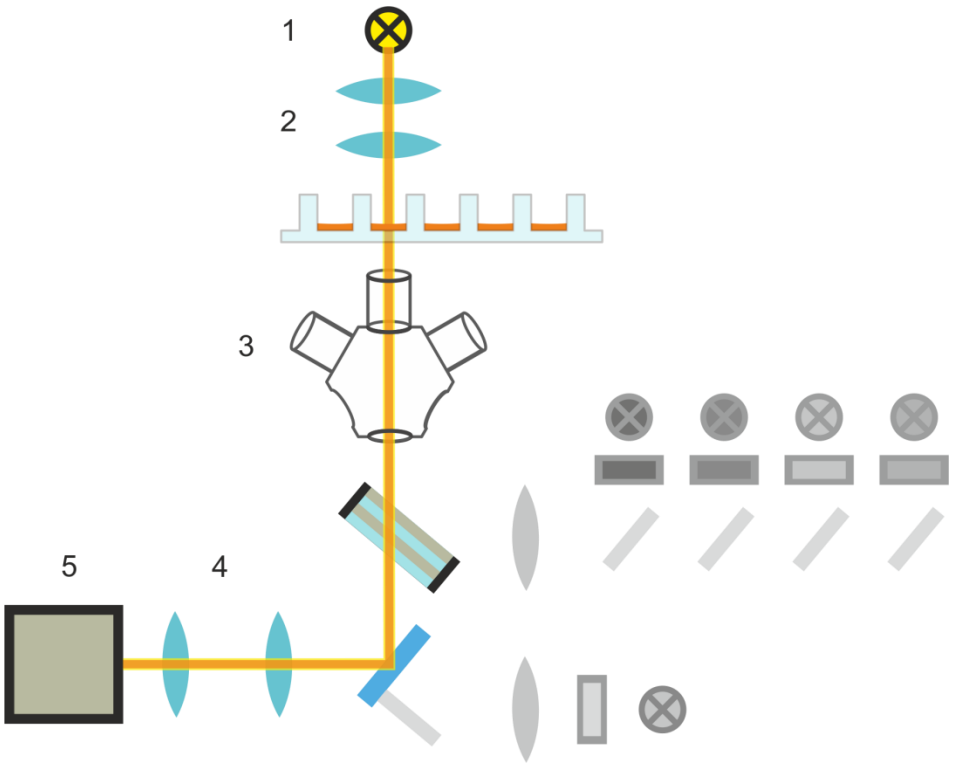


Figure 29: Schematic diagram of the bright field illumination system

13.2.2 Detection

An improved astigmatism-based autofocusing procedure (for a schematic diagram of the autofocusing system see figure below) allows for a stable, reliable and time efficient detection of objects in a microplate.

An LED (1) produces light, which is guided to the objective (2) and imaged onto the sample (3). The partial reflection of the autofocus light at the sample interfaces is imaged by the same objective, passes through the multiband dichroic filter (4) and is further transmitted via a tube lens (5) to the camera (6). For each measurement, a scan is performed along the optical axis to find the optimal position.

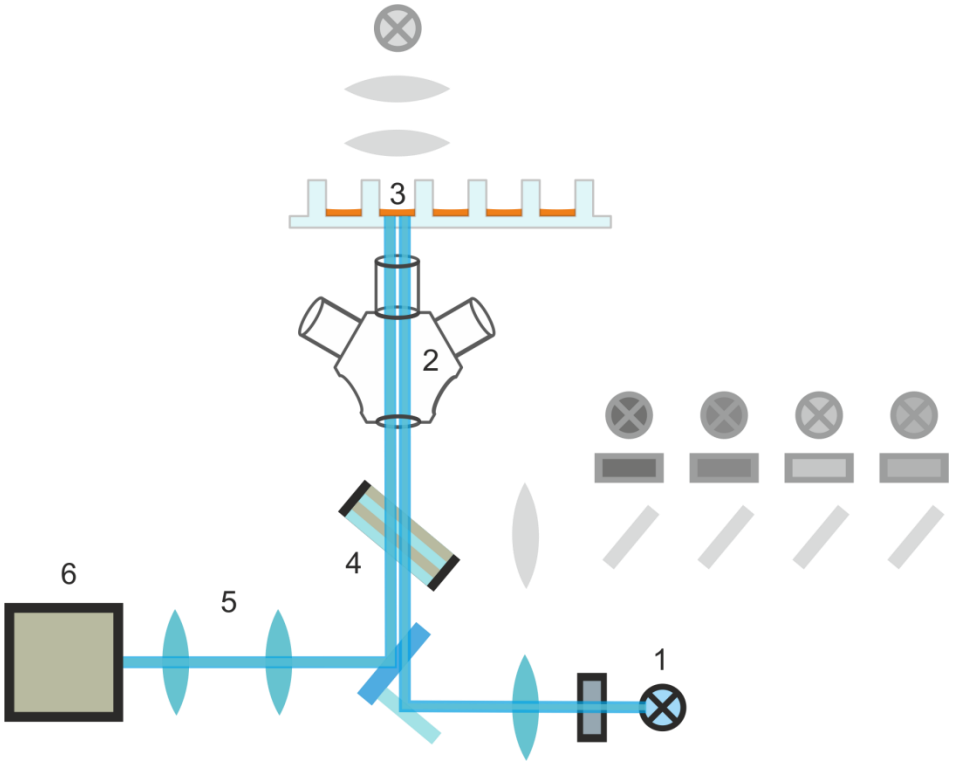


Figure 30: Schematic diagram of the auto-focusing system

13.2.3 Applications for Bright Field Imaging

Confluence Assessment

In order to create reproducible results in live-cell experiments, it is necessary to use comparable confluence-levels before starting an experimental procedure. In general, confluence is defined as the surface area of a well, petri dish or tissue culture flask that is covered with adherent cells.

In SparkControl, confluence values refer to the surface of the well that is covered by cells, given as a percentage of the whole.

Roughness Factor

The roughness factor provides a simple measure for cytotoxicity, cell division or even for the level of bacterial or yeast contamination within the cell population in a well, without the necessity of fluorescent staining.

SparkControl calculates the roughness factor as the normalized mean standard deviation of pixel intensities over all separated areas. An area may contain one or more cells. The dimensionless roughness factor can have a value between 0 and infinity.

NOTE: The roughness factor provides additional information about the cellular texture in a well. Changes in roughness factor are up to the user's interpretation.

Cell Counting in Microplates

Bright field cell counting is a label-free method for counting cells in a bright field image without any additional staining with fluorescent dyes. This enables researchers to quantitatively monitor cell proliferation and cell health without time-consuming workflows that may disrupt cell viability.

13.3 Fluorescence Imaging

The fluorescence module utilizes four color channels, that correspond to the most commonly used dye classes DAPI/Hoechst, FITC, TIRTC and Cy5.

Owing to the Cell Imager module's innovative hardware architecture, samples are analyzed and both fluorescence and bright field images are acquired using the same astigmatism-based autofocus system, the same objectives and the same camera. However, in contrast to the bright field module, fluorescent samples are illuminated and detected from the bottom.

13.3.1 Fluorescence Channels and their Excitation- and Emission-Profiles

Four different LEDs and their corresponding excitation filters may be selected in SparkControl.

The following table provides information about excitation and emission wavelengths, provided by the fluorescence module:

Channel	λ_{ex}	λ_{em}
Blue	381 - 400 nm	414 - 450 nm
Green	461 - 487 nm	500 - 530 nm
Red	543 - 566 nm	580 - 611 nm
Far-red	626 - 644 nm	661 - 800 nm

Exposure times and autofocus offset can be optimized by employing SparkControl's microscope mode, the Live Viewer.

13.3.2 Image Acquisition

If excited at the appropriate wavelength, the sample (1) emits a fluorescence signal, which is again passed through the multiband dichroic filter (2) and transmitted via the tube lens (3) to the camera (4).

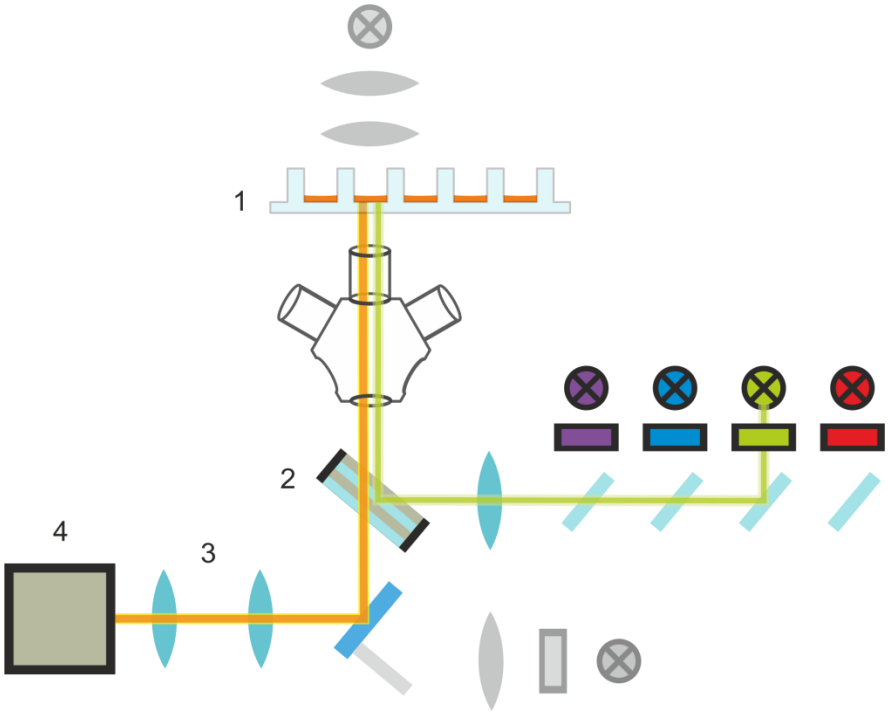


Figure 31: Schematic diagram of the fluorescence illumination system

13.4 Specifications for Cell Imager

13.4.1 General

Camera	Sony IMX264 CMOS chip, 2456 x 2054 pixels (= 5 megapixel), pixel size 3.45 µm
Illumination	Bright field LED, four sets (LED + excitation filter) of different excitation- and emission wavelengths for fluorescence imaging
Image	Wide-field bright field, digital phase contrast and wide-field fluorescence
Supported plate-formats	6-, 12-, 24-, 48-, 96- and 384-well plates

13.4.2 Objectives

The following table summarizes the optical properties of the various selectable Olympus objectives:

Objective	**2x**	**4x**	**10x**
Numerical aperture	0.08	0.13	0.30
Pixel resolution	3.45 µm	1.72 µm	0.69 µm
Optical resolution	4.50 µm	2.77 µm	1.20 µm
Field of view	8.47 mm x 7.09 mm	4.24 mm x 3.54 mm	1.69 mm x 1.42 mm

13.4.3 Full-multiband Filter Set

A Semrock full-multiband filter set, consisting of a full-multiband dichroic (FF409/493/573/652-Di01) and a specific emission filter set (FF01-432/515/595/730-25) is ideal for use with Hoechst, FITC, GFP, TRITC and Cy5.

Figure 32: Transmission profile of the full-multiband filter set, built-in T-image (obtained from the official Semrock website: www.semrock.com.

13.4.4 Measurement Times

Image Acquisition	Specified Measurement Time
96-well, whole-well bright field & digital phase imaging, 2x objective	≤ 12 min
96-well, center, bright field, digital phase and one fluorescence channel, 10x objective, default exposure time	≤ 15 min
Image Acquisition and Analysis for Standard Applications	**Specified Measurement Time**
Confluence, 96-well, whole-well, 2x objective, sample within 60-80% confluence range	≤ 20 min (incl. analysis)
Nuclei Counting, 384-well, whole-well, 4x objective, sample within 60-80% confluence range, optimized acquisition and analyzing settings	≤ 45 min (incl. analysis)
Viability, 24-well, center, 10x objective, sample within 60-80% confluence range, optimized acquisition and analyzing settings	≤ 10 min (incl. analysis)

13.5 Standard Applications

The Cell Imager supports a wide range of applications in the area of imaging-based cytometry. Some examples for common applications are given below. For details about defining the image analysis, see the Analysis Plugins instructions.

NOTE: In order to avoid fogged lids and indeterminate outcomes, use SPARK's temperature control to adjust the ambient temperature of the microplate to pre-measurement conditions.

NOTE: The given working-concentrations for the fluorescent dyes are only meant as guidelines and must be optimized for different cell lines by the user.

NOTE: For each application, an incubation time of 30 minutes is recommended to gain optimal fluorescence signals from treated cells. Additionally, incubation times must be optimized for different cells lines.

NOTE: Make sure to process samples in the dark, as fluorescence dyes may photobleach.

13.5.1 Nuclei Counting

Nuclei counting applications typically involve counting of cells in the blue fluorescent channel after staining their nuclei with a blue fluorescent dye such as Hoechst 33342, a cell-permeable dye that binds to DNA regions rich with adenine and thymine. Once bound, the Hoechst 33342 dye emits blue fluorescence after excitation by ultraviolet light. A dye concentration of 1 µg/ml is recommended.

13.5.2 Transfection Efficiency

Transfection Efficiency applications are usually based on cells producing a fluorescence protein as a reporter of gene expression, e.g. Green Fluorescence Protein (eGFP) and stained with a counterstain such as Hoechst 33342. Images are acquired in both color channels and the counterstain signal is used for the evaluation of the transfection efficiency rate.

13.5.3 Viability

Cell viability or cytotoxicity assays are common applications in the field of drug discovery, gene engineering and cell cultivation studies. Their goal is to determine the number of live and dead cells in a cell sample and thus to assess the impact of, for example, drugs, genetic modification or medium conditions on the living cells. Several Viability applications are based on esterase activity and cell permeability of cells stained with calcein AM and propidium iodide (PI). Calcein AM is used as a marker for live cells. It can pass the cell membrane of live and dead cells, but only the esterase activity of the live cells can convert the non-fluorescent calcein AM to a calcein with green fluorescence emission. Conversely, propidium iodide acts as a marker for dead cells. It can permeate only the cell membranes of dead cells that have lost their membrane integrity. Its intercalation into DNA results in an emission of red fluorescence. When working with SPARK CYTO, the following concentrations of the respective stain are recommended: 100 ng/ml of calcein AM and 1 µg/ml of propidium iodide.

13.5.4 Cell Death

Apoptosis and necrosis are two biochemical processes that lead to destruction of a cell. Apoptosis is the natural, genetically programmed cell death that results in elimination of old and unhealthy cells. On the other hand, necrosis or the unprogrammed, accidental cell death is initiated after exposure of cells to harmful external forces such as toxins, heat or infection.

For Cell Death analysis apoptotic and necrotic cells can be stained with Hoechst 33342, propidium iodide (PI) and Annexin V- AlexaFluor-488. The Hoechst dye is used as a counterstain and thus stains the nuclei of all cells in a sample. Its binding to DNA results in emission of blue fluorescence after excitation in the UV range. Propidium iodide is used as a red stain for necrotic cells. It also binds to the cellular DNA but it can permeate only the perforated membrane of the necrotic cells and not the intact membrane of the apoptotic cells. Finally, Annexin V-AlexaFluor488 targets the apoptotic cells. It binds in a calcium-dependent manner to phosphatidylserine, a phospholipid, that is exposed just on the outer surface membrane of the apoptotic cells. This binding event can be detected as green fluorescence.

The following concentrations are recommended for each fluorescent dye:

- 0.5 µL Annexin V – Alexa Fluor 488 conjugate (Invitrogen™, REF: A13201) per 100 µl 1x Annexin V binding buffer
- 1 µg/ml Hoechst 33342
- 1 µg/ml propidium iodide

13.5.5 Confluence

Confluence applications are typically based on bright field imaging and provide a quick overview of cell density and cell proliferation. The cell confluence is calculated as a percentage of the well area covered by adherent cells. See chapter 13.2.3 Applications for Bright Field Imaging for further details.

When using a small numeric aperture objective like the 2x objective, Tecan recommends using 200 µl volume/well to achieve best results (see Figure 33).

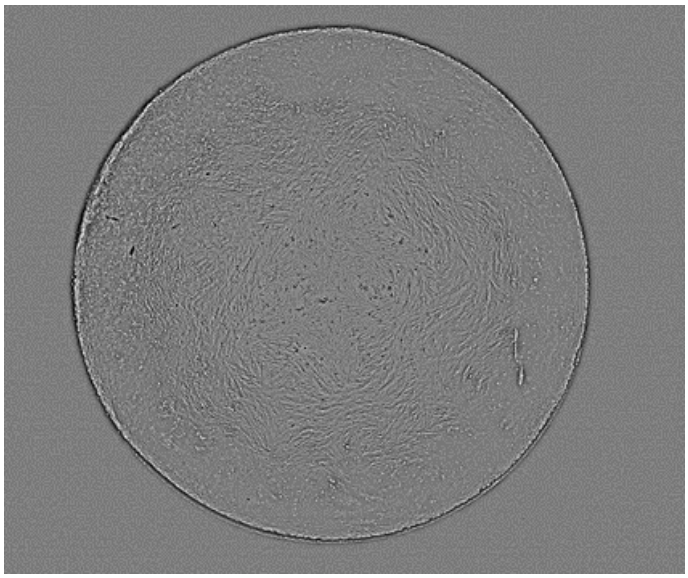


Figure 33: A processed bright field image of a well (96-well microplate) filled with 200 µl of medium as recommended.

When low liquid levels (e.g. 100 µl) are used in a well of a 96-well microplate, a blurred ring-shaped structure (yellow arrow) and a central overexposure of the well can be observed in raw images (Figure 34). This is due to reflection and refraction of the illuminating light at the liquid surface and at the well border. As a consequence, the resulting processed images may make further image analysis difficult.

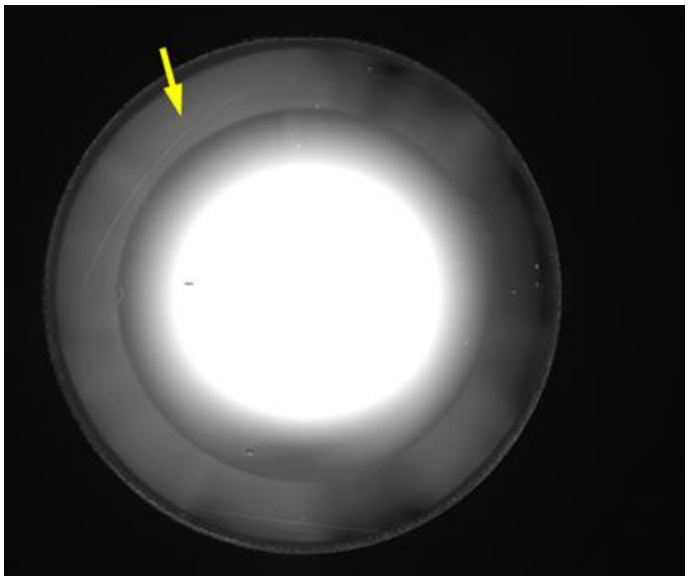


Figure 34: Bright field image of a well (96-well microplate) filled with 100 µl of medium.

Tecan has developed an innovative proprietary image capturing and processing technique that includes HDR to overcome this problem and thus deliver usable images, e.g. for confluence assessment (Figure 35). When using the 2x objective and low liquid levels in a 96-well microplate, there is a limitation to this approach resulting in a deviation of about 4 – 6 % in confluence assessment.

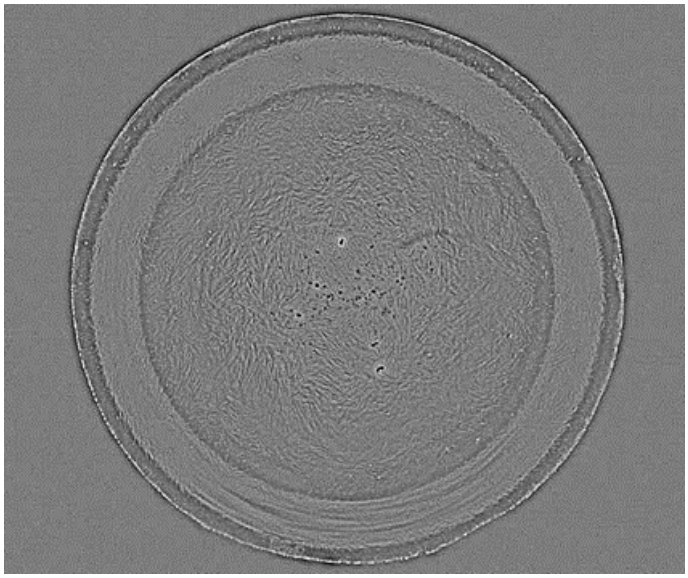


Figure 35: Processed bright field image of a well (96 well microplate) filled with 100 µl of medium.

Therefore when performing confluence assessment in 96-well plate, use of the 4x objective is recommended or when the 2x objective is used, perform the assessment with larger liquid volumes (≥ 200 µl) in the well to avoid the influence of the ring-structure.

NOTE: If the 2x objective is used in combination with the Confluence application in a 96-well format, a filling volume of ≥ 200 µl is recommended, as otherwise the meniscus may cause undesirable circular artifacts.

NOTE: For whole-well confluence analysis, a border offset of 150 µm is recommended.

13.5.6 Cell Counting in Bright Field Images

SPARK CYTO and its analysis software also support cell counting in a bright field image. The cell count reflects changes in the growth of a cell culture and thus provides insights into cell proliferation and cell viability (e.g. cytotoxicity studies). The cell number can also be correlated with other assay readouts (e.g. luminescence, absorbance) and used in the process of result normalization.

Using the machine learning approach, the teaching of the algorithm was performed with four selected cell lines (HeLa, A432, CHO and 3T3) that differ in their morphology and contrast. Therefore the best results are to be expected with these or similar cell lines.

NOTE: If the 2x objective is used in combination with the cell counting in a bright field image, reduced cell detection might be observed in the well border area due to decreased cell visibility in that area. For more details see chapter 13.5.5 Confluence.

NOTE: Low contrast of the unstained cells is one of the major obstacles for the bright field cell counting. Better results might be achieved by using dedicated imaging plates.

NOTE: Experimental conditions such as medium (composition, volume/well), autofocus offset values, plate bottom artefacts may have an impact on the bright field image and thus cell segmentation. For the best results use optimal experimental and acquisition settings.

13.5.7 3D Imaging

The 3D imaging application allows for basic analysis of spheroids and organoids in bright filed and/or fluorescence images. The capability of z-stacking with subsequent z-projection of all stacks, captures more information of the whole 3D object. The analysis results include the total object count, area, dimension, and eccentricity for the detected object (s). The fluorescence intensity signal of an object in a single-color channel or multiple-color channels can be measured in addition. In case of multiple objects per well, the total object count can be correlated with other assay readouts (e.g., luminescence) to normalize the result.

To detect the above-mentioned parameters, teaching of a deep learning algorithm for spheroids was performed on four selected cell lines (HeLa, A549, MCF-7 and MDA-MB-231). Single spheroids and multiple spheroids grown with and without matrix (Matrigel) were trained to allow for obtaining best possible results for these and similar cell lines. Teaching with organoids was conducted to identify in particular colon, lung and liver organoids. The main focus at teaching was set on segmentation of spherical objects.

14 Spark-Stack Microplate Stacker Module

The Spark-Stack is an integrated microplate stacker module, which is available as an option for the SPARK multimode reader. It is designed for automated loading, unloading and restacking of plates for walk-away automation of up to 50 non-lidded microplates per run.

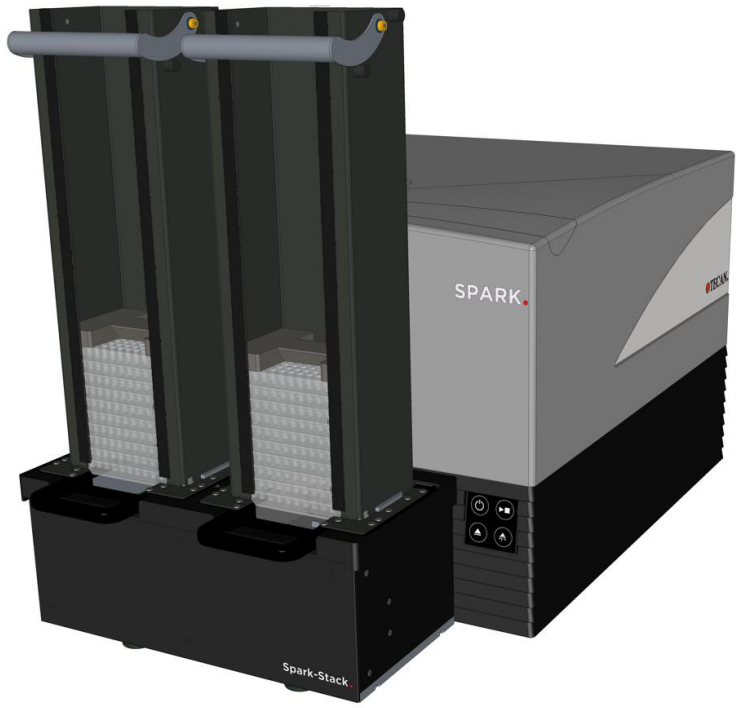


Figure 36: The built-in Spark-Stack microplate stacker for automated loading, unloading and restacking of up to 50 plates per run.

The built-in microplate stacker module uses plate magazines (stacks) as storage containers. The plate magazines are compatible with non-lidded 6- to 1536-well plates and are provided with light-protection covers for light-sensitive assays.

The microplates in the plate magazine located at the INPUT position of the Spark-Stack module are loaded into the SPARK reader one after the other. After the measurement has been performed, the processed plates are collected in the plate magazine at the OUTPUT position.

The grippers of the plate magazines are spring loaded to remain closed in the event of a power failure, holding the plates in position inside the plate magazines despite the lack of power.

Two different heights of plate magazines are available:

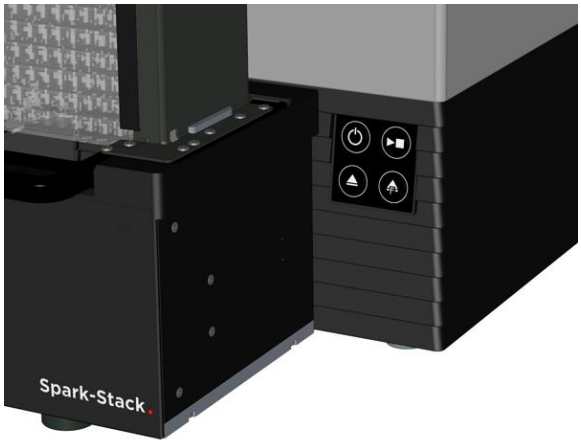
- Two short stacks with a capacity of up to 30 plates (standard 96-well plates) per run
- Two long stacks with a capacity of up to 50 plates (standard 96-well plates) per run

14.1 Access to the Front Panel

By removing the plate magazines from the stacker module, the operator has full access to the front panel of the SPARK multimode reader, for:

- Exchanging dichroic mirrors
- Exchanging filter slides
- Manual loading of a single plate onto the plate carrier of the SPARK reader
- Manual loading of the Spark MultiCheck plate for performing IQ/OQ

14.1.1 Onboard Control Buttons



If no plate magazines are installed on the Spark-Stack, all onboard control buttons are active; for more information, see chapter 2.6 Onboard Control Buttons.

When a plate magazine is installed on the Spark-Stack, only the Stop function of the Onboard-Start button  remains active. All other onboard control buttons are inactive. Pressing the Onboard-Start button during a stacker run will stop the stacker run after the current action is completed.

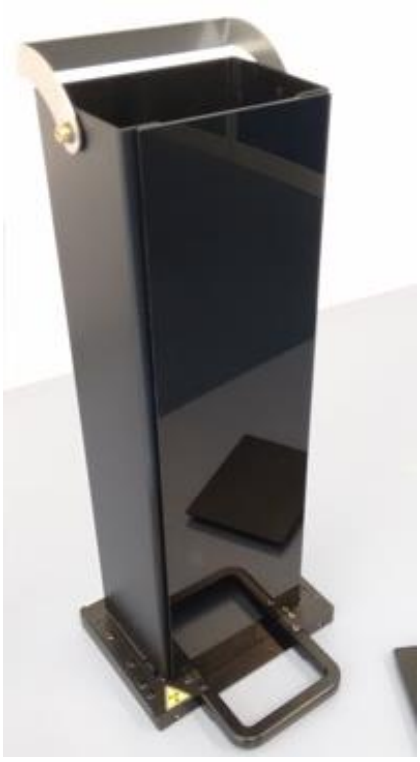
CAUTION: If a stacker run is interrupted using the Onboard-Start button  , a microplate may remain in the reader. Make sure to remove the microplate from the reader before starting another stacker run.

CAUTION: In the event of a power failure, make sure to remove the microplate from the reader and to remove all processed plates from the OUTPUT plate magazine, before starting a new stacker run.

14.1.2 Light Protection for Sensitive Assays/Dark Covers

The Spark-Stack microplate stacker includes a set of light-protective front and top covers, which can be quickly inserted into place on the plate magazines.

These elements help to shield microplates containing light-sensitive contents, such as GFP-transfected cells, AlphaScreen, AlphaLISA, AlphaPlex assay plates, etc. from ambient light in the laboratory.

		
1. Place the front cover onto the magnetic strips of the plate magazine.	2. Slide the front cover down into position.	3. Place the top cover on the plate magazine.

14.2 Microplate Requirements for the Spark-Stack

Any common microplates (without lids) ranging from 6 to 1536-well formats conforming to ANSI / SLAS standards may be used for performing stacker runs with the Spark-Stack module:

CAUTION: Do not use microplates with lids in the Spark-Stack module.

WARNING: Do not use humidity cassettes in the Spark-Stack module.

CAUTION: Make sure that the microplate matches the plate definition in the method to prevent problems during a stacker run. Always use microplates of the same type and color.

Specifications Spark-Stack

Parameters	Characteristics
Microplates (without lids)	from 6 to 1536-well formats conforming to ANSI / SLAS standards
Restacking time	15 seconds per plate (96-well microplate without smooth mode)

Required Microplate Dimensions

Parameters	Characteristics
Overall plate height	From 10 mm to 23 mm
Footprint	Length = 127.76 mm ± 0.5 mm Width = 85.48 mm ± 0.5 mm
Minimum difference between plate height and skirt height	≥ 6.7 mm

WARNING: Do not touch the inside of the input magazine or the output magazine during a stacker run.

WARNING: Do not insert or remove plates manually during a stacker run.

Microplates with Barcodes

Microplates with barcodes for identifying plate IDs are especially helpful for stacker kinetic runs.
A SPARK microplate reader equipped with the optional, integrated barcode reader module is required for barcode reading.

For more information, see chapter 2.5.2 Microplates with Barcode.

Automated Processing of Cell Chips with the Spark-Stack Module:

The plate magazines of the Spark-Stack are compatible with the cell chip adapter of the SPARK reader.

Therefore automated loading of Cell chips inserted in the cell chip adapter is possible using the Spark-Stack microplate stacker module.

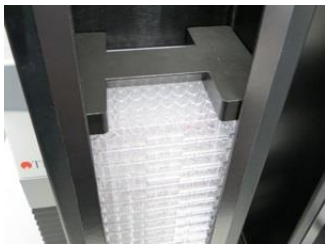
For more information, see chapter Cell Counting in Cell Chips in the SparkControl software manual.

Stabilizer Weights

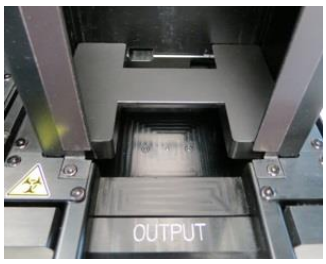
The stacker comes with two H-shaped stabilizing weight elements (one for each plate magazine). These elements are designed to weigh down the microplates in the plate magazines for reliable stacking.

NOTE: The plate magazines recognize the stabilizer weight, therefore the stabilizer weights will not be loaded into the SPARK reader and do not need to be removed when restacking. Make sure that the stabilizer weight is always on top of the plates in the plate magazine.

1. Place a stabilizer weight on the plates in the input magazine (The middle section is wedge-shaped. The wider part of the wedge should be facing up for easier gripping).



2. Place a stabilizer weight at the bottom of the output magazine.

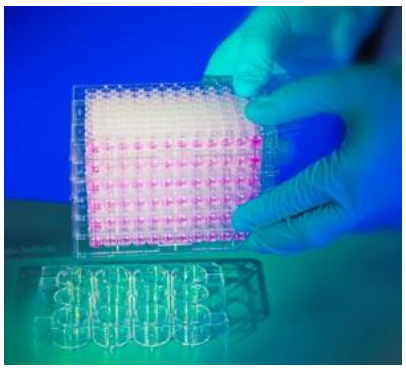


3. The Spark-Stack is now ready for operation.

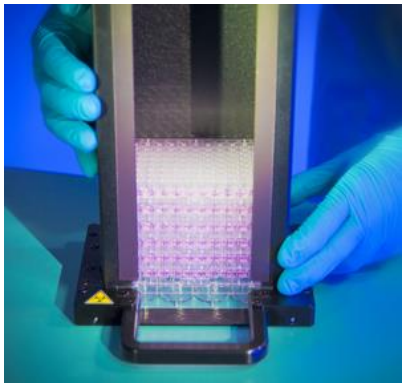
14.2.1 Loading Multiple Microplates into a Plate Magazine

It is possible to load several microplates at once into a plate magazine of the Spark-Stack module by using a standard 6-, 12-, or 24-well microplate, two 96-well microplates, or a standard half-deepwell or deepwell plate as a platform for the stack of microplates to be loaded into the plate magazine.

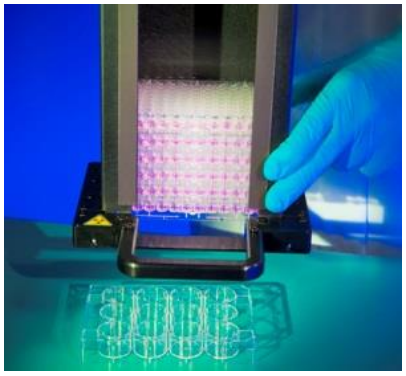
1. Prepare the full amount of microplates to be loaded into the plate magazine for the stacker run.
2. Place a portion of these microplates onto the platform plate. In this example, a standard 12-well plate is used as a platform plate. Make sure that the microplates are the same type and color and that well **A1** is in the upper left hand corner (closest to the **A1** label on the back left hand corner of the plate magazine).



3. Slide the plate magazine over the stack of microplates all the way down until the plate magazine makes contact with the surface of the lab bench.



4. Lift up the plate magazine. The stack of microplates has been added to the plate magazine. The platform plate remains on the lab bench.



Load the remaining microplates using the same procedure.

CAUTION: Ensure that no microplates are inserted upside-down.

CAUTION: Ensure that all microplates are inserted with the A1 label in the upper left hand corner.

CAUTION: Always wear gloves when manually inserting microplates into the plate magazine. Fingerprints or smudges on the optical (bottom) surface of the microplate can adversely affect reader measurements.

CAUTION: Use only compatible plates. Flexible, and non-level plates, such as PCR-plates are not suitable.

CAUTION: Do not use microplates that show any form of damage.

CAUTION: Do not use microplates with lids in the Spark-Stack module.

CAUTION: If the sealing films or foils are removed from microplates before measurement: Make sure that the top of the microplates are not sticky from residual adhesive, otherwise the plates can become stuck together and cause problems with retrieval from the plate magazine.

Additionally, make sure the microplates are level and have not become bent during the sealing process.

NOTE: To minimize evaporation during long time stacker kinetic runs, use empty plates at the first and the last position (top and bottom of the stack of plates to be measured) when loading microplates into the plate magazine.

NOTE: Condensation might have an impact on measurement quality. To avoid/minimize condensation effects on the sealing foil and/or bottom of the plate

- work in a temperature-controlled room
- equilibrate plates to room temperature
- centrifuge plates with sealing foils
- use the SPARK's heating option and define measurement workflows with plate incubation before measuring each plate.

14.2.2 Loading a Single Microplate into a Plate Magazine

Before loading a single microplate into a plate magazine, please ensure that:

- well **A1** of the microplate is closest to the **A1** label on the back left hand corner of the plate magazine,
- the microplate is not upside down, its type and color correspond to the plate definition used in the method, and
- the microplate is not obviously damaged.

CAUTION: Always wear gloves when manually inserting microplates into the plate magazine. Fingerprints or smudges on the optical (bottom) surface of the microplate can adversely affect reader measurements.

1. Manually insert the microplate into the top of the plate magazine and carefully lower it towards the bottom of the plate magazine.

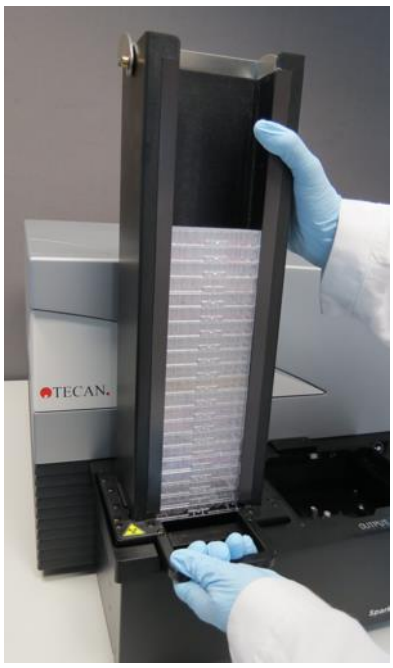
2. Gently release the microplate at the bottom of the plate magazine.

14.2.3 Loading the Plate Magazines onto the Spark-Stack Module

Lift the plate magazines using the handles at the bottom as shown below.

Position the plate magazine above the corresponding position on the Spark-Stack module and push it straight down.

1. Load the plate magazine with the microplates to be processed at the position labelled INPUT.
2. Press the plate magazine down firmly to click it into position.



3. Load the empty plate magazine at the position labelled OUTPUT.

CAUTION: If a plate magazine has not been inserted properly onto the Spark-Stack module, the Start Stacker button will be disabled. If this happens, press the plate magazine down to click it into place. Then start the stacker run.

CAUTION: If the output magazine is not empty, an error message will appear at the start of a stacker run. If this happens, clear the plates from the output magazine and restart the stacker run in the software.

CAUTION: If a plate has been forgotten on the plate carrier inside the SPARK reader, an error message will appear at the start of a stacker run. If this happens, remove the plate magazines from the Spark-Stack module. Move out the plate carrier from the SPARK reader and remove the microplate. Then move the empty plate carrier back into the SPARK reader. Re-load the plate magazine onto the Spark-Stack module and restart the stacker run.

CAUTION: Do not load additional microplates into the input magazine while a stacker run is in progress.

14.2.4 Inserting Microplates Directly into the SPARK Reader

By removing both plate magazines from the Spark-Stack module, it is possible to perform single plate measurements using standard microplates, the Spark MultiCheck plate or the NanoQuant Plate.

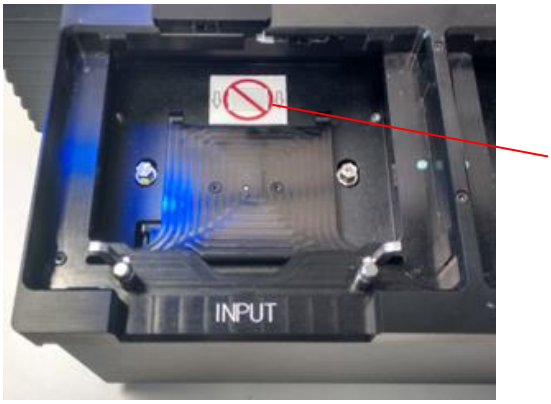
WARNING: Move the plate transport out first, before inserting a microplate. Place the microplate directly on the plate transport of the SPARK reader. Do not place the plate on the lifting table of the stacker when the **No microplates** label is visible, indicating that the plate transport is still located inside the reader. Otherwise this will a cause a collision with the plate, when the plate transport is moved out of the reader.

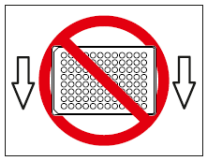
If this happens press the onboard Start/Stop button  , or press the stop button in the software.

WARNING: Treat bio-hazardous material according to applicable safety standards and regulations.

When loading a single plate by hand:

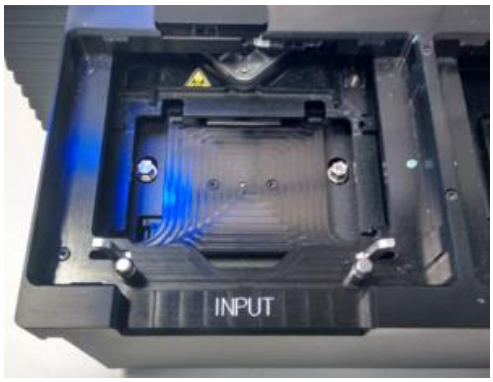
1. If the **No Microplates** label is visible when you try to load a single plate by hand, this indicates that the plate carrier is still inside the reader. Do not insert a microplate if this label is visible!



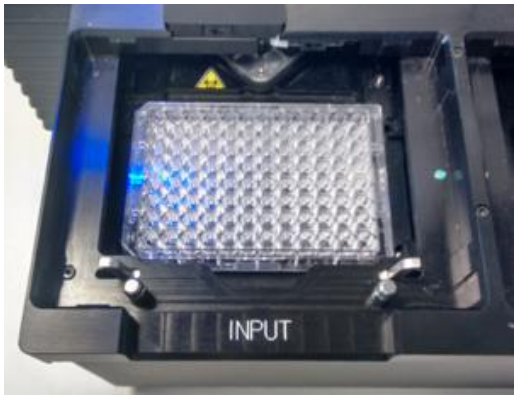


No Microplates label

2. Move the plate transport out first, before inserting a microplate. The **No Microplates** label is not visible when the plate carrier is moved out.



3. Place the microplate on the center of plate transport. Ensure that the **A1** label on the microplate is in the upper left hand corner. Always place the microplate on the plate transport – never directly onto the lifting table of the stacker.



14.2.5 Unloading Processed Microplates Individually

CAUTION: Always wear gloves when unloading microplates from the plate magazine.

1. Gently remove the plate magazine from the Spark-Stack module. Avoid any tilting of the plate magazine. Place the plate magazine on the work bench.

2. Carefully grip the microplate at the top of the stack of plates in the plate magazine.

3. Gently slide the microplate towards the top of the plate magazine, and then remove the microplate.
   Avoid any spilling.

4. Discard the plate according your laboratory procedures.

14.2.6 Unloading a Group of Processed Microplates

CAUTION: Always wear gloves when unloading microplates from the plate magazine.

1. Gently remove the plate magazine from the Spark-Stack module. Avoid any tilting of the plate column.
2. Place the plate magazine on the work bench.

3. Carefully slide one hand underneath the microplate at the bottom of the plate magazine, and stabilize the group of microplates you want to unload, with the other hand.

4. Gently slide the group of microplates towards the top of the plate magazine, and then remove the group of microplates. Avoid any spilling.

5. Discard the microplates according your laboratory procedures.

14.2.7 Cleaning and Maintenance of the Spark-Stack

Liquid Spills

WARNING: Always switch off the instrument before removing any kind of spills on the microplate stacker. All spills must be treated as potentially infectious. Therefore, always adhere to applicable safety precautions, (including the wearing of powder-free gloves, safety glasses and protective clothing) to avoid potential infectious disease contamination.

Additionally, all resulting waste from the clean-up must be treated as potentially infectious and the disposal must be performed according to the information given in chapter 7.4 Disposal.

Cleaning and Disinfection Procedure (including Liquid Spills)

The procedure for cleaning and disinfecting the Spark-Stack, including for liquid spills inside a plate magazine or on the Spark-Stack module is as follows:

1. Wear protective gloves, protective glasses and protective clothing.
2. Prepare a suitable container for all disposables used during the disinfection procedure
3. Switch off the SPARK reader to shut down the instrument and the built-in Spark-Stack module.
4. Remove the plate magazines.
5. Remove the microplates from the plate magazine or remove the microplate from the lifting table of the Spark-Stack module.
6. Wipe up any spills immediately with absorbent material.
7. Clean the surfaces of plate magazines and the Spark-Stack module.
8. For biohazardous spills, carefully wipe all outside surfaces of the instrument with a lint-free paper towel soaked in the disinfection solution (B33 [Orochemie, Germany] or 70% ethanol).
9. Wipe cleaned areas dry.
10. Dispose of contaminated material appropriately.

Preventative Maintenance

No special preventative maintenance is required for the Spark-Stack module. For further information, see chapter 7 Cleaning and Maintenance.

15 Injectors

The injector module consists of one or two syringes contained in external units with lightproof covers. There are different syringe volumes, 500 µl, 1000 µl and 2500 µl. The injector needles are designed to inject liquid into any well of a 1 to 384-well microplate that complies with SBS standards (with the exception of small volume 384-well plates).

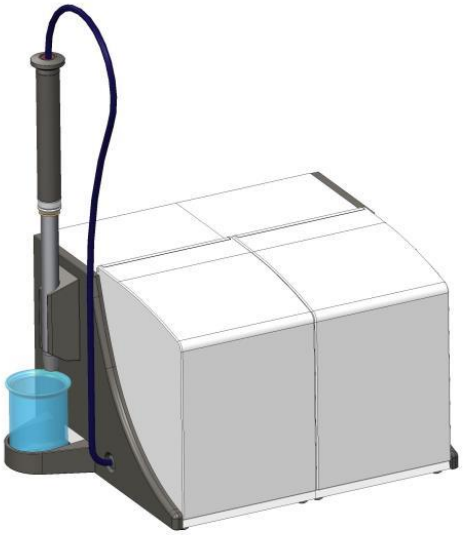


Figure 37: Injector module consisting of two injector boxes

CAUTION: Switch off the instrument before plugging in or unplugging the injector module.

15.1 Injector Carrier

The injector carrier can be easily removed (by the customer) from the instrument for actions such as injector priming, rinsing or optimizing injection speed.

When using the injector during a measurement procedure, the injector carrier must be inserted correctly into the instrument. Remove the injector dummy and insert the injector carrier into the injector port. Press the injector carrier gently into the port to lock it in place.

The instrument is equipped with an injector sensor that checks the position of the injector carrier. If the injector is inserted into the instrument incorrectly, the sensor will not recognize the inserted injector carrier and injection will be disabled; however, actions such as rinsing and priming will remain enabled. Performing rinse or prime procedures with an incorrectly inserted injector carrier can damage the instrument. Therefore, always make sure that the injector carrier is in the service position for rinsing and priming (see figure below).

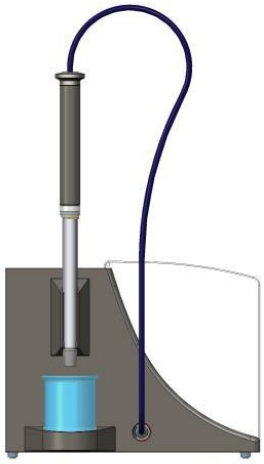


Figure 38: Injector carrier in service position

CAUTION: Never touch the syringes during operation.

CAUTION: The injector carrier must be in the service position for rinsing and priming. Do not perform rinse or prime procedures if the injector is inserted in the instrument. Performing rinse or prime procedures with an incorrectly inserted injector carrier can damage the instrument.

CAUTION: The injector carrier must be inserted correctly into the injector port, otherwise the injector will not be detected and prime and rinse functions will remain enabled. Performing rinse or prime procedures with an incorrectly inserted injector carrier can damage the instrument.

The injection speed can be adjusted via the software. The optimal injection speed is dependent on the assay characteristics, such as the plate format and the viscosity and measuring behavior of the liquids. The removable injector carrier makes it possible to optimize this process outside of the instrument where a visual inspection can be easily performed.

15.1.1 Injector Dummy

All instruments possessing injector ports (instruments with injectors or instruments prepared to be upgraded for injectors) are delivered with injector dummies. The injector dummy replaces the injector if the injector itself is not in use. In such cases, the injector dummy ensures that the desired atmosphere in the instrument remains stable (temperature, gas concentration).

Always reinsert the injector dummy into the injector port after the injector carrier has been removed. Press the injector dummy gently into the port to lock it in place and close the lid. The injector dummy activates the injector sensor only if it is positioned correctly in the injector port.

CAUTION: Make sure that the injector dummy is inserted in the injector port every time the injector is not in use.

CAUTION: Be aware that the injector dummy also activates the injector sensor if correctly inserted into the injector port. Injection steps can be performed with the injector dummy inserted, however the results will be unusable.

15.1.2 Storage Bottles and Bottle Holders

One injector box can accommodate a storage bottle up to 125 ml volume. The standard bottle set for each injector box consist of one 125 ml and one 15 ml bottle. Each injector box includes a bottle holder designed for different sizes and volumes. The bottles and tubes containing the fluids that are to be injected can be attached securely to the holder using flexible PVC clasps. The tubes from the injector syringe can be inserted into a carbon needle that reaches down to the bottom of the flask to ensure the optimal aspiration of even small volumes of fluid (see Figure 40: Bottle holders).

If not in use the carbon needle carrying the tubes can be easily stored inside the injector box. To avoid any liquid drops in the injector box, the storage position of the needle is equipped with a liquid collection container (1 ml).

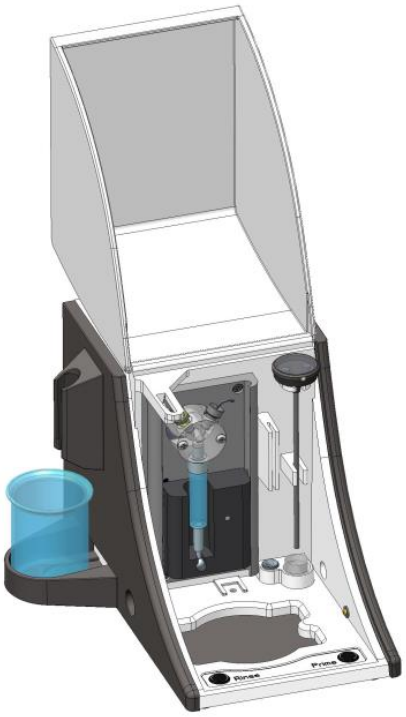


Figure 39: Injector box with carbon needle and storage tub

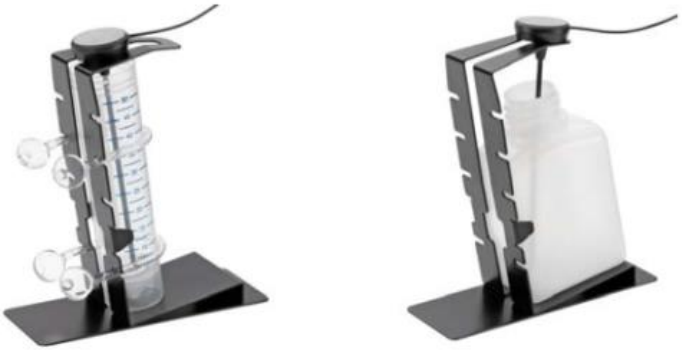


Figure 40: Bottle holders

15.2 Priming and Rinsing

CAUTION: The injector carrier must be in the service position for rinsing and priming. Prime and rinse must not be performed when the injector carrier is in the injector port.

The initial filling step of the injector system (priming) as well as the cleaning step of the injector system (rinsing) must take place outside of the injector port. For these procedures the injector carrier is removed from the instrument and put into the service position of the injector module. For priming and rinsing steps of the injector system, a default setting for injection speed and volume dispensed is provided. If required the priming parameters can be adjusted in the Injector Control window of the software.

The prime volume depends on the tubing length. Two types of injector tubing are available: Short = 100 cm (39.37 in.) and Long = 200 cm (78.74 in.).

The minimal priming volume is 1000 µl for an injector with short tubing and 1500 µl for an injector with long tubing.

CAUTION: Prime volumes that are too small may result in incomplete filling of the system, and therefore may negatively affect assay performance.

CAUTION: Do not touch the injector needles! They can become easily bent or misaligned, which can cause injection problems or damage the instrument.

15.2.1 Priming

Before the injection system can be used, an initial filling step (priming) is needed to remove all air and to completely fill the system with liquid.

It is recommended to perform a rinsing step before priming.

Priming can be performed by using the software or by using the hardware button ‘prime’ on the injector box.

To perform the priming procedure:

1. Fill the storage bottles with the necessary reagents and insert the feeding tube(s). Make sure, that the tube(s) reaches the bottom of the bottle.
2. Remove the injector from the injector carrier port of the instrument and insert it into the service position of the injector module.
3. Put an empty container under the injector.

4. Adjust parameters via the Injector/Prime window in the Dashboard or the Method Editor.

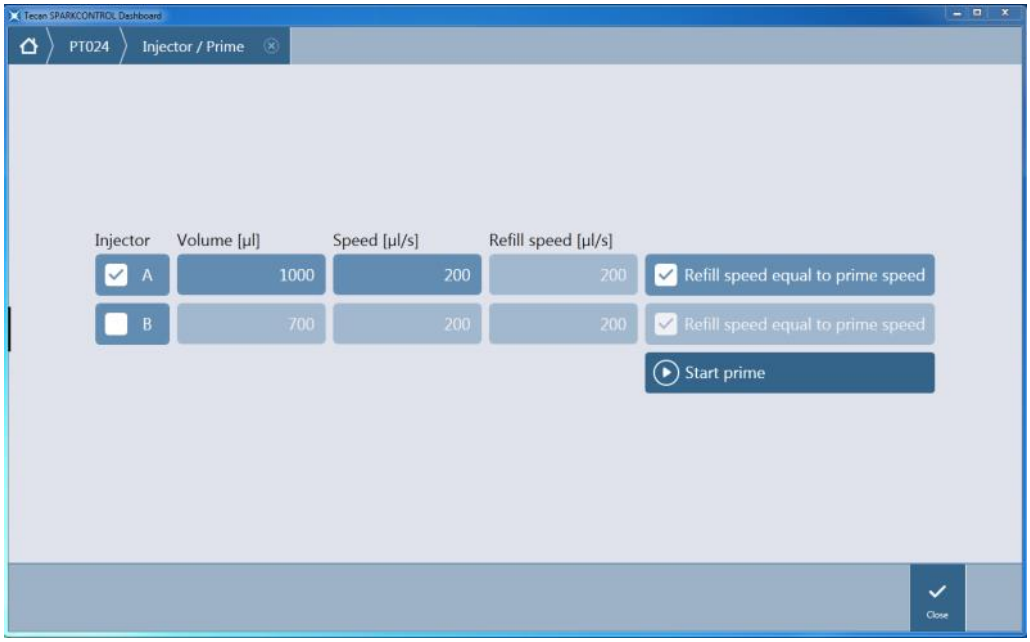


Figure 41: Priming parameters

5. Visually inspect the liquid jet, the syringes for air bubbles and the tube(s) for leaks and kinks. Any bubbles should be removed after priming to ensure good injection performance.
6. Select the required injector(s).
7. Define the prime volume.
8. Define the prime speed (values depend on selected syringe size).
9. Define the refill speed (values depend on selected syringe size) or select **Refill speed equal to prime speed**.
10. Start prime by clicking the **Start prime** button.
11. Select **Close** to exit the Injector/Prime window.

NOTE: The selected settings for priming can be saved to the hardware buttons on the injector box by choosing the option Save as default. This function is only available via the Method Editor. Press the Prime button on the injector box to start the priming procedure.

After a successful priming procedure, reinsert the injector carrier into the instrument and close the lid of the injector module completely before starting a measurement. The injectors are now ready to use.

15.2.2 Reagent Backflush

Prior to the cleaning of the injector system, reagent backflushing allows the remaining reagent in the liquid system (injector needles, syringes, valves and tubing) to be pumped back into the storage bottles. This procedure is a cost effective solution for minimizing reagent consumption. The dead volume of the injection system is approximately 100 µl.

To perform a reagent backflush procedure:

1. Remove the injector from the carrier port and insert it into the service position of the injector box.

WARNING: Hold the injector carrier only by the handle provided for this purpose.

2. Insert the feeding tubing into the appropriate storage bottle.
3. Adjust parameters via the Injector/Backflush window in the Dashboard or the Method Editor.

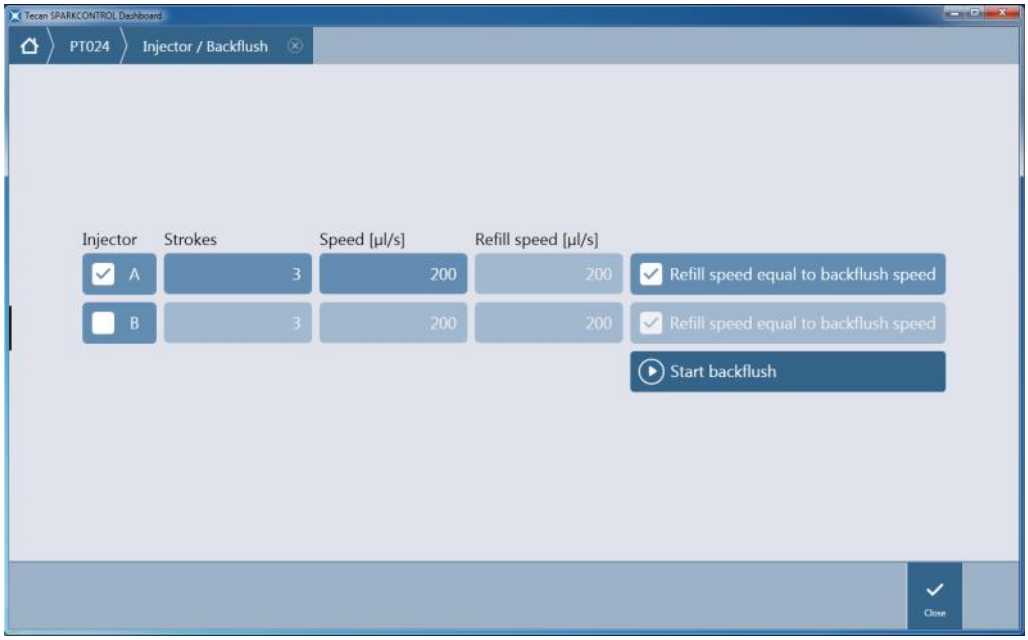


Figure 42: Backflush parameters

CAUTION: The injector must be in the service position for the Backflush action. Do not perform Backflush when the injector is in the instrument.

4. Select the required injector(s). Only primed injectors are available for ‘backflush’.
5. Define the number of piston strokes.
6. Define the backflush speed (values depend on selected syringe size).
7. Define the refill speed (values depend on selected syringe size) or select **Refill speed equal to backflush speed**.
8. Start the reagent backflush procedure by clicking **Start backflush**.
9. Click **Close** to exit the Injector/Backflush window.

Rinse

Before the instrument is switched off, it is recommended to perform a rinse procedure to clean the injector system. Rinsing can be started via the software or by using the hardware button ‘Rinse’ on the injector box.

To perform a typical rinse procedure:

1. Remove injector carrier and bring it into the ‘service position’.
2. Optionally, perform a backflush procedure to feed unused reagent back into the storage bottle.
3. Fill the storage bottles with the appropriate wash reagents (distilled or deionized water, 70 % ethanol, …) and insert feeding tubes of the injector system.
4. Put an empty container under the injector.
5. Adjust parameters via the Injector/Rinse window in the Dashboard or the Method Editor.

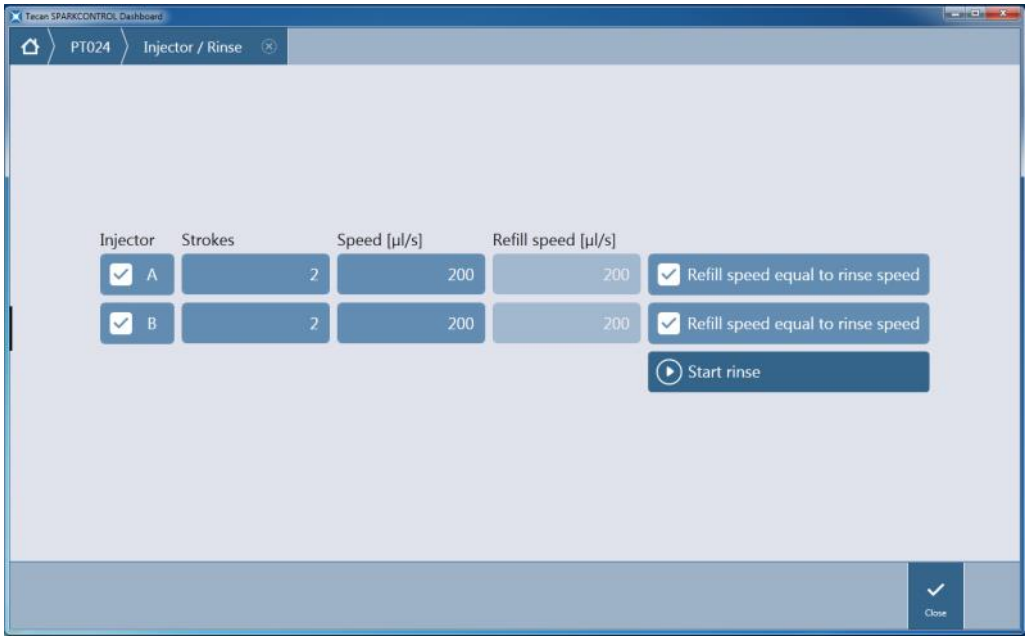


Figure 43: Rinse parameters

6. Select the required injector(s).
7. Define the number of piston strokes (1-60).
8. Define the rinse speed (depend on the syringe size).
9. Define the refill speed (depend on the syringe size) or select **Refill speed equal to rinse speed** check box.
10. Click **Start rinse** to start the rinse procedure. After the rinse procedure the syringe will remain filled, first step in the priming procedure is to empty the syringe.
11. Select **Close** to exit the Injector/Rinse window.

NOTE: The selected settings for rinsing can be saved to the hardware buttons on the injector box by choosing the option Save as default. This function is only available via the Method Editor. Press the Rinse button on the injector box to start the rinsing procedure.

CAUTION: The injector carrier must be in the service position for the Rinse action. Do not perform Rinse when the injector is in the instrument.

CAUTION: Be sure to run a final rinse procedure with distilled water.

CAUTION: Take good care of the injectors! If they are damaged, the accuracy of dispensing may be affected. This can result in damage to the instrument.

15.3 Injector Cleaning and Maintenance

The required maintenance may vary with your application. The following procedures are recommended for optimal performance and maximum life of the injector system.

CAUTION: To avoid reagent mixing and cross-contamination, rinse the whole injector system thoroughly between different applications requiring the injector(s).

Daily Maintenance:

If not otherwise stated by the manufacturer of the kit used, the following tasks must be performed daily:

- Inspect the syringes(s) and tubing for leaks.
- Flush the whole system thoroughly with distilled or deionized water after each use and when the syringe is not in use. Failure to do so can result in crystallization of reagents. These crystals can damage the syringe seal and valve plug, which can result in leakage.

CAUTION: Do not allow the syringes(s) to run dry for more than a few cycles.

Weekly/Periodical Maintenance:

The injector system (tubing, syringes, inject needles) must be cleaned weekly to remove precipitates such as salts and eliminate bacterial growth.

Follow these steps to clean the syringe/injector system with 70 % EtOH (ethanol):

1. Depending on the user's application thoroughly flush the system with buffer or distilled water before rinsing with 70 % EtOH.
2. Rinse the syringe with 70 % EtOH with syringes fully lowered for 30 minutes.
3. After the 30-minute period, pump all fluid from the syringe and tubing into a waste container.
4. Rinse the syringe/injector system with 70 % EtOH.
5. Rinse the syringe/injector system with distilled or deionized water. Leave the fluid pathway filled for storage.
6. Clean the end of the injector needles carefully with a cotton swab soaked in 70 % ethanol or isopropanol.

WARNING: Risk of fire and explosion!

Ethanol is flammable and when improperly handled can lead to explosions.
Proper laboratory safety precautions must be observed.

15.3.1 Syringe Replacement

CAUTION: Syringes must only be replaced by a service technician, otherwise the performance of the instrument cannot be guaranteed.

15.4 Injector: Reagent Compatibility

The injector system consists of the following materials:

- PTFE, TFE, FEP: Tubing, valve plug, seal
- PEEK: Needle head, coupler tubing/injector
- KelF: Valve body
- Parylene coating: Injector needles

See the following list for reagent compatibility. Rating ‘A’ indicates a good compatibility with the injector system. Chemicals with the rating ‘D’ must not be used with the injector system. They will severely damage the injector system.

‘A’ Rated Chemicals	‘D’ Rated Chemicals
Acetic Acid < 60%	Acetonitrile
Dimethyl Formamide	Butyl Amine
Ethanol	Chloroform
Methanol (Methyl Alcohol)	Carbon Tetrachloride (dry)
Water, Deionized	Diethyl Ether
Water, Distilled	Ethanolamine
Water, Fresh	Ethylene Diamine
Potassium Hydroxide (Caustic Potash)	Furfural
Potassium Hypochlorite (aqueous)	Hexane
Sodium Hydroxide (< 60%, aqueous)	Hydrofluoric Acid
Sodium Hypochlorite	Monoethanolamine
	Sulfuric Acid (diluted or concentrated)
	Tetrahydrofuran

CAUTION: Use only ‘A’ rated reagents with the injector system. ‘D’ rated reagents must not be used with the injector system.

The information listed in this table has been created by Tecan Austria according to available material compatibility information and provides only a general guideline for the selection of compatible reagents.

WARNING: Approved chemicals must be stored and handled properly. Environmental factors such as temperature, pressure and concentration can result in undesired chemical behavior, which can damage the instrument.

WARNING: Be aware that the improper handling of chemicals may result in serious injury. Follow safe laboratory practices and wear protective clothing when handling chemicals.

15.5 Heater and Magnetic Stirrer

The injector module can additionally be equipped with a heater and magnetic stirrer option. The heater is designed to keep the injection solution at the desired temperature. The stirring function keeps the injection solution in motion in order to avoid the accumulation of precipitates or debris on the bottom of the solution container. Concurrent to the heating and stirring functions, the injection solution is also protected from light during the entire experiment by the injector cover. The heater and stirrer functions are independently regulated with control knobs positioned on the module itself without the use of the SparkControl software.

Figure 44: Heater and magnetic stirrer module

The heater and magnetic stirrer option is available for both the one-syringe and the two syringe injector option. The basic heater and stirrer module consists of control knobs for temperature and stirring speed regulation.

For the two syringe injector option, the basic heater and magnetic stirrer module is expanded by an additional module. The temperature and stirring speed of the expansion module is simultaneously regulated by the basic module.

Figure 45: Injector module with two syringes and heater and magnetic stirrer option

15.5.1 Installing the Heater/Stirrer

To install the Heater & Stirrer module it is necessary to remove the bottom plate(s) of the injector module (see figure below). The appropriate tool to loosen the screws is delivered with the instrument. When finished, simply place the injector on top of the Heater & Stirrer module. Plug-in the power cable of the Heater & Stirrer and the module is ready to use.

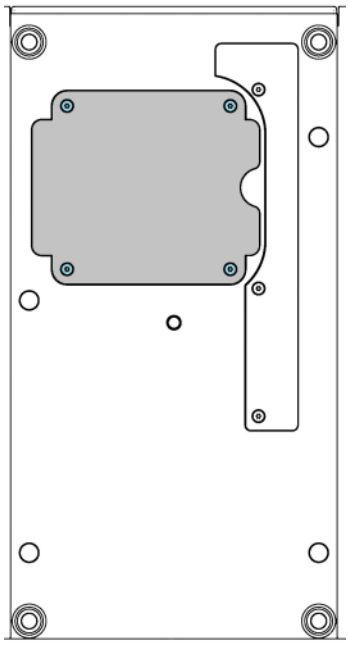


Figure 46: Bottom of the injector box.

15.5.2 Temperature Regulation

Temperature regulation is possible between 20 °C and 42 °C. If the temperature regulation is switched on, the status lamp is lit.

- A constant green light indicates that the target temperature is achieved.
- A constant yellow light indicates that the target temperature is higher than the current temperature.
- A yellow blinking light indicates that the target temperature is lower than the current temperature.
- A red or red blinking light indicates that an error occurred. (Contact your local Tecan service representative).

NOTE: The selected temperature equates the temperature of the surface of the heating plate. The temperature of the injection solution in the container has to be controlled explicitly by the user.

CAUTION: If heating is activated be aware that the basic as well as the expansion module are equally tempered!

15.5.3 Setting the Stirring Speed

The stirring speed can be set from 50 to 1000 rpm (revolutions per minute). When stirring is switched on, the status lamp is lit.

- A constant green light indicates that the motor is running.
- A red or red blinking light indicates that an error occurred. (Contact your local Tecan service representative).

15.5.4 Laboratory Flask and Magnetic Stir Bar

The heating plate is designed to accommodate a laboratory flask of up to 100 ml volume. The standard set for each heater and magnetic stirrer module consists of one 100 ml laboratory flask and an appropriate magnetic stir bar.

15.6 Injector Specifications

NOTE: All specifications are subject to change without prior notification.

15.6.1 Technical Specifications for the Injector

Parameters	Characteristics
Plate types	1- to 384-well plates
Injector syringe volumes	500 µl, 1000 µl, 2500 µl

15.6.2 Performance Specifications for the Injector

500 µl syringe		
Inject Volume	**Accuracy**	**Precision**
10 µl	≤ 5 %	≤ 5 %
100 µl	≤ 1 %	≤ 1 %
450 µl	≤ 0.5 %	≤ 0.5 %
1000 µl syringe		
Inject Volume	**Accuracy**	**Precision**
20 µl	≤ 5 %	≤ 5 %
200 µl	≤ 1 %	≤ 1 %
900 µl	≤ 0.5 %	≤ 0.5 %
2500 µl syringe		
Inject Volume	**Accuracy**	**Precision**
50 µl	≤ 5 %	≤ 5 %
500 µl	≤ 1 %	≤ 1 %
2250 µl	≤ 0.5 %	≤ 0.5 %

15.6.3 Specifications for the Heater / Stirrer

Parameters	Characteristics
Power supply	24 V, max. 60 Watt, external plug-in
Temperature regulation	20-42 °C
Stirring speed regulation	50-1000 rpm

15.7 Quality Control of the Injector Module

15.7.1 Periodic Quality Control Tests

Depending on usage and application, we recommend a periodic evaluation of the instrument on Tecan site.

The tests described in the following chapters do not replace a full evaluation by the manufacturer or authorized dealers. But the tests may be performed periodically by the user to check significant aspects of the instrument performance.

The results are strongly influenced by errors in pipetting and the setting of the parameters in the instrument. Therefore, please follow the instructions carefully. The user should determine the appropriate intervals for this testing based on how frequently the instrument is operated.

CAUTION: Before starting measurements, make sure that the microplate is inserted correctly; well A1 has to be on the upper left side.

WARNING: The following instructions explain how to perform the Quality Control to check the specifications of the instrument. If the results of these control tests do not conform to the specifications of the instrument given in this manual, please contact your local service center for further advice.

15.7.2 Injector Accuracy

Accuracy is the ability of a system to give responses close to a true value. The accuracy is calculated as percentage deviation from the true value.

Material:

- Distilled water
- Greiner 96-well plate, flat bottom, transparent
- Scales with accuracy specification of 1 mg

Procedure:

Prime the injector with distilled water. Weigh the empty plate and note. Inject 20 µl into 20 wells of a Greiner 96-well plate (flat bottom, transparent) plate and immediately weigh the plate again (take care of evaporation effects). Perform procedure at room temperature (25 °C).

Injection Parameters:

Injector	Select Injector A or B
Speed	200 µl/s
Refill speed	Same as injection speed
Refill mode	Standard
Refill volume	Default
Plate definition file	GRE96ft
Part of plate	D2-E10

Evaluation:

The weight of 400 µl distilled water (20 x 20 µl) at 25 °C is 398.8 mg (mass density of water is 0.997 mg/µl). Calculate the Accuracy (%) as follows:

$$\text{Accuracy (\%)} = \frac{398.8 - measured}{(398.8/100)}$$

16 Environmental Control

The heating, gas and humidity control of Tecan's multimode reader, SPARK, provides an optimal system for the regulation of environmental conditions during a measurement run. Stable environmental conditions are demanded by many assays to ensure optimal performance. Especially when performing live-cell experiments – a constant temperature, pH-value and humidity are required to maintain cell health and to minimize cellular response to environmental changes.

16.1 Heating Module

The heating module enables temperature control within a range from 3 °C above ambient temperature to 42 °C. Heating of the measurement chamber will take some time. Please check the temperature control display. If not incubated externally, the microplate should be left for equilibration before the measurement is started.

NOTE: To keep the temperature constant and provide uniformity across the plate, the plate must be placed in incubation position while shaking or waiting. When the heating function is used during shaking, the temperature may vary slightly.

The temperature control in the software can be activated manually or during the execution of a method.

16.2 Cooling System

The cooling system of the SPARK multimode reader enables temperature control in a range from 18 °C up to ambient temperature.

Preparing the instrument for cooling and the cooling of the measurement chamber itself will take some time. Please follow these instructions and check the temperature control display. If not incubated externally, the microplate should be left for equilibration before the measurement is started.

The cooling system consists of two main components: the external liquid cooling device and the integrated cooling module (Te-Cool). The two components form a closed circulation system.

The liquid cooling device is an external unit, which pumps cooled liquid into the integrated cooling module to cool the air and the heated water returns to the external liquid cooling device to be cooled again.

The integrated cooling module is mounted on the bottom of the SPARK multimode reader. It cools the air and blows the air into the measurement chamber of the reader. The warm air flows back to the integrated cooling module to be cooled again.

Tecan recommends and supports the following liquid cooling device exclusively: **Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies GmbH, Germany)**. Tecan assumes no responsibility for any other product or liquid cooling solution. Prior to operating the SPARK reader in combination with the integrated cooling module and the liquid cooling device, read and follow the instructions provided by the manufacturer of the liquid cooling device (Laird Technologies, operating manual).

WARNING: Tecan assumes no responsibility for any liquid cooling system other than the one recommended in this document.

WARNING: Carefully read and follow the instructions given in the operating manual of the external liquid cooling device as well.

CAUTION: To ensure optimal operation of the cooling system, an annual maintenance procedure must be performed by a Tecan service technician.

16.2.1 Setting up the Liquid Cooling System

CAUTION: If the external liquid cooling device is used after it has been placed in storage or transported, it should be left to stand for at least 3 hours, to allow for temperature adjustment.

Before activating the cooling control option, ensure that the designated site meets the following requirements: Select a location for the external liquid cooling device that is flat, level, vibration free, away from direct sunlight or heat sources, and free from dust, solvents and acid vapors. Leave sufficient distance behind the instrument for access to the rear panel.

NOTE: The ambient temperature sensor is located on the inside of the rear panel of the instrument and may be influenced by nearby heat sources.

The external liquid cooling device has an air-cooled refrigeration system. The liquid cooling device must be positioned so that the air flow is not restricted. Supply and return flow connections must be easily accessible and all tubes must be installed without sharp bends. A minimum clearance of 0.3 meters on all vented sides is necessary for adequate ventilation.

CAUTION: Leave enough space between the liquid cooling device and adjacent objects, 0.3 meters on all vented sides. Inadequate ventilation will cause a reduction in cooling capacity and compressor failure.

Coolant

Only distilled water-propylene-glycol mixture may be used as a coolant. A propylene-glycol concentrate is obtainable from Tecan. This concentrate (0.25L concentrate) must be diluted with 0.75L distilled water prior to use to obtain 1L of coolant. Never use any other coolant or tap water to avoid instrument damages caused by pollution and corrosion!

CAUTION: Only use the recommended coolant in the cooling system, otherwise the integrated cooling module or the external liquid cooling device could become damaged (lime scale, impermeability of tubing).

CAUTION: Never operate the liquid cooling device without coolant in the reservoir!

16.2.2 Connection Procedure

CAUTION: Use only cooling tubing with no signs of damage.

The following information details the connection procedure:

- SPARK reader and external liquid cooling device: Make sure that the main power cables are unplugged and the main power switch is in the OFF position.
- Connect the coolant OUTLET of the liquid supply of the external liquid cooling device to the instrument's SUPPLY port on the back of the integrated cooling module. Use the provided tube. (See figure below and Figure 48).
- Connect the coolant INLET of liquid return of the external liquid cooling device to the instrument's RETURN port on the back of the cooling module. Use the provided tube.
- Connect the integrated cooling module to the cooling port of the SPARK reader using the provided CAN cable. (See figure below and Figure 50).
- Connect the condensate tube from the CONDENSATE OUTLET on the rear panel of the instrument (integrated cooling module). Place a condensate collector at the end of the tube. A condensate collector is not delivered with the instrument. (See figure below and Figure 49).
- Open the coolant reservoir of the external liquid cooling device by removing the cap. (See figure below).
- Fill the coolant reservoir about 2/3 full with coolant.
- Close the coolant reservoir of the external liquid cooling device by replacing the cap. (See figure below).

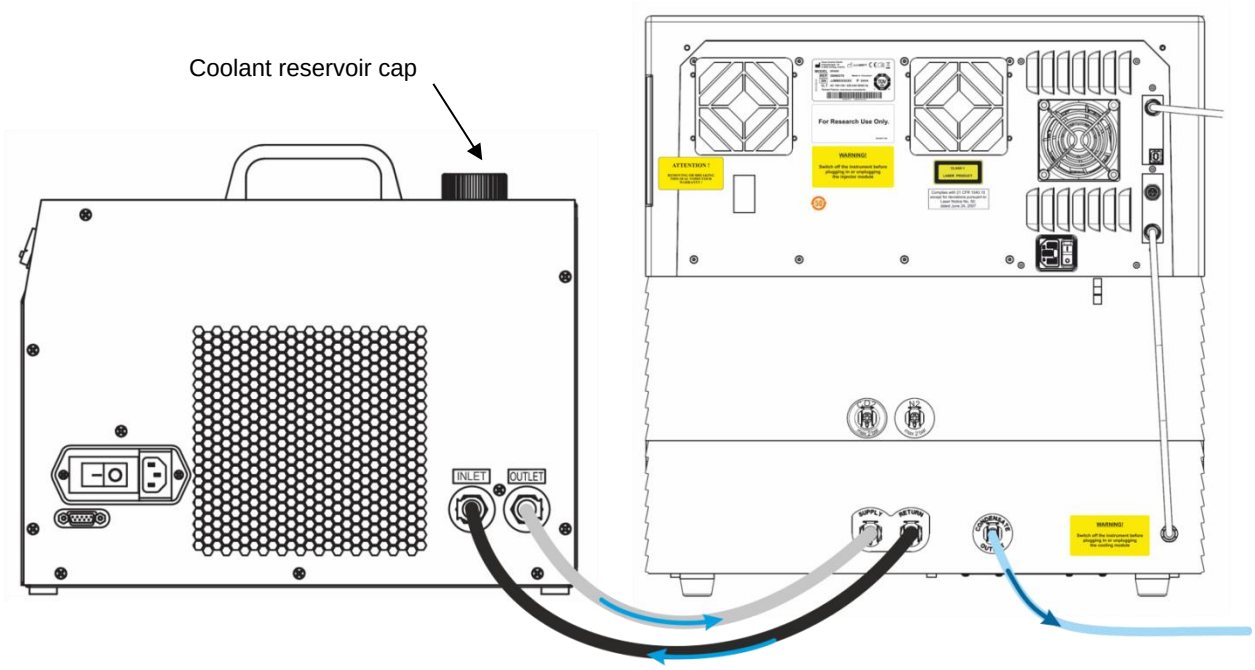


Figure 47: SPARK with integrated cooling module connected to the external liquid cooling device

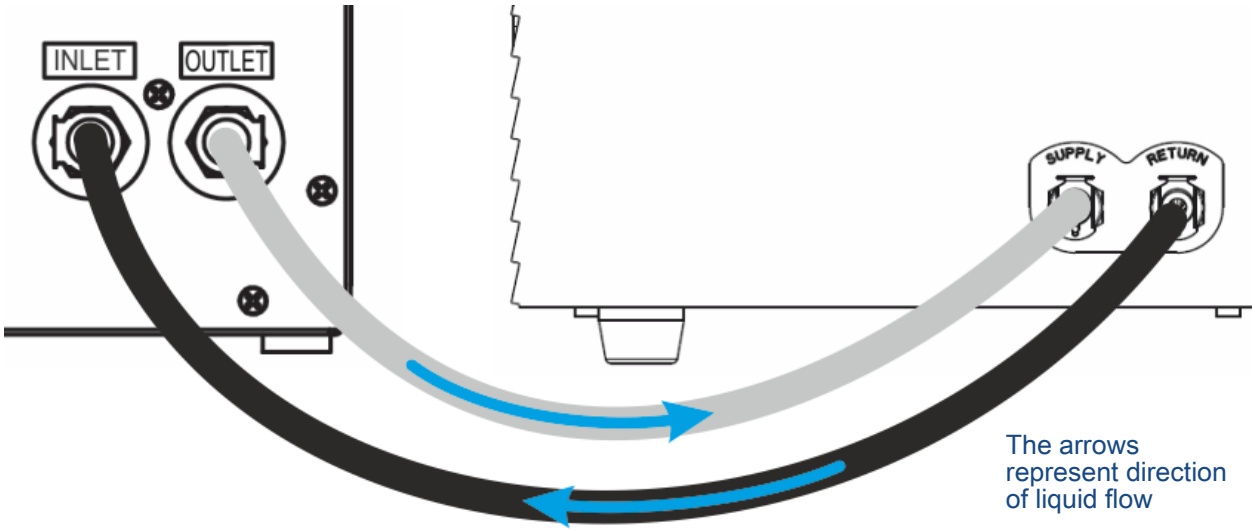


Figure 48: Connections between the integrated cooling module and the external liquid cooling device

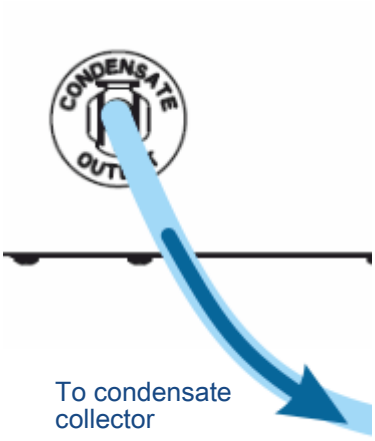


Figure 49: Condensate outlet

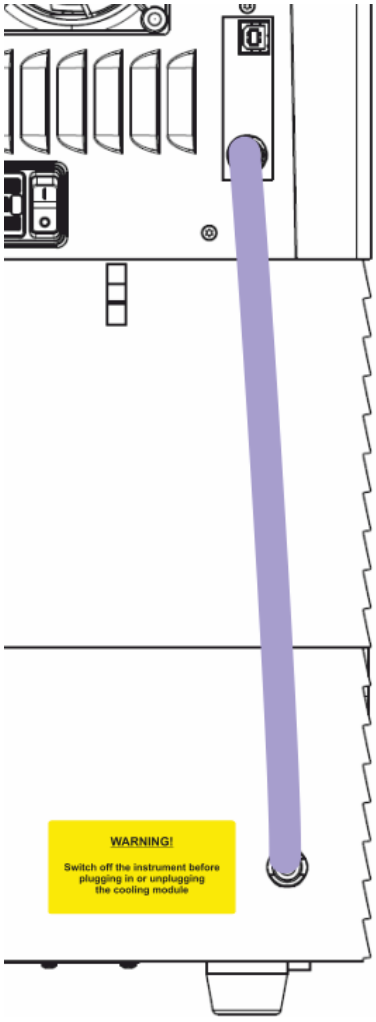


Figure 50: CAN cable

16.2.3 Switching on the External Liquid Cooling Device

1. Make sure that the coolant reservoir is about 2/3 full of coolant.
2. Connect the main power cable of the liquid cooling device to an appropriate AC power source.
3. Switch on the device and let it run for about 10 minutes in order to fill and vent the cooling system. Continuously check the filling level during this procedure. If required, add coolant.
5. Check the compliance with the operational parameters (see the operating manual of the liquid cooling device).
6. Set the digital controller to 12 °C (see the operating manual of the liquid cooling device).
7. Replace the cap on the coolant reservoir.
8. The device is now ready for operation.

NOTE: For daily start-up, switch on the liquid cooling device for an appropriate amount of time prior to usage, depending on the ambient temperature of the laboratory.

CAUTION: Place the liquid cooling device near the instrument being cooled so that the tubing is straight and without bends or kinks.

16.2.4 Operating the Integrated Cooling Module (Te-Cool)

Switch on the main power switch of the external liquid cooling device and set target temperature to 12 °C. To set the temperature, see the operating manual, Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies).

Wait for coolant equilibration before starting a measurement by using the cooling function of the SparkControl software. Depending on target temperature settings, the ambient conditions and the current temperature of the measurement chamber, this will take 30 to 90 minutes.

Two condensation prevention stoppers are delivered with the instrument (see figure below). They fit into the slots on the left and the right side of the integrated cooling module. They should not be installed by default. If installed, the cooling module will heat up and target cooling temperature may not be achieved. They must be installed if the cooling function operates at full capacity (large difference between ambient temperature and target temperature) to prevent condensation. Otherwise an accumulation of water might be observed.

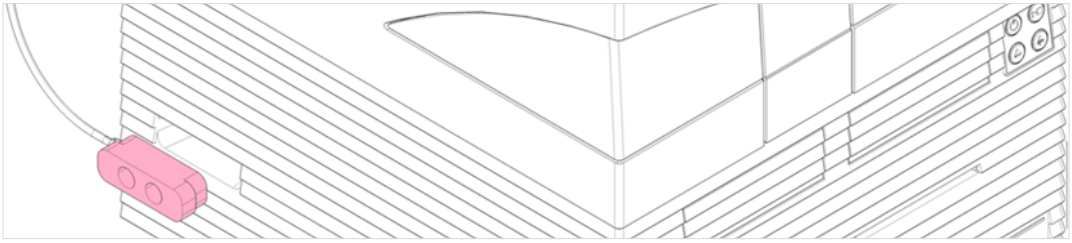


Figure 51: Condensation prevention stoppers (both sides of the instrument)

NOTE: The condensation prevention stoppers must only be installed by the user if a large difference between ambient temperature and target temperature is expected.

16.2.5 Alarm Function/Troubleshooting

For the external liquid cooling device alarm functions and for troubleshooting, see the operating manual, Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies GmbH).

For further technical support and services, contact your local Tecan Customer Support organization.

16.2.6 Maintenance

For the external liquid cooling device maintenance, see the operating manual, Thermoelectric Re-circulating Liquid Chiller MRC 150/300 (Laird Technologies GmbH).

For daily maintenance, inspect the tubes for kinks and leaks and check that all tubes are connected properly. Check that the external liquid cooling device is filled up with coolant. Check the level of the condensate collector and empty if required.

16.3 Gas Control

The gas control module offers a comprehensive solution for a variety of cell-based applications for the SPARK multimode reader. Two integrated gas inlets allow the control of CO_2 and O_2 to help maintain stable culture conditions and improve cell growth. Carbon dioxide concentration is regulated by an inflow of CO_2 gas, whereas oxygen reduction is achieved by supplying N_2 gas.

When equipped with the gas control module, the instrument can be used for in vitro studies of eukaryotic cell lines as well as for the study of anaerobic or facultative anaerobic bacteria.

The gas control module is available in two configurations:

CO_2 configuration:	CO_2 concentration can be regulated inside the measurement chamber
CO_2 and O_2 configuration:	CO_2 and/or O_2 concentrations can be regulated inside the measurement chamber.

16.3.1 Gas Safety

Adhere to the following guidelines:

- Always follow basic safety precautions when using the gas control module to reduce the risk of injury, fire, or electrical shock.
- Read and understand all information in this chapter. Failure to read, understand, and follow the instructions in this chapter may result in damage to the instrument or gas control module, injury to operating personnel or poor instrument performance.
- Observe all WARNING and CAUTION statements in this chapter. Ensure that this safety information is accessible for every employee working with the gas control module.
- Furthermore, it is assumed that instrument operators, due to their vocational experience, are familiar with the necessary safety precautions for handling gas and biohazardous substances.
- Precautions must be taken when working with potentially infectious material. Make sure to treat biohazardous material according to applicable safety standards and regulations as well as good laboratory practice guidelines.
- Wear protective glasses when using compressed gases outside of the instrument when the instrument is open.

WARNING: The gas control option is designed for CO_2 (carbon dioxide) and N_2 (nitrogen) supply only. The gas control option must only be used by trained personnel.

Never use a flammable or cryogenic gas supply!

WARNING: Adequate ventilation must be provided for the room in which CO_2 and N_2 are used.

WARNING: Follow the security measures for working with compressed gas (transportation, storage, handling and use)!

The CO_2 and N_2 gas cylinders must be securely fastened upright to a large, stationary object at all times.

Always protect the gas cylinder from falling! A compressed gas cylinder which falls and is damaged can easily become a lethal projectile!

16.3.2 Gas Connection

Before activating the gas control option, ensure that the designated site meets the following requirements. Operate the gas control module in a well-ventilated, temperature and humidity controlled (air-conditioned) environment.

Temperature: 15 °C (59 °F) – 35 °C (86 °F)

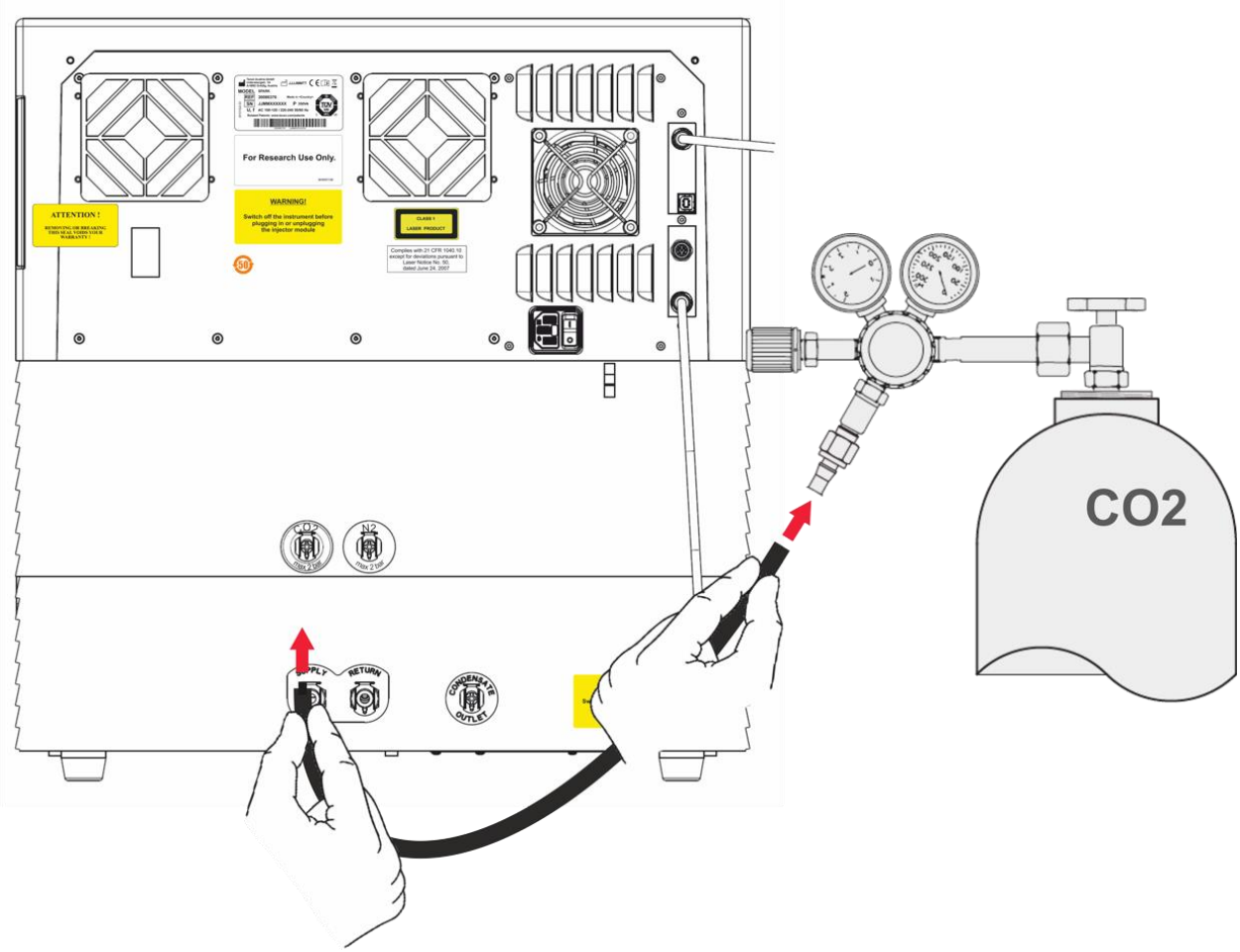
Do not expose or locate the instrument near direct sunlight or heat sources.
Maintain a low-dust environment. Keep liquids and vapors away from the instrument.
Leave sufficient distance behind the instrument for access to the rear panel. Make sure that all gas tubes are accessible and in no way obstructed.

WARNING: Follow appropriate gas handling precautions and safety regulations when setting up the CO_2 and/or N_2 supply. Read all label information and material safety data sheets (MSDS) from the manufacturer or supplier.

WARNING: Always use a regulator approved for the specific gas with high- and low-pressure gauges.

The following information describes the gas connection procedure:

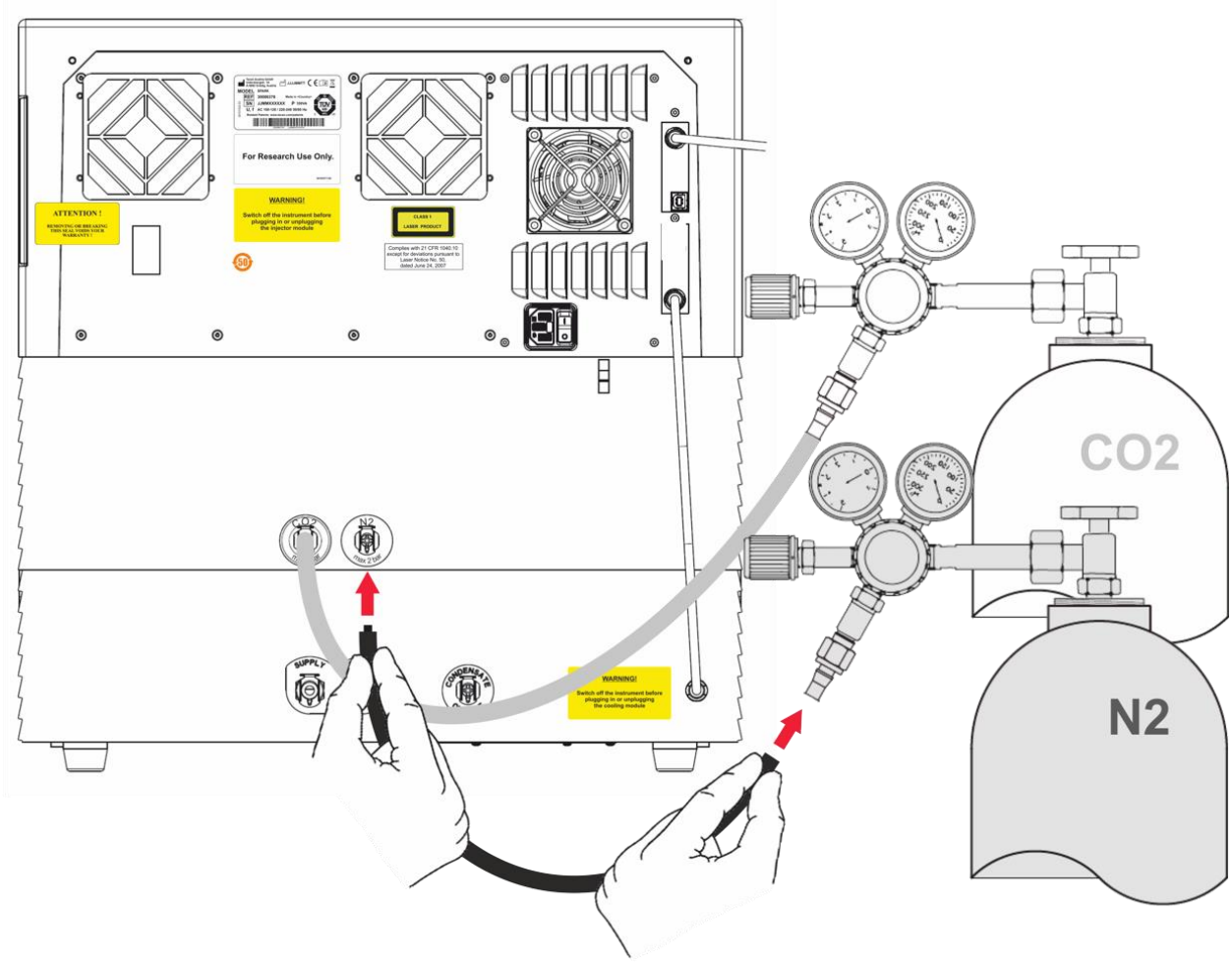
Connect the pressure regulator's outlet of the CO_2 gas cylinder or laboratory gas handling system to the instrument's inlet port ('CO_2') on the back. Use the provided tube with quick connector and attach the tube to the regulator of the cylinder with a plastic clamp, as depicted in the figure below.



Start the SparkControl software and enter the sea level height of your location. See chapter SparkControl Settings – Instrument in the SparkControl software manual).

NOTE: Before starting work with the Gas module, the sea level height of your location must be entered via the SparkControl software.

If the gas control module is configured for **CO_2 and O_2**, nitrogen gas can be used to regulate the amount of oxygen, in addition to CO_2 regulation. Connect the pressure regulator's outlet of the N_2 gas cylinder or central gas supply to the instrument's inlet port (N_2) on the back. Use the provided tube with quick connector and attach the tube to the regulator of the cylinder with a plastic clamp, as depicted in the figure below.



16.3.3 CO_2 and N_2 Gas Cylinders (Not Supplied)

To control the gas concentration, gas cylinder(s) or a laboratory gas handling system with pressure reduction valves are required.

Gases: Carbon Dioxide (CO_2) to regulate CO_2 concentration; Nitrogen (N_2) for the reduction of O_2 concentration (e.g. 50 Liter cylinder). It is recommended that the gases meet the following gas purity levels:

Gas	Gas Purity
CO_2	≥ 99.0 %
N_2	≥ 99.9 %

The pressure reduction valve must have two gauges – one for the pressure in the bottle (high pressure gauge) and one for the reduced pressure of max 2 bar (max 29 psi; low pressure gauge). Take care that the display for regulating the pressure has a range of 5 bar (72.5 psi) or maximum 15 bar (217.5 psi) to allow regulation from 1 – 2 bar. Make sure that the pressure reduction valve is designed for use with biological applications (ask manufacturer).

The connection from gas cylinder to pressure reduction valve is different for each country. **Check with a gas cylinder company in your country for the proper connection!** Check that the connection piece of pressure reduction valve matches the inner diameter of the gas tube to the instrument. (The inner diameter of this tube is approx. 6 mm). The tube on the connector to the pressure reduction valve must be secured with a plastic clip; a pair of pliers will be necessary to perform this task.

Make sure that there are no bends or kinks in the tubing.

If necessary, convert bar into psi: bar x 14.5 = psi (pounds per square inch), e.g. 2 bar = 29.0 psi.

To protect the gas cylinder from falling, a cylinder stand or table mount (with a securing chain or strap), or gas cylinder cradle can be bought from a gas cylinder company or ordered from a laboratory catalog.

WARNING: Before opening the main valve, ensure that the regulator and the shut-off valves are closed.

WARNING: Make sure that the gas (CO_2 and N_2) to the instrument does not exceed a maximum pressure of 2 bar.

WARNING: Keep the injector port closed during gas supply. Insert the injector dummy if the injector is not in use.

WARNING: Before running a method with gas supply check gas tubes and connectors for leaks and ensure that tubes and connectors are fixed properly.

16.3.4 Acoustic Alarm

If the target concentration is not reached within 20 minutes after the initial activation of a gas mode or when it deviates for more than 10 minutes during operation, i.e. with a deviation (> +/- 20 %), an acoustic alarm will sound. This will help you to recognize, for example, when the gas supply has run out (tank is empty). A message appears specifying which gas is affected and to check the corresponding gas cylinder. Click **OK** to stop the acoustic alarm and continue the method.

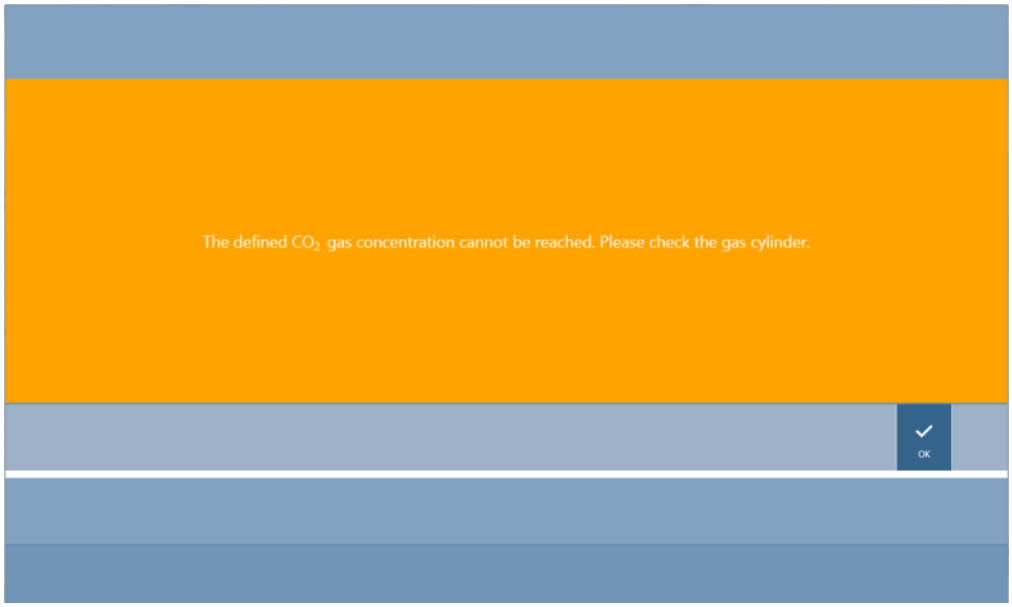


Figure 52: Stopping the gas alarm

If power is lost, the gas valves will close automatically.

16.4 Humidity Control

Evaporation is most pronounced when carrying out long-term studies (3 days or more). Especially when performing live-cell experiments over long periods of time significant evaporation effects may occur, affecting the outer wells of the microplate and the corner wells in particular. When the water evaporates the concentrations of substances in the medium will increase, which can influence cell growth and performance, causing heterogeneous or biased results.

The humidity cassette passively stabilizes the humidity and reduces evaporation for lengthy incubations. The humidity cassette is combinable with all plate formats from 1 to 384 wells complying with the SBS standard. It also allows for simultaneous incubation, signal detection in all measurement modes. Gas exchange (ventilation), signal detection as well as injection steps are supported in combination with the lid lifting option. Shaking in combination with the humidity cassette is restricted to orbital and double orbital mode.

NOTE: The humidity cassette is always combined with the lid lifting option.

SPARK CYTO configurations require specific humidity cassettes with altered dimensions, which are marked as **Cyto** on the packaging label (see Figure 53). In comparison to standard humidity cassettes, the maximum filling levels for the reservoirs are different. All plate formats (6-well to 384-well) are compatible and user handling remains the same.

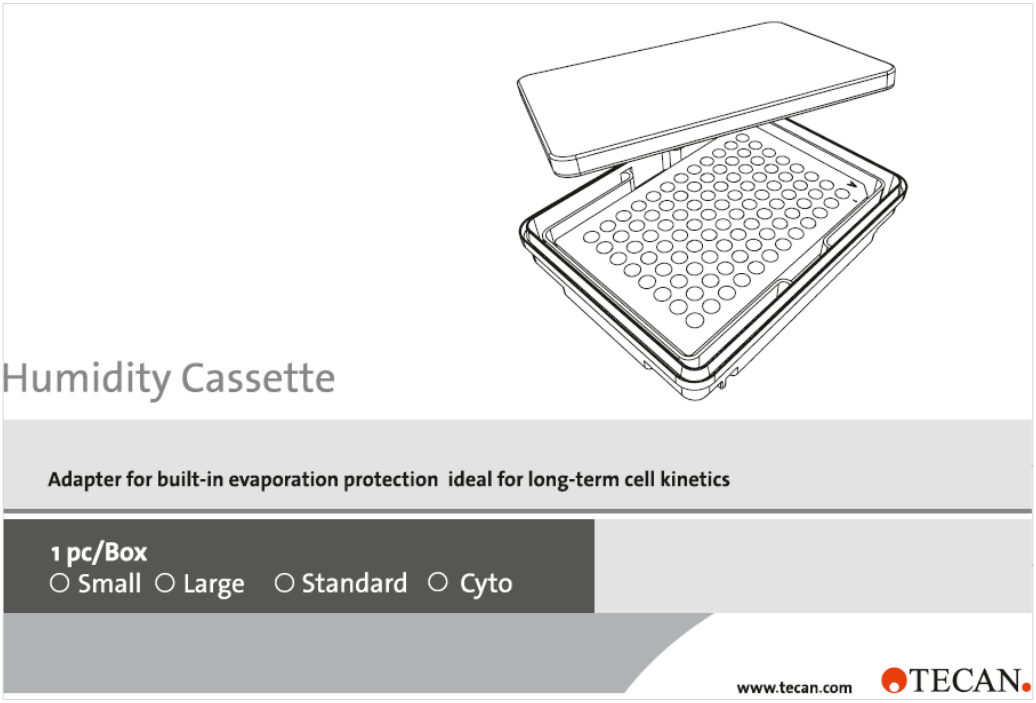


Figure 53: Label for supplied humidity cassettes

WARNING: Always use the Cyto humidity cassettes in combination with the Cell Imager module, otherwise the instrument could become damaged.

16.4.1 Humidity Cassette Standard / Cyto

The humidity cassette consists of water reservoirs and a lid with magnetic foil to facilitate lid lifting. The lid is closed to prevent evaporation. To allow gas exchange, the lid lifting option (ventilation) must first be selected in the software.

WARNING: Do not use humidity cassettes in the Spark-Stack module.

Figure 54: Humidity cassette

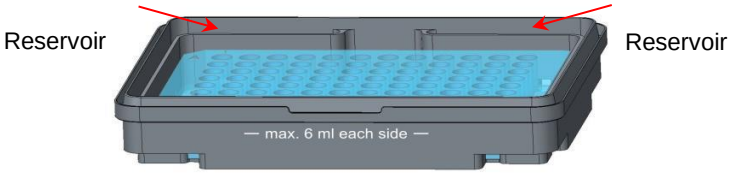


Figure 55: Basic part of the humidity cassette which holds the microplate and contains the water reservoirs

Standard Humidity Cassettes

Two different cassette types, a large one and a small one, are offered to shelter different types of microplates.

Humidity Cassette – Small: Usable for 96- and 384-well plates without a plate lid. The maximum height is 16 mm. By using the lid lifting option in the software, all detection modes can be combined with the low humidity cassette. Maximum filling level of 4 ml in each reservoir.

Humidity Cassette – Large: Usable for 6- to 384-well plates with or without a plate lid with a maximum height of 23 mm (including lid). By using the lid lifting option in the software, all detection modes except luminescence can be combined with the high humidity cassette. Maximum filling level of 6 ml in each reservoir.

Cyto Humidity Cassettes

The humidity cassettes delivered with the Cell Imager module have different maximum filling levels compared to the standard humidity cassettes.

Humidity Cassette – Cyto Small: Usable with 96- and 384-well plates without a plate lid. The maximum height is 16 mm. By using the lid lifting option in the software, all detection modes can be combined with the low humidity cassette. Maximum filling level of 3 ml in each reservoir.

Humidity Cassette – Cyto Large: Usable with 6- to 384-well plates with or without a plate lid with a maximum height of 23 mm (including lid). By using the lid lifting option in the software, all detection modes except luminescence can be combined with the high humidity cassette. Maximum filling level of 5.2 ml in each reservoir.

WARNING: Select the correct humidity cassette type (small or large) in the software to avoid instrument damages.

16.4.2 Handling Procedure

1. Fill the reservoir of the humidity cassette according to the corresponding filling levels as follows:
   - Small cassette Standard: 4 ml
   - Large cassette Standard: 6 ml
   - Small cassette Cell Imager: 3 ml
   - Large cassette Cell Imager: 5.2 ml
2. Insert the microplate (with or without lid) containing samples to be investigated into the basic part of the humidity cassette. Check that the orientation is correct; the cassette is labeled accordingly.
3. Place the lid on the cassette to properly close the humidity cassette, match A1 position of the microplate with the A1 position of the cassette lid.
4. Put the humidity cassette on the plate carrier. Take care of correct orientation the position of well A1 has to be on the upper left side.

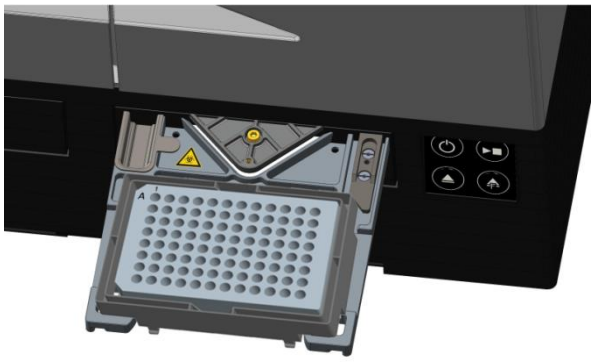


Figure 56: Microplate on the plate carrier with the A1 well in the upper left-hand corner

5. Start method.

CAUTION: Before starting measurements by using the humidity cassette, make sure that the microplate position and the cassette position A1 is inserted correctly. The position of well A1 has to be on the upper left side.

WARNING: Do not fill more water into the reservoirs than recommended to avoid spill over.

WARNING: Before the humidity cassette is placed on the plate transport ensure that the cassette lid closes properly.

6. After the end of the run and the plate carrier has been moved out, the humidity cassette containing the sample microplate can be easily removed from the plate carrier. Remove the lid of the cassette and put the lower part of the cassette containing the microplate on the unloading tool to easily remove the plate from the cassette.

The humidity cassette can be cleaned by using 70 % ethanol or sterilized at maximum 125 °C.

The unloading tool is located in the original packaging of the humidity cassette underneath the lower part of the humidity cassette. It has been cut from the material of the packaging, but not removed. Remove the foam piece by pushing it out.

Figure 57: Unloading tool (Part of packaging)

16.5 Environmental Control Specifications

NOTE: All specifications are subject to change without prior notification.

16.5.1 Heating

Parameters	Characteristics
Heating range	+3 °C above ambient up to +42 °C
Heating range with active gas control	+3 °C above ambient up to +42 °C
Heating uniformity	< 0.5 °C between 30 °C and 37 °C at incubation position
Environmental operating conditions	+15 °C to +35 °C

16.5.2 Cooling

Parameters	Characteristics
Cooling range	+18 °C up to 42 °C
Cooling uniformity over a 96-well plate	< 1.0 °C at a plate temperature between 18 °C and 37 °C
Environmental operating conditions	+18 °C to +30 °C

16.5.3 Gas Control

Parameters	Characteristics
CO_2 concentration range	0.04 % to 10 % volume
CO_2 concentration accuracy	< 1 %
O_2 concentration range	0.1 % to 21 % volume (imprecise regulation below 0.5 % and below 0.8 % with active cooling)
O_2 concentration accuracy	< 0.5 %

NOTE: Sensitivity of the CO_2 sensor below 0.1 % is imprecise.

16.5.4 Humidity Control

Parameters	Characteristics
96-well plate with lid, 4 days incubation at +37 °C with 5 % CO_2	Evaporation < 10 % (excluding the outside wells; first and last column, first and last row)
Operating condition	+18 °C to +42 °C

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Tecan Customer Support

If you have any questions or need technical support for your Tecan product, contact your local Tecan Customer Support organization. Go to: http://www.tecan.com/customersupport for contact information.

Prior to contacting Tecan for product support, prepare the following information for the best possible technical support (see name plate):

- Model name of your product
- Serial number (SN) of your product
- Software and software version (if applicable)
- Description of the problem and contact person
- Date and time when the problem occurred
- Steps that you have already taken to correct the problem
- Your contact information (phone number, fax number, e-mail address, etc.)

TECAN AUSTRIA GMBH, Untersbergstrasse 1a, A-5082 Grödig / Salzburg, Austria
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Declaration of Conformity

We, TECAN Austria GmbH herewith declare under our sole responsibility that the product identified as:

Product Type: **Microplate Reader**
Model Designation: **SPARK**

Article Numbers: **30086376**

Address: Tecan Austria GmbH
Untersbergstr. 1A
A-5082 Grödig, Austria

is in conformity with the provisions of the following European Directive(s) when installed in accordance with the installation instructions contained in the product documentation:

- **EMC Directive**
- **Machinery Directive**
- **RoHS Directive**

is in conformity with the relevant U.K. legislation for UKCA-marking when installed in accordance with the installation instructions contained in the product documentation:

- **Electromagnetic Compatibility (EMC) Regulations**
- **Supply of Machinery (Safety) Regulations**
- **The Restriction of the Use of Certain Hazardous Substances in Electrical and Electronic Equipment Regulations**

The current applicable versions of the directives and regulations as well as the list of applied standards which were taken in consideration can be found in separate CE & UK declarations of conformity.