Instructions for Use for

INFINITE 200 PRO Reader Family

INFINITE M Nano
INFINITE Lumi
INFINITE M Nano+
INFINITE M Plex
INFINITE F Nano+
INFINITE F Plex

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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

Declaration for EU Certificate

See the last page of these Instructions for Use.

About the Instructions for Use

Original Instructions. This document describes the INFINITE 200 PRO Reader Family multifunctional microplate readers. It is intended as reference and instructions for use. This document instructs 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 application has changed.

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- Infinite® 200 PRO series
- Infinite® F Nano+
- Infinite® F Plex
- Infinite® Lumi
- Infinite® M Nano
- Infinite® M Nano+
- Infinite® M Plex
- Infinite® M1000 PRO
- i-control™
- Magellan™
- NanoQuant Plate™
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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.

ATTENTION

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.**

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

	Manufacturer
	Date of manufacture
	CE Conformity Marking
	United Kingdom Conformity Assessed marking shows that the labeled product is following the applicable regulation in Great Britain.
	Consult Instructions for Use
	Catalog number
	Serial Number
	Unique Device Identification The UDI symbol identifies the data carrier on the label.
	USB label
	WEEE symbol
	China RoHS symbol
	TÜV SÜD MARK

Table of Contents

1. Safety

1.1 Instrument Safety

1. Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.
2. 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.
3. Observe all WARNING and CAUTION statements in this document.
4. Never open the housing of the Infinite 200 PRO while the instrument is plugged into a power source.
5. Never force a microplate into the instrument.
6. The Infinite 200 PRO is intended as a general purpose laboratory instrument for professional use. Observe proper laboratory safety precautions, such as wearing protective clothing and using approved laboratory safety procedures.

CAUTION

TECAN AUSTRIA GMBH HAS TAKEN GREAT CARE IN CREATING THE STORED PLATE DEFINITION FILES THAT ARE RECEIVED WITH THE INSTRUMENT SOFTWARE.
WE HAVE TAKEN EVERY PRECAUTION TO ENSURE THAT THE PLATE HEIGHTS AND WELL DEPTHS ARE CORRECT ACCORDING TO THE DEFINED PLATE TYPE. THIS PARAMETER IS USED TO DETERMINE THE MINIMUM DISTANCE BETWEEN THE TOP OF THE PLATE AND THE CEILING OF THE MEASUREMENT CHAMBER. ADDITIONALLY, TECAN AUSTRIA HAS ADDED A VERY SMALL SAFETY GAP TO PREVENT ANY DAMAGE OCCURRING TO THE MEASUREMENT CHAMBER AS A RESULT OF SMALL CHANGES IN PLATE HEIGHT. THIS DOES NOT AFFECT THE PERFORMANCE OF THE INSTRUMENT.
USERS MUST ENSURE THAT THE PLATE DEFINITION FILE SELECTED CORRESPONDS TO THE ACTUAL PLATE BEING USED. USERS SHOULD ALSO TAKE CARE THAT NO POTENTIAL FLUORESCENT OR LUMINESCENT CONTAMINATION LIES ON TOP OF THE PLATE. BE AWARE THAT SOME PLATE SEALERS LEAVE BEHIND A STICKY RESIDUE THAT MUST BE COMPLETELY REMOVED BEFORE STARTING MEASUREMENTS.

CAUTION

BEFORE STARTING MEASUREMENTS, MAKE SURE THAT THE MICROPLATE POSITION A1 IS INSERTED CORRECTLY. THE POSITION OF WELL A1 MUST BE ON THE UPPER LEFT SIDE.

CAUTION

TO ENSURE THE OPTIMAL WORKING OF TECAN INSTRUMENTS WE RECOMMEND A SERVICE INTERVAL OF 6 MONTHS.

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:

1. National industrial protection law
2. Accident prevention regulations
3. Safety data sheets of the reagent manufacturers

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF 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.

2. General Description

2.1 Instrument

The Tecan Infinite 200 PRO is a multifunctional microplate reader designed for the entry-level life science market. The Infinite 200 PRO provides high performance for the vast majority of today's microplate applications and research and is robotic compatible.

Based on the technological concept of the established Infinite reader, six configurations are available within the Infinite 200 reader family. The six configurations, respective capabilities and options are summarized below:

	Monochromator (M) Configurations				**Filter (F) Configurations**	
Capabilities	M Nano	M Nano+	Lumi	M Plex	F Nano+	F Plex
Absorbance - monochromator	x	x		x		
Absorbance - filter					x	x
Fluorescence - monochromator		x		x		
Fluorescence - filter					x	x
Fluoresence - top reading		x		x	x	x
Fluoresence - bottom reading		x		x	x	x
Fluorescence-polarisation - filter						x
Luminescence			x	x		x
Options						
1 injector	x	x	x	x	x	x
2 injectors	x	x	x	x	x	x
Cuvette	x	x		x		
NanoQuant Plate	x	x		x	x	x

2.1.1 Intended Use

The Infinite 200 PRO has been designed as a general purpose laboratory instrument for professional use, supporting common 6 to 384-well microplates conforming to the ANSI/SBS standards (see 5.12.2 Recommended Types of Microplates for further details).

Note
System Validation by Operating Authority is required. The Infinite 200 PRO has been validated on a selected set of assays only. It is the responsibility of any operating authority to ensure that the Infinite 200 PRO has been validated for every specific assay used on the instrument.

2.1.2 Multifunctionality

The following measurement techniques are supported by the Infinite reader, depending on the selected configuration of the reader:

- Fluorescence Intensity (FI) Top
- Fluorescence Intensity (FI) Bottom
- Time-Resolved Fluorescence (TRF)
- Fluorescence Resonance Energy Transfer (FRET)
- Flash Fluorescence (with injectors)
- Fluorescence Polarization (FP)
- Absorbance
- Absorbance (with injectors)
- Absorbance in cuvettes
- Glow Luminescence
- Flash Luminescence
- Bioluminescence Resonance Energy Transfer (BRET)

Any common microplate ranging from 6 to 384 well formats conforming to the ANSI/SBS standards (ANSI/SBS 1-2004; ANSI/SBS 2-2004, ANSI/SBS 3-2004 and ANSI/SBS 4-2004) may be measured with any of the above measurement techniques. Switching between measurement techniques or plate formats is fully automated via software. It is not necessary to manually reconfigure the optics to switch between the reading modes supported by the Infinite reader.

2.1.3 Filling Volumes

CAUTION

THE FOLLOWING MICROPLATES CAN BE PROCESSED ONLY WITH THE SUBSEQUENT FILLING VOLUMES:

- **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**

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.

2.1.4 Performance

The Infinite reader has been designed to meet the requirements of a general-purpose laboratory instrument.

The Infinite reader provides a range of parameters for optimizing the measurement results according to the specific configuration, assay type (cell-based or homogeneous), the microplate type, and the dispensed volumes per well and dispensing speeds.

2.1.5 User Friendliness

Infinite readers with monochromator configurations offer unparalleled flexibility in wavelength selection for fluorescence intensity and absorbance measurements. Via software any wavelength can be easily adjusted within the specified wavelength range. In addition to single wavelength measurements, absorbance and fluorescence spectra can be recorded. When running a spectrum there is no restriction due to cut-off filters.

Infinite readers with filter configurations offer high flexibility for the customization of fluorescence and absorbance measurements; slides containing fluorescence and absorbance interference filters are easily accessible to the user.

Note
If the instructions given in this document are not correctly performed, the instrument will either be damaged, or the procedures will not be performed correctly, and the safety of the instrument is not guaranteed.

2.1.6 Onboard Control Button

The Infinite reader possesses an onboard control button to control plate movements without the need to be connected to the software. Upon pressing the **Plate In/Out** button, the current position of the plate carrier is automatically recognized, and the plate is moved into or out of the instrument.

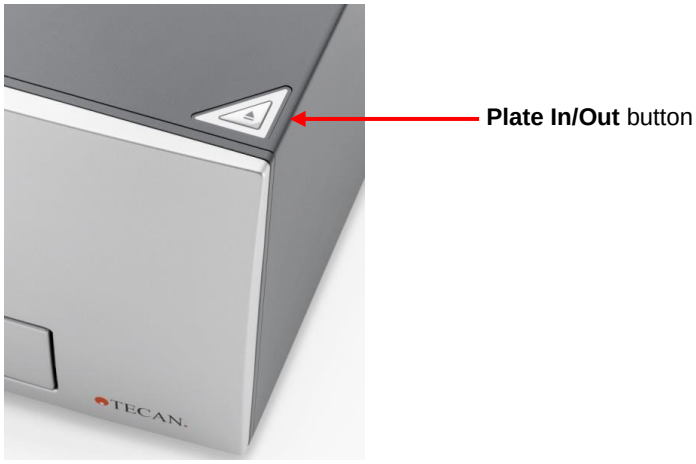


*Figure 1: Onboard of the Infinite reader. The **Plate In/Out** button is in the front right corner of the top cover.*

2.1.7 Rear View

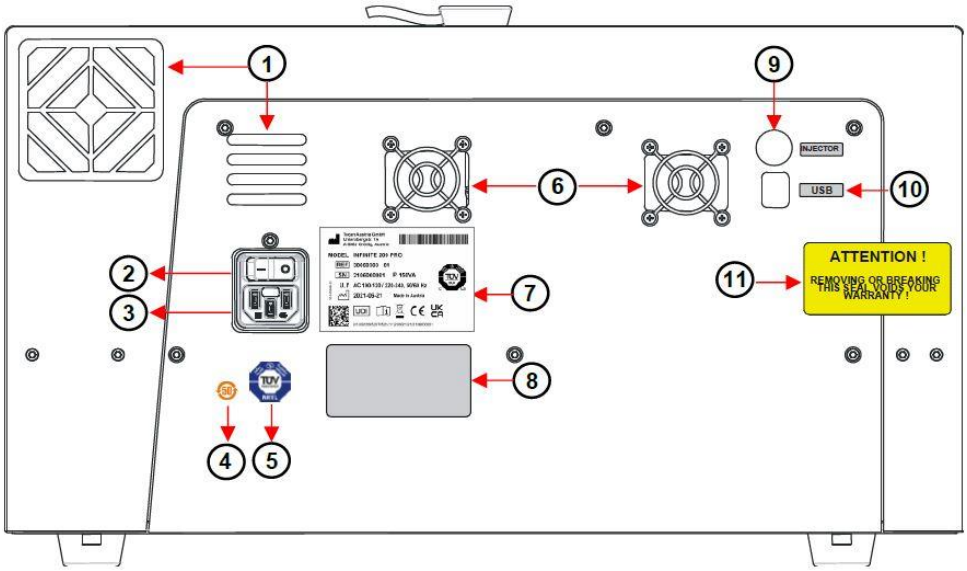


Figure 2: Rear panel

1	Instrument Fan	
2	Main Power Switch	
3	Main Power Socket	
4	Label – China RoHS symbol	
5	Label – Technical Inspection Agency (TÜV)	
6	Power Supply Fan	
7	Name Plate	
8	Label – Options/Configuration	
9	Injector Connection	
10	USB Connection	
11	Warranty Label	ATTENTION REMOVING OR BREAKING THIS SEAL VOIDS YOUR WATTENTY!

CAUTION

ONLY TECAN AUTHORIZED SERVICE TECHNICIANS ARE ALLOWED TO OPEN THE INSTRUMENT. REMOVING OR BREAKING THE WARRANTY SEAL VOIDS THE WARRANTY.

2.2 Software

The Infinite reader is delivered with the **i-control** software, for operating the instrument and includes a help file and printed Instructions for Use. The software is formatted as a self-extracting archive on the software storage media. (For information about the system requirements, refer to the Instructions for Use for **i-control** software. The Instructions for Use for **i-control** software can be found on the software storage media.)

For advanced data reduction, the **Magellan** software can be used to control the Infinite reader. Magellan offers all functionality for compliance with the FDA regulation 21 CFR part 11 for electronic records and signatures (for more information, contact your local Tecan representative).

2.3 Injectors (Optional)

The Infinite reader can be optionally equipped with an injector module consisting of one or two syringe pumps (XE-1000, Tecan Systems) located in a separate box, which feed one or two injector needles.

The injector needles are designed to inject liquid in any SBS-conform microplate well types, in which the well-size is equal to or larger than an SBS standard 384-well plate.

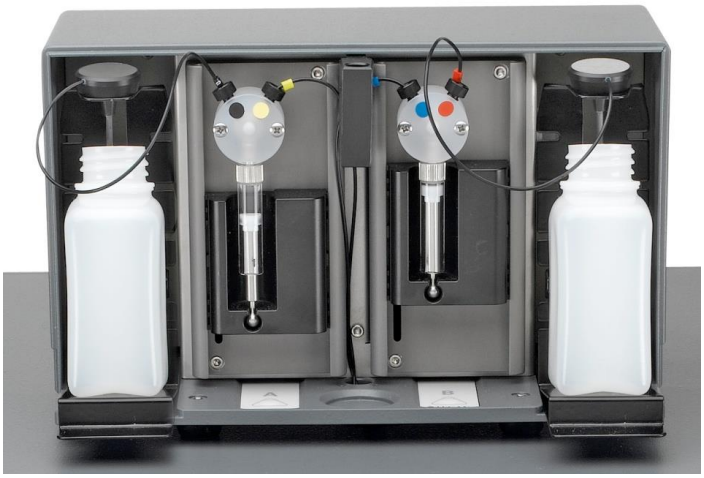


Figure 3: Injector-box with bottle holders

2.3.1 Injector Measurement Modes

The injectors of the Infinite reader can be used with the following measurement modes:

- Fluorescence Intensity top and bottom
- Time Resolved Fluorescence
- Absorbance
- Flash Luminescence
- Glow Luminescence
- Dual Color Luminescence

As the measurement position is not the same as the injector position, a short time delay (approx. < 0.5 s) between injection and reading occurs.

For details on how to set up a measurement with injectors, please refer to chapter 4.10.4 Injector Modes (i-control).

2.3.2 Injector Module Diagram

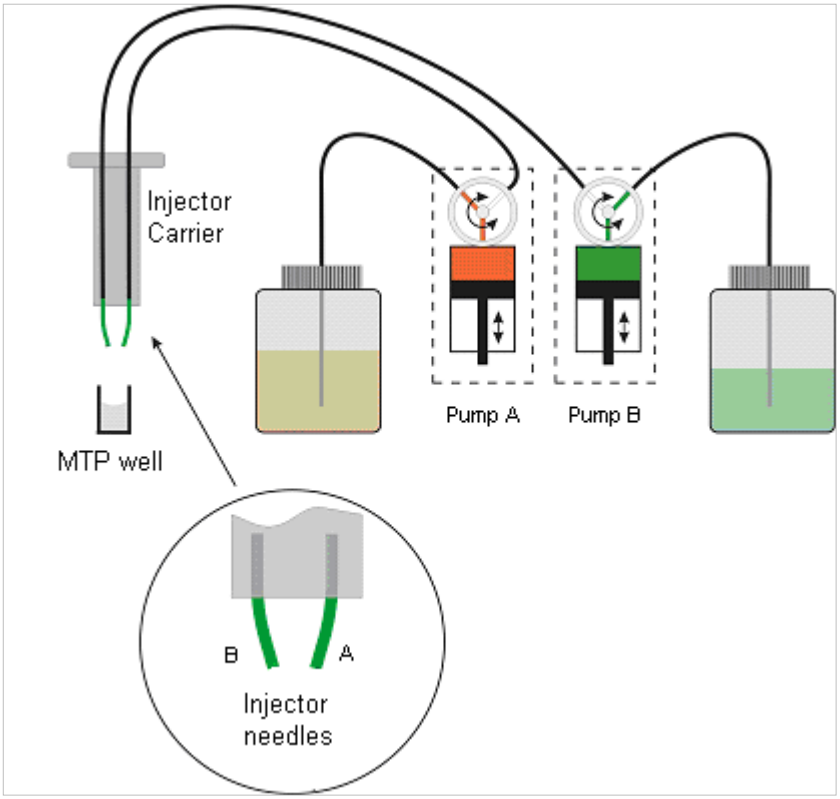


Figure 4: Schematic view of the injector module

2.3.3 Injector Pump Options

There are up to two pumps available for the Infinite reader (see Figure 4 above):

- Pump A feeds injector needle A
- Pump B feeds injector needle B

The Infinite reader can be equipped with one pump (pump A) or two pumps (pumps A and B):

- **One Injector Option (one pump):** An Infinite reader equipped with one pump allows injections in any SBS-conform microplate well types, in which the well-size is equal to or larger than an SBS standard 384-well plate.
- **Two Injector Option (two pumps):** Some applications, such as flash luminescence reactions or dual reporter gene assays require the injection of two independent liquids into the same well; therefore, Tecan Austria offers a two-injector option.

2.3.4 Storage Bottles and Bottle Holders

The injector box can accommodate up to two 125 ml bottles.

The standard bottle set supplied with the Injector option consists of:

- One 125 ml bottle and one 15 ml bottle for the **One Injector option** (one pump) or
- One 125 ml bottles and two 15 ml bottles for the **Two Injectors option** (two pumps).

The injector option includes up to two bottle holders that are designed for tubes of 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 reaching down to the bottom of the flask to ensure the optimal aspiration of even small volumes of fluid.

Figure 5: Bottle holders

2.3.5 Injector Carrier

The injector carrier, which includes the injector needles, can be easily removed from the instrument for priming or washing the system and for optimizing the injection speed.

Figure 6: Injector carrier

When using the injector during a measurement or for just dispensing a plate the injector carrier must be inserted correctly into the instrument. Remove the injector dummy and insert the carrier into the injector port. Press the carrier softly into the injector port until you hear a clicking noise.

The instrument contains an injector sensor that checks that the position of the injector carrier for the actions **inject** and **dispense** is correct.

If the injector carrier is not inserted correctly, the injector sensor does not recognize the inserted carrier and neither dispensing nor injection is possible. On the other hand, actions like washing and priming are enabled although the injector carrier is inserted; therefore, always make sure that the injector carrier is in the service position for washing and priming.

Every delivered instrument is equipped with an injector-ready option to be upgraded with an injector in the field.

CAUTION

THE INJECTOR CARRIER MUST BE IN THE SERVICE POSITION FOR WASHING UND PRIMING.

PRIME AND WASH MUST NOT BE PERFORMED WHEN THE INJECTOR IS IN THE INSTRUMENT!

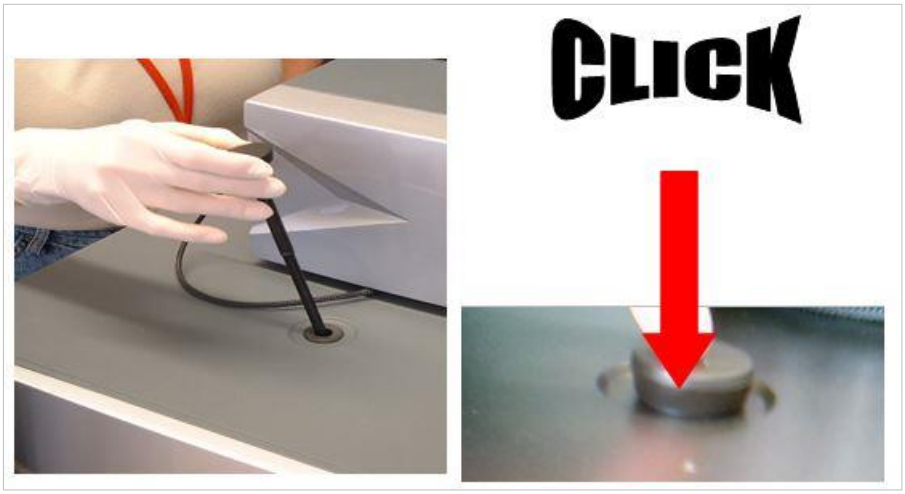


Figure 7: Inserting the injector carrier into the injector port

CAUTION

IF THE INJECTOR CARRIER IS NOT INSERTED CORRECTLY IN THE INJECTOR PORT, THE INJECTOR SENSOR WILL NOT DETECT THE INSERTED INJECTOR AND THEREFORE WASHING AND PRIMING WILL BE ENABLED, WHICH CAN DAMAGE THE INSTRUMENT.

2.4 Measurement Techniques

The following sections provide an introduction to the Infinite reader measurement techniques when fully equipped. To keep this compact, a few simplifications have been made. For details see the references.

2.4.1 Fluorescence

The Infinite reader offers the basic fluorescence measurement technique and some even more sophisticated variants:

- Fluorescence Intensity (FI) (or simply Fluorescence)
- Fluorescence Resonance Energy Transfer (FRET)
- Fluorescence Time Resolved (TRF)
- Fluorescence Polarization (FP – Infinite F Plex only)

FI may also be used to measure Fluorescence Resonance Energy Transfer (FRET). For some microplate applications, FRET offers advantages over FI and TRF, because they simplify assay preparation. These preferably apply for **mix and measure** binding studies. Compared to FP, FRET requires both binding partners to be labeled in a suitable way. On the other hand, FRET may utilize TRF labels for increased sensitivity, then being referenced as HTRF (Homogeneous TRF).

TRF should not be confused with Fluorescence Lifetime measurements.

Fluorescent molecules emit light of specific wavelength when struck by light of shorter wavelength (Stokes Shift). In particular, a single fluorescent molecule can contribute one fluorescence photon (quantum of light). This is a part of the energy, which has been absorbed before (electronic excitation), but could not be released quickly enough into thermal energy.

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.

For a detailed treatise on fluorescence techniques and applications see:

Principles of Fluorescence Spectroscopy by Joseph R. Lakowicz, Plenum Press.

A) Fluorescence Intensity (FI)

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

B) Fluorescence Resonance Energy Transfer (FRET)

Some microplate applications utilize a sophisticated dual labeling strategy. The FRET effect enables you to measure how many of two differently labeled compounds are in close proximity. This makes it suitable for binding studies.

Basically, FRET is a fluorescence intensity measurement of one of the two fluorescent labels (acceptor). However, the acceptor is not susceptible to the excitation wavelength of the light source being used. Instead, the acceptor may receive excitation energy from the other fluorescent label (donor) if both are spatially close together. As a prerequisite, the excitation wavelength has to apply to the donor. Secondly, the emission spectrum of the donor must overlap the excitation spectrum of the acceptor (resonance condition). Nevertheless, the transfer of excitation energy from donor to the acceptor is radiation free.

Some FRET-based applications utilize suitable pairs from the fluorescent protein family, like GFP/YFP (Green/Yellow Fluorescent Protein, (ref. **Using GFP in FRET-based applications** by Brian A. Pollok and Roger Heim – trends in Cell Biology [Vol.9] February 1999). Overview is given in the Review Article – **Application of Fluorescence Resonance Energy Transfer in the Clinical Laboratory: Routine and Research** by J. Szöllösi, et al. in Cytometry 34, page 159-179 (1998).

Other FRET-based applications take advantage from using TRF labels as the donor. For example, see, **High Throughput Screening** – Marcel Dekker Inc. 1997, New York, Basel, Hong Kong, section 19 Homogeneous, Time-Resolved Fluorescence Method for Drug Discovery by Alfred J. Kolb, et al.

C) Time Resolved Fluorescence (TRF)

TRF applies to a class of fluorescent labels (chelates of lanthanides like Europium, [ref. **Europium and Samarium in Time-Resolved Fluoroimmunoassays** by T. Stâhlberg, et. al. - American Laboratory, December 1993 page 15]), some of them having fluorescence lifetimes in excess of 100 microseconds. The Infinite reader uses a Flash lamp light source with flash duration much shorter than fluorescence lifetime of these species. This offers the opportunity to measure fluorescence emission at some time, when stray light and prompt fluorescence have already vanished (Lag Time). Thus, background can be significantly lowered while sensitivity is improved.

The benefits of TRF consequently apply to assays using multiple labels with different fluorescence lifetimes.

D) Fluorescence Polarization (FP)

Fluorescence Polarization (FP) measures rotational mobility of a fluorescent labeled compound. FP is therefore particular suitable for binding studies, because the tumbling motion of small molecules may be dramatically slowed down after binding to a larger molecule.

Fluorescence polarization measurements are based on the detection of the depolarization of fluorescence emission after excitation of a fluorescent molecule by polarized light. A fluorescent molecule can be visualized as an antenna. Such a molecule can absorb energy if and only if the polarization of the excitation light matches the orientation of the antenna. During the fluorescence lifetime, i.e., the time a molecule remains in the excited state; small molecules diffuse rotationally relatively rapidly. Hence, they re-orient before they emit their photon. As a result,

and due to the random character of diffusion, a linearly polarized excitation light will be translated into a less polarized emission light. Thus, a high resultant mP value denotes the slow rotation of the labeled molecule, indicating that binding probably did occur. A resultant low mP value denotes a fast rotation of a molecule, indicating that binding probably did not occur.

The FP measurement result is calculated from two successive fluorescence intensity measurements. They differ in the mutual orientation of polarizing filters, one being placed behind the excitation filter, another ahead of the emission filter. By processing both data sets, it is possible to measure the extent of how much the fluorescent label has changed orientation in the time span between excitation and emission.

2.4.2 Absorbance

Absorbance is a measure for the attenuation of monochromatic light when transmitted through a sample. Absorbance is defined as:

$A = LOG_{10} (I_0 / I_{SAMPLE})$,

Where I_{SAMPLE} is the intensity of the light being transmitted, I_0 the light intensity not attenuated by sample. The unit is assigned with Optical Density (OD)

Thus, 2.0 OD means $10^{2.0}$ or 100-fold attenuation (1% transmission),

1.0 OD means $10^{1.0}$ or 10-fold attenuation (10% transmission), and

0.1 OD means $10^{0.1}$ or 1.26-fold attenuation (79.4% transmission).

If the sample contains only one species absorbing in that narrow band of wavelengths, the background corrected absorbance (A) is proportional to the corresponding concentration of that species (Lambert-Beer's Law).

2.4.3 Luminescence

Glow Type Chemi- or Bioluminescence

The Infinite reader provides measurement of glow type chemi- or bioluminescence. Glow type means that the luminescence assay glows much longer than a minute. Luminescence substrates are available, which provide stable enough light output over hours.

As an example, luminescence can be measured to determine the activity of an enzyme labeled compound (-peroxidase, -phosphatase). Light emission results from a luminescence substrate being decomposed by the enzyme. Under excess of substrate the luminescence signal can be assumed to be proportional to the abundance of the enzyme labeled compound. As with enzyme-based assays, control of environmental conditions is rather critical (temperature, pH-value).

For practical aspects of luminescence assays see the following example:

Bioluminescence Methods and Protocols, ed. R.A. LaRossa, Methods in Molecular Biology 102, Humana Press, 1998

Bioluminescence Resonance Energy Transfer (BRET)

BRET is an advanced, non-destructive, cell-based assay technology that is perfectly suited for proteomics applications, including receptor research and the mapping of signal transduction pathways. BRET is based on energy transfer between fusion proteins containing *Renilla luciferase* (Rluc) and a mutant of the Green Fluorescent Protein (GFP). The BRET signal is generated by the oxidation of p.a. DeepBlueC, a coelenterazine derivative that maximizes spectral resolution for superior sensitivity. This homogeneous assay technology provides a simple, robust and versatile platform with applications in basic academic as well as applied research.

Flash Luminescence

In flash type luminescence assays, the measurement is only done during the dispensing of the activating reagent or after a short delay time (for Flash luminescence measurements with the Infinite reader, see also 2.3.1 Injector Measurement Modes).

Over the past years luminescence substrates have been improved towards providing more stable signals. In so-called glow type luminescence assays the luminescence signal is spread over a wide time scale (e.g., a half-life of 30 min.)

2.5 Optical System

2.5.1 Fluorescence Intensity System (Infinite M configurations)

The optical system of the fluorescence top and bottom system of the Infinite M configurations is sketched below.

The system consists of:

- Light source system
- Excitation double monochromator
- Fluorescence top optics
- Emission double monochromator
- and fluorescence detection

The solid arrows indicate the light path of the excitation light; the dashed arrows indicate the emission light path.

To simplify the system, the **Flash Monitor** (see section Flash Monitor, page 28) is not shown. Each monochromator unit, (2) and (4), is built of two gratings and a schematic view is displayed in more detail in the figures below.

Fluorescence Intensity Top Diagram

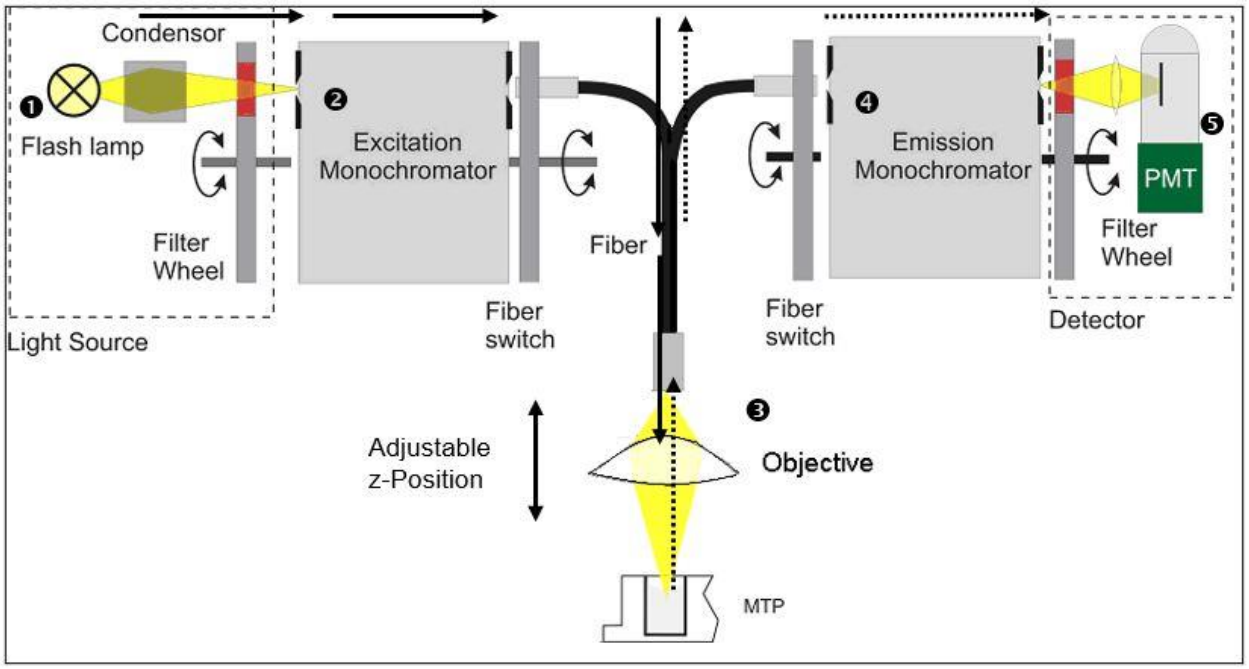


Figure 8: Optical System Fluorescence Top

Fluorescence Intensity Bottom Diagram

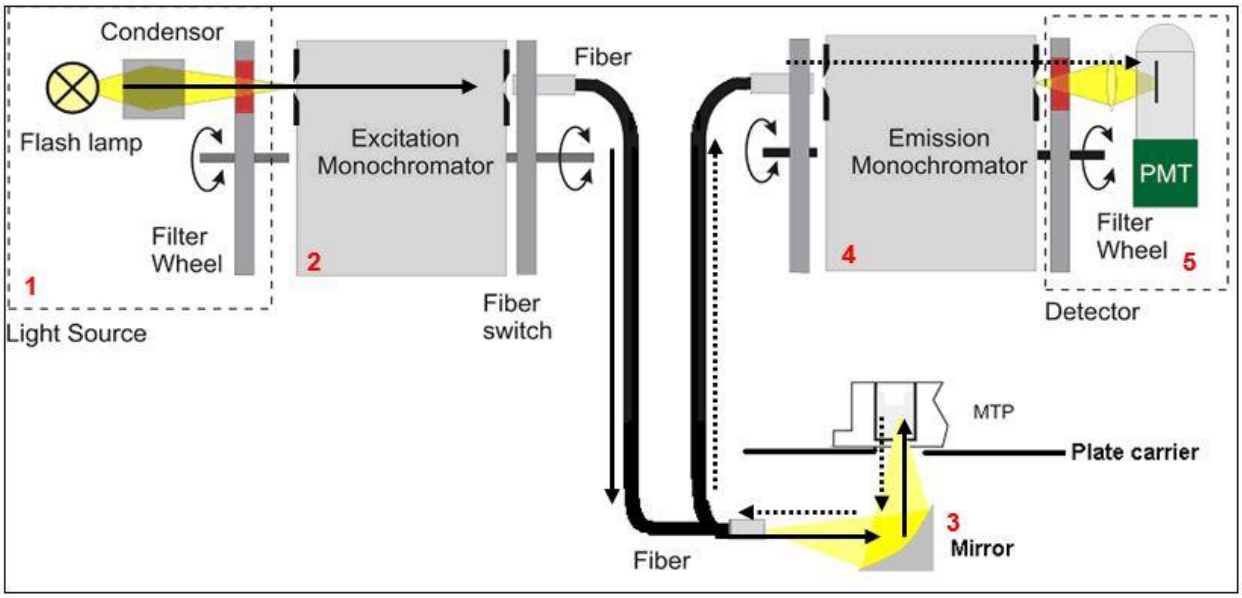


Figure 9: Optical System Fluorescence Bottom

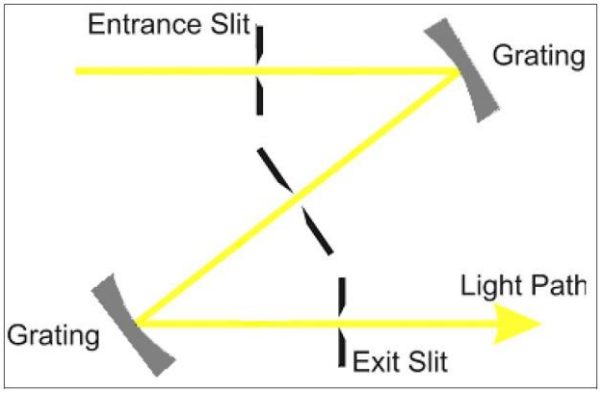


Figure 10: Detailed view of excitation and emission double monochromator unit

Fluorescence Intensity Light Source System

Fluorescence applications usually require a specific range of excitation wavelengths. Additionally, pulsed excitation light may be required (Time Resolved Fluorescence [TRF]).

The Infinite M configurations light source system is built from the following components:

- Flash Lamp
- Condensing Optics
- Filter Wheel
- Excitation Double Monochromator
- Fiber Optic Bundle
- Flash lamp Monitor

Flash Lamp

The Infinite M configuration utilizes a high energy Xenon arc discharge lamp (flash lamp). The flash sparks across a small gap between two electrodes. The lamp bulb contains a high-pressure Xenon atmosphere. The flash decays within a few microseconds. The flash frequency is 40 Hz.

The Infinite M configuration uses the flash lamp for fluorescence and for absorbance measurements, although pulsed illumination is a must only for TRF. The main benefits of this singular kind of lamp are:

- High intensity from the deep UV to the near IR
- Very long lifetime
- Many applications - only one kind of lamp
- No warmup time required

Condenser

Condenser type optics from fused silica focus the flashlight onto the entrance slit of the excitation monochromator.

Filter Wheel

A filter wheel is located between the condenser and the excitation monochromator. The filter wheel contains wavelength specific optical filters, which are necessary to block undesired diffraction orders produced by the optical gratings. The filters are set automatically.

Excitation Double Monochromator

In both fluorescence and absorbance applications, the Excitation Double Monochromator is used to select any desired wavelengths from the flash lamp spectrum in the range from 230 to 850 nm (spectrally enhanced version) for fluorescence intensity and from 230 nm to 1000 nm for absorbance applications.

In many cases, fluorescence emission spectra do not depend on the exact excitation wavelength; therefore, to achieve a maximum total fluorescence signal, a broad excitation bandwidth should be used.

The bandwidth of the Infinite M configurations monochromator system is < 9 nm for wavelengths > 315 nm and < 5 nm for wavelengths ≤ 315 nm.

For a more detailed description of how a monochromator works, see below.

Description of 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 and an exit slit. The dispersive element diffracts the light into the optical spectrum and projects it onto the exit slit. A dispersive element can be realized by using a glass prism or an optical grating. Modern monochromators such as those used in the Infinite M configurations are designed with optical gratings.

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 (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 detector 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, since 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.

In the Infinite M configurations, a double monochromator is installed on both the excitation and detection side. This opens the opportunity for easy selection of excitation and fluorescence wavelengths with no limitations by cut off filters.

Fiber Optic Bundle

Light from the exit slit of the Excitation Monochromator is coupled into a fiber optic bundle, which guides the light either to the top measuring optics or the bottom measuring optics. The lower end of each fiber bundle acts as a color specific light source. In both cases, a small portion of the light is always guided to the flash lamp monitor diode.

Flash Monitor

The light energy of single flashes may fluctuate slightly. To take these variations into account, a silicon photodiode monitors the energy of every single flash. Fluorescence and Absorbance measurement results are compensated correspondingly.

Fluorescence Top/Bottom Optics

Flashlight enters the optical system and is focused by the condenser onto the entrance slit of the Excitation Monochromator. The wavelength of the excitation light is selected within the monochromator. After passing the monochromator, the excitation light is coupled into a fiber bundle, which guides the light to the top or bottom measuring head. The light is then focused into the sample by the top/bottom lens system.

The fluorescence light is collected by the top/bottom lens system again, coupled into the fluorescence fiber bundles and guided to the detection system.

The Fluorescence Measuring Optics Top is built from the following components:

- Fluorescence Intensity Lens System Top
- Fluorescence Fiber Bundle
- The bottom optics consists of the following components:
- Fluorescence Bottom Mirror
- Fluorescence Fiber Bundle

Fluorescence Intensity Lens System Top

The exit side of the bundle acts as a color specific light source. The lens system at the end of the excitation top fiber is designed to focus the excitation light into the sample, and collect the fluorescence light and focus it back onto the fluorescence fiber bundle.

The objective lenses are made from fused silica. This material provides high UV transmission and is virtually void of auto-fluorescence.

Excitation Spot Size

The size of the fiber bundle cross section determines the diameter of the beam waist (spot size) in the microplate well. The spot diameter for the M-series is about 3 mm for the top optics and 2 mm for the bottom optics.

Fluorescence Fiber Bundle Top and Bottom

The fiber bundle plugged into the top/bottom measuring head contains a homogeneous mixture of both excitation and emission fibers. The emission fibers guide the fluorescence light to the emission monochromator head where a lens system focus the light onto the entrance slit of the Emission Monochromator.

Fluorescence Bottom Mirror

The exit side of the bundle acts as a color specific light source. The mirror at the end of the excitation bottom fiber is designed to focus the excitation light into the sample and collects the fluorescence light and focuses it back onto the fluorescence fiber bundle.

Z-Positioning (Fluorescence Top on Infinite M configurations only)

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, z-adjustment helps to maximize the signal-to-noise ratio. For further details about z-positioning see chapter 4.5.2 Z-Optimization (FI Top measurements with Infinite M configurations only).

Fluorescence Intensity Detection

The fluorescence detection system is used for both measuring modes: fluorescence from above (top) and below the microplate wells (bottom).

The fluorescence light is focused onto the entrance slit of the Emission Monochromator. After passing the monochromator the light is focused onto the detector (PMT). A filter wheel is located between the monochromator and the PMT.

The Fluorescence Detection system is built from the following components:

- Emission Double Monochromator
- Filter Wheel PMT
- PMT Detector

Emission Double Monochromator

Like the Excitation Double Monochromator, the Emission Double Monochromator is used to select any wavelength of the fluorescence signal.
It acts like an adjustable filter to discriminate scatter of excitation light and nonspecific fluorescence. The wavelength range is selectable from 280 – 850 nm in the spectrally enhanced instrument. The bandwidth is 20 nm.

Filter Wheel PMT

The filter wheel contains wavelength specific optical filters, which are necessary to block undesired diffraction orders produced by the optical gratings. The filters are set automatically.

PMT Detector

A photo-multiplier tube (PMT) is used for the detection of such low light levels associated with fluorescence. The PMT of the spectrally enhanced version of the Infinite M configurations is sensitive up to the near infrared (NIR) while still having low dark current. Electronic circuitry uses analog to digital conversion of PMT output current. Adjusting the PMT gain enables measurement of a wide range of concentrations in lower or higher concentration domains. For details, see Section 4.5.1 Instrument Parameters.

2.5.2 Fluorescence Intensity System (Infinite F configurations)

The following parts constitute the fluorescence intensity system of the Infinite F configurations instrument:

- Light Source
- Fluorescence Optics
- Fluorescence Detection System

The fluorescence top system is shown in Figure 11, the bottom system in Figure 12. The solid arrows indicate the excitation light path; the dashed arrows determine the emission light path.

Fluorescence Intensity Top Diagram

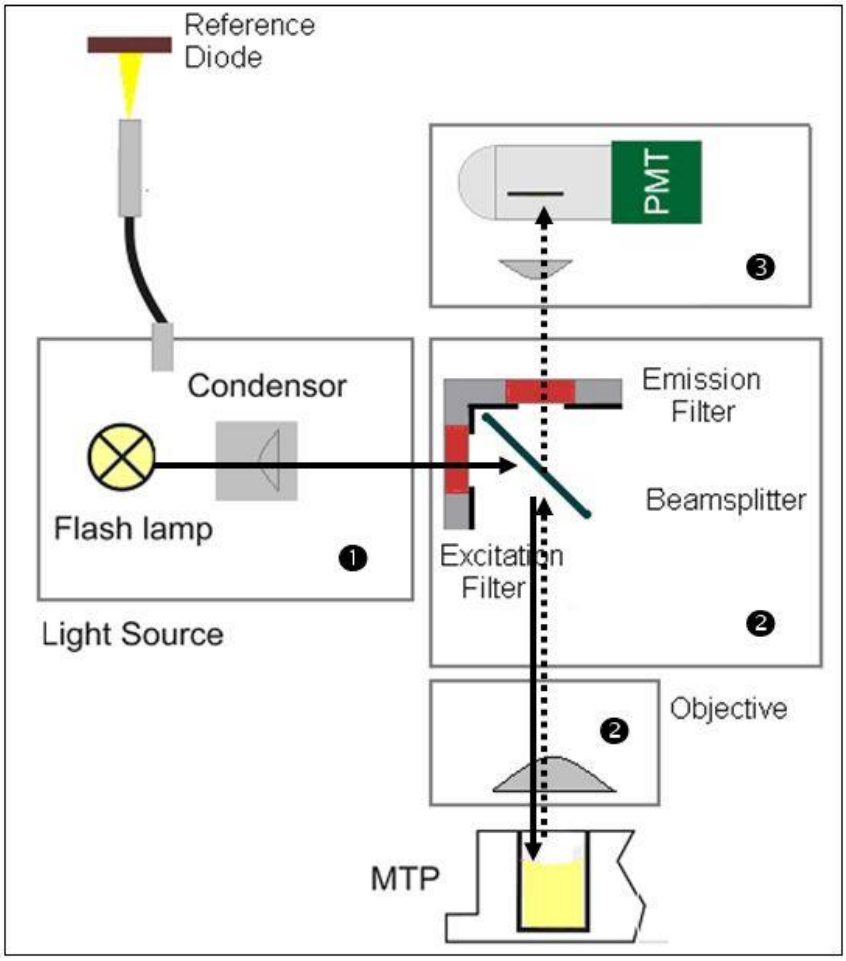


Figure 11: Fluorescence intensity top system of the Infinite F configurations

Fluorescence Intensity Bottom Diagram

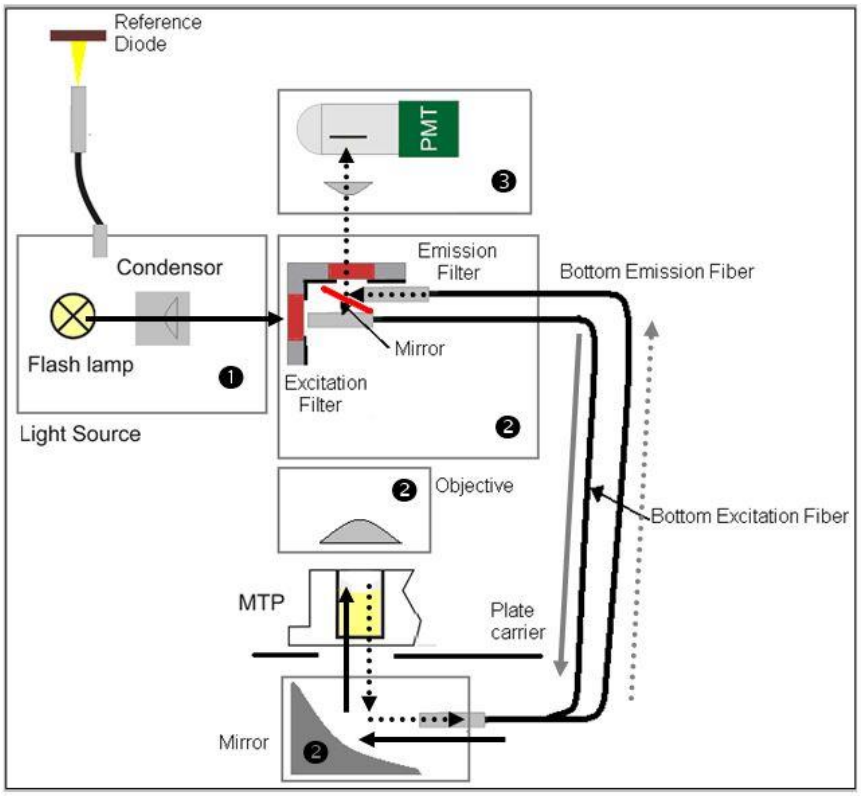


Figure 12: Fluorescence intensity bottom system of the Infinite F configurations

Light Source System

Flashlight enters the optical system by being focused through a slit containing the filter. This opening acts as a color specific light source.

The Infinite F configurations light source system is built from the following components:

- Flash lamp
- Condensing Optics
- Excitation Filters
- Flash lamp Monitor

Flash lamp

The Infinite F configuration utilizes a high energy Xenon arc discharge lamp (Flash lamp). The flash sparks across a small gap between two electrodes. The lamp bulb contains a high-pressure Xenon atmosphere. The flash decays within some microseconds.
The flash frequency is 40 Hz.

The Infinite F configuration uses the Flash lamp for fluorescence and for absorbance measurements, although pulsed illumination is a must only for TRF. The main benefits of this singular kind of lamp are:

- High intensity from the deep UV to the near IR
- Very long lifetime
- Many applications - only one kind of lamp
- No warmup time required

Condenser

Condenser type optics focus the light through the entrance slit to the fluorescence optical system.

Excitation Filter

Wavelength-specific bandpass filters serve to select the wavelength range of interest from the whole spectrum of excitation light coming from the flash lamp. Filters are installed in removable slides and are user exchangeable.

Flash Monitor

The light energy of single flashes may fluctuate slightly. To take these variations into account, a reference silicon photodiode monitors the energy of every single flash. Fluorescence measurement results are compensated correspondingly.

Fluorescence Optics Top

Flashlight enters the optical system by being focused through a slit and then through the excitation filter. Depending on the measured wavelength either a semi-transparent (50%) or a special dichroic mirror reflects the light towards the microplate. The objective lens system focuses the light into the sample.

Fluorescence Emission is measured from above the well. Fluorescence light is collected by the objective, directed through the appropriate mirror, and focused through the exit slit for detection.

Objective Lens System

The objective is designed to collect the fluorescent light emitted from a well and focus it through the exit slit to the detection system.

The objective lenses are made from fused silica. This material provides high UV transmission and is virtually void of auto-fluorescence.

Mirror Selection - Fluorescence Top (Infinite F configurations only)

The Infinite F configuration is equipped with a mirror carrier, which houses a 50% mirror as well as a 510 dichroic mirror.

The advantage of the 50% mirror is that is works with any pair of excitation and emission wavelengths. However, 50% of excitation light that is directed into the sample and, subsequently, 50% of the emission light coming out of the sample are lost.

Dichroic mirrors are wavelength dependent and are designed to reflect a certain range of wavelengths almost entirely. Dichroic mirrors exhibit a high reflection of excitation light and a high transmission of emission light and usually give a better signal-to-noise ratio compared to 50% mirrors.

Available for plate formats up to 384 wells.

Note
A dichroic mirror needs to match the selected fluorescence excitation and emission wavelengths.

Mirror Type	Reflection (Excitation)	Transmission (Emission)
50% mirror	230 – 900 nm	230 – 900 nm
510 dichroic (e.g., fluorescein)	320 – 500 nm	520 – 780 nm

According to the wavelengths defined in the measurement script, the dichroic mirror is selected automatically if both excitation and emission wavelength match the specified range of that mirror. If either the excitation or the emission wavelength does not match the ranges of the dichroic mirror, the 50% mirror is chosen automatically for the measurement.

Fluorescence Optics Bottom

Flashlight enters the optical system by being focused through a slit and then through the excitation filter. The excitation bottom fiber guides the light to the bottom optics probe, which consists of an elliptical mirror which focuses the light through the bottom of the microplate into the well. The emitted light is focused onto the excitation bottom fiber, which guides the light over a mirror through the emission filter to the fluorescence detection system.

Fluorescence Detection

Emission Filter

Wavelength-specific bandpass filters serve to discriminate unspecific fluorescence signals from the sample-specific emission light of interest. Filters are installed in removable slides and are user exchangeable.

Fluorescence filters may be used interchangeably as excitation or emission filters, depending on the measurement requirements.

The spot diameter for the Infinite F configurations is about 2 mm.

PMT Detector

A photomultiplier tube (PMT) is used for the detection of such low light levels as involved with fluorescence. For details, see section Fluorescence Intensity Detection, page 29.

2.5.3 Fluorescence Polarization System (Infinite F Plex only)

For technical details please refer to chapter 2.5.2 Fluorescence Intensity System (Infinite F configurations).

The Infinite F Plex, the Infiinite F configuration for Fluorescence Polarization (FP) measurements, is delivered with a standard FP filter slide. The filter slide is equipped with filters and polarizers for excitation and emission, at 485 and 535 nm respectively, and can be applied for measuring, for example, fluorescein-based FP applications.

For details on how to mount polarizers and FP filters please refer to chapter 4.4 Defining Filter Slides (Infinite F configurations).

2.5.4 Absorbance System (Infinite F configurations)

For absorbance measurements a similar optical path is used as for fluorescence excitation. The absorbance measurement module is located underneath the plate carrier. It measures the light being transmitted through the sample. Before measurement of the microplate, a reference measurement is performed with the plate carrier moved out of the light beam (see also 2.4.2 Absorbance).

The absorbance system is shown in Figure 13 and consists of the following components:

- Light Source
- Absorbance Optics
- Absorbance Detection Unit

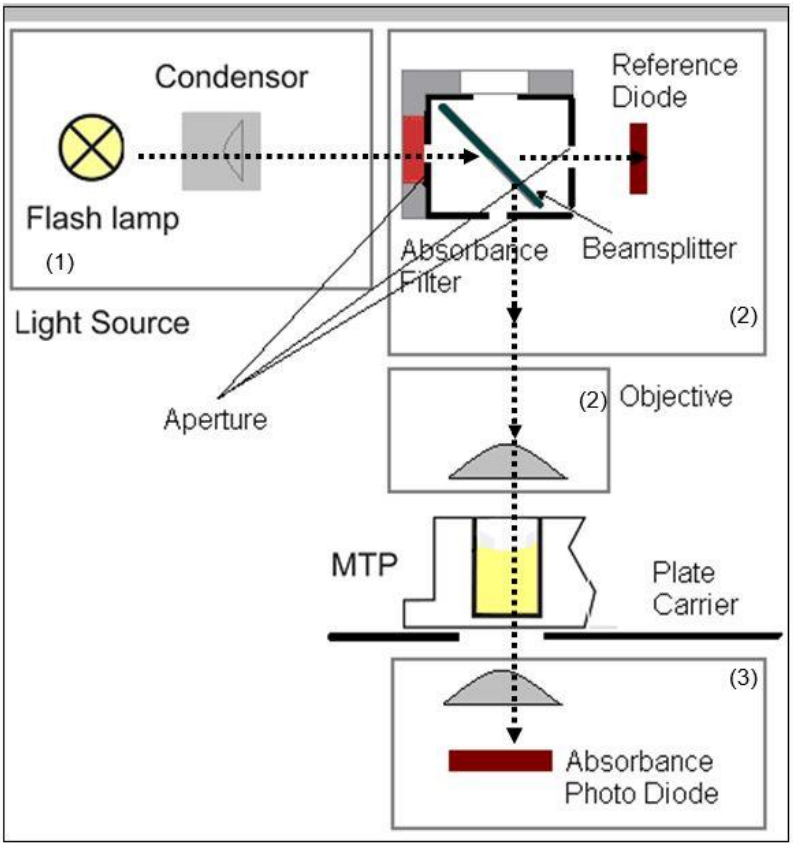


Figure 13: Absorbance System of the Infinite F configurations

Light Source System

The absorbance light source system is similar to the fluorescence top system.

Band Pass Filter

In absorbance applications, optical filters of band pass type are necessary to select the useful wavelengths from the flash lamp spectrum. Filters are mounted in removable slides.

Absorbance Filter

Absorbance measurements require relatively narrow band pass filters (2 – 10 nm) with steep slopes.

Absorbance Optics

The mirror carriage has an absorbance position. A pair of small slits forms a narrow and more collimated light beam when compared with fluorescence excitation.

Light focused through the dispensed liquid is slightly refracted at the interfaces between air, liquid, and plate bottom. To accomplish a reliable measurement in the presence of the meniscus, a focusing lens recollects the rays of light, which might have been refracted too far away from the optical axis.

The spot size of the absorbance light beam is 0.5 mm (diameter).

Absorbance Detection

A silicon photodiode is used for the measurement of the light beam. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels being encountered with absorbance measurements up to 4 OD.

Note
For absorbance measurement 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.

For further information please contact your local Tecan distributor or visit: www.tecan.com.

2.5.5 Absorbance System (Infinite M configurations)

For absorbance measurements, a similar optical path is used as for fluorescence excitation.

The absorbance system consists of:

- light source
- excitation monochromator
- absorbance MTP optics
- absorbance MTP measurement module

Condenser type optics focus the light through the excitation filters and then through the entrance slit to the excitation monochromator. A fiber bundle then guides the light from the excitation monochromator to the absorbance MTP optics, which focuses the light into the wells. The absorbance MTP measurement module is located underneath the plate carrier. These modules measure the light being transmitted through the sample.

Before measurement of the microplate (MTP), a reference measurement is performed with the plate carrier moved out of the light beam.

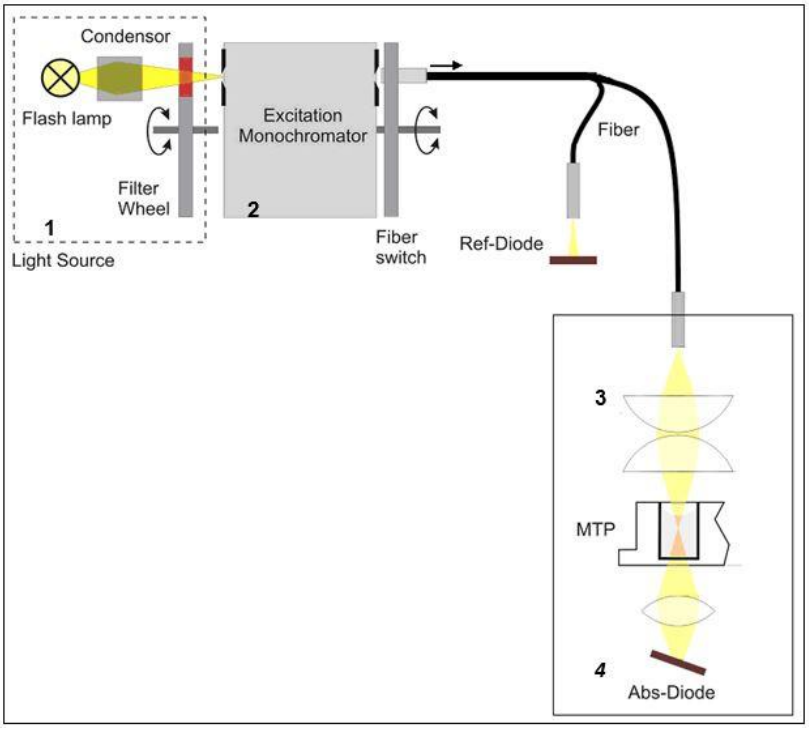


Figure 14: Optical System Absorbance Infinite M configurations

For details about the light source (1) and the excitation monochromator (2), please refer to Fluorescence Intensity Light Source System, page 26.

Absorbance Optics MTP

A fiber bundle guides the light from the excitation monochromator system to the absorbance MTP optics.

The absorbance optics consists of a pair of lenses focusing the light beam into the well of the microplate.

The spot size of the absorbance light beam is 0.7 mm in diameter.

Absorbance Detection MTP

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 being encountered with absorbance measurements up to 4 OD.

Note
For absorbance measurement of nucleic acids in small volumes (2 µl) use Tecan's NanoQuant Plate™.

With this device it is possible to measure 16 different samples within one measurement.

For further information please contact your local Tecan distributor or visit: www.tecan.com

Pathlength Correction

The Pathlength Correction, for Infinite readers with monochromator configurations, can be used to correct the measured absorbance values of samples in microplates to 1 cm pathlength, to compare the measurement results to those read with cuvettes or to perform quantitative analysis of samples based on their extinction coefficient.

According to the Lambert-Beer's law, the amount of absorbed light is proportional to the concentration of the sample and to the pathlength of the light passing the sample. Unlike a standard cuvette with a pathlength of 1 cm, the path of the light in a microplate is unknown and depends on the filling volume of wells. For aqueous solutions, the pathlength can be calculated from the absorbance values for water recorded in the near infrared wavelength range (900 nm to 1000 nm) by using a cuvette and the respective microplate.

Note
The absorption of water is temperature dependent. Please make sure that all measurements are performed at exactly the same temperature.

Note
Any light absorption of assay components between 900 and 1000 nm will interfere with pathlength correction.

Note
Please be aware that buffer (salt concentration), organic solvents, meniscus and plate characteristics can affect the pathlength correction measurement.

CAUTION

TURBID SAMPLES CAN LEAD TO SHORTENING OF ESTIMATED PATHLENGTH DUE TO SCATTERING OF LIGHT. THE PATHLENGTH CORRECTION WITH CUVETTE WILL NOT COMPENSATE FOR THIS EFFECT.

The **Pathlength** calculation of the sample is performed as follows:

$Pathlength_{Sample} = (A_{TW} – A_{RW})/(A_{Water}) * 1\ cm$

A_{TW} = Absorption of aqueous sample at Test wavelength

A_{RW} = Absorption of aqueous sample at Reference wavelength

A_{Water}= A_{TW} minus A_{RW} of water in a 1 cm cuvette (= Correction factor)

The calculated pathlength is finally used to correct the absorbance of sample (A_{Sample}) at specific dye wavelength to 1 cm ($A_{SampleCorrected}$):

$A_{SampleCorrected} = A_{Sample}/Pathlength_{Sample}$

2.5.6 Luminescence System

The Infinite reader Luminescence System consists of the following parts:

- Luminescence Optics
- Detection Unit (single photon counting PMT)

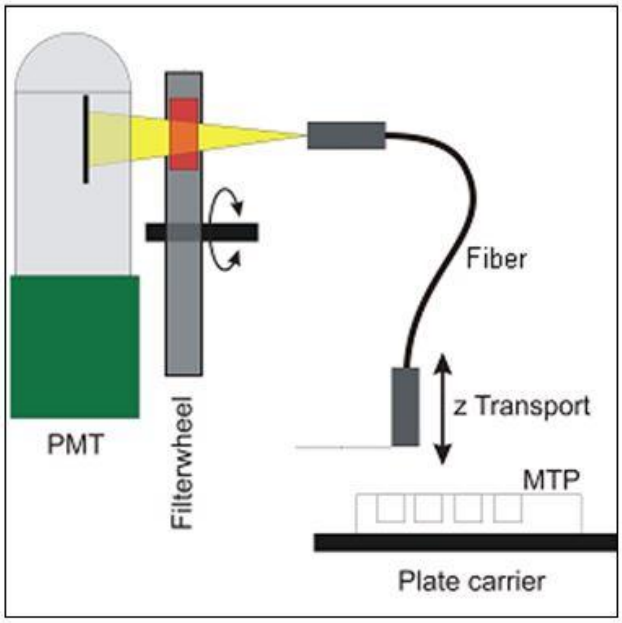


Figure 15: Optical System Luminescence

The luminescence fiber bundle guides the light from the sample to the detection unit (PMT) passing through a filter wheel. The photon counting PMT (photomultiplier tube) is designed for applications in chemo- and bioluminescence providing a high dynamic range. The exceptionally low noise and high sensitivity allows the detection of very low light levels.

The z-position of the luminescence fiber bundle fixed onto the optics carrier is adjusted automatically by the software and depends on the selected plate definition file. As light is refracted at the sample liquid surface, z-adjustment helps to maximize signal to noise and minimize cross-talk.

Luminescence Optics

In luminescence measurement mode, the Infinite reader uses fixed microplate position and a moveable luminescence measurement head (see Figure 15: Optical System Luminescence). The plate thickness is defined by selecting the corresponding plate type in the software (see i-control Instructions for Use).

Fiber

A glass fiber guides the light from the sample to the detection unit. The fiber is designed to measure from 6-well up to 384-well plates.

Filter Wheel

A filter wheel with 6 filter positions in front of the PMT window is switched to the required luminescence channel. The sensitivity of the detection system makes it necessary to attenuate high luminescence light levels; therefore, the filter wheel can also switch a neutral density filter across the selected fiber exit.

Filter Wheel Position	Filter
Position 1	Lumi Green*
Position 2	Lumi Magenta*
Position 3	OD2 neutral density filter
Position 4	No attenuation
Position 5	Red NB **
Position 6	Blue2 NB **

* recommended for the BRET^2 assay and the ChromaGlo - Luciferase Assay

** recommended for the NanoBRET™ assay

The OD2 neutral density filter serves to attenuate high light levels by a factor of 100 (corresponding to 2 OD absorbance). The resulting values are automatically scaled to counts per second and displayed accordingly in the software results output.

See Figure 16 to Figure 19 for transmission spectra of luminescence filters.

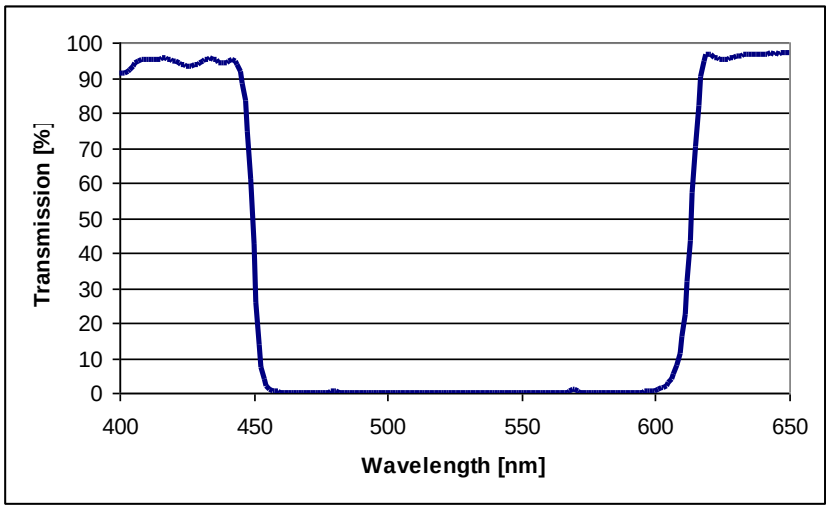


*Figure 16: Transmission spectrum of filter **Lumi Magenta***

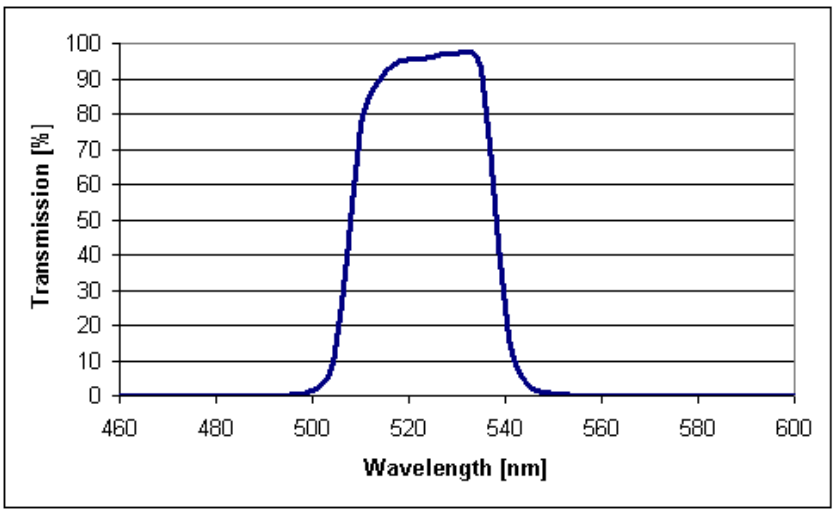


*Figure 17: Transmission spectrum of filter **Lumi Green***

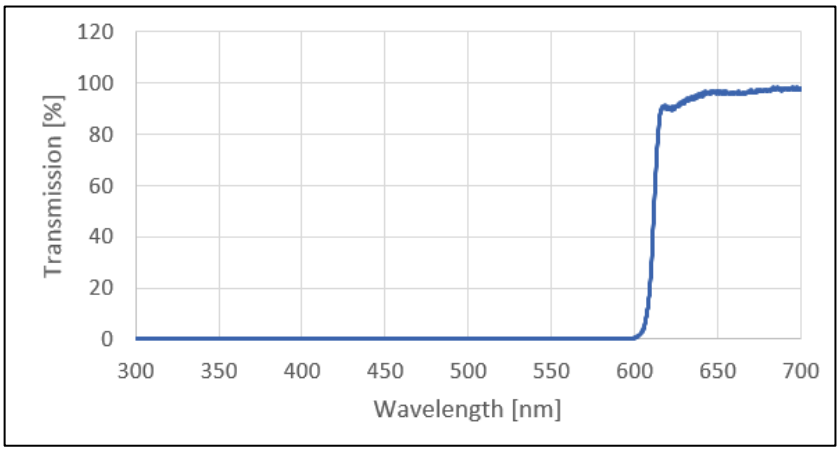


*Figure 18: Transmission spectrum of filter **Red NB***

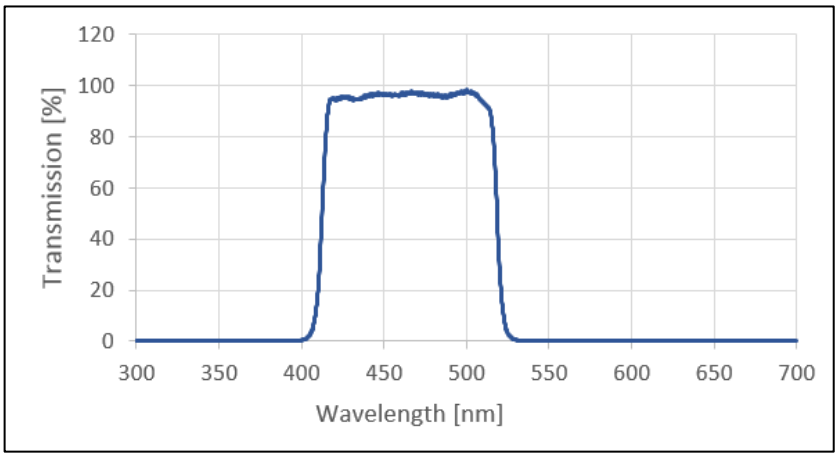


*Figure 19: Transmission spectrum of filter **Blue2 NB***

2.5.7 Cuvette Port (Infinite M Configurations)

The Infinite M configurations may be optionally equipped with a cuvette port for absorbance measurements.

For absorbance measurements with the cuvette port of Infinite M configurations a similar optical path is used as for fluorescence excitation.

The absorbance system consists of:

- light source
- excitation monochromator
- absorbance cuvette measurement module
- absorbance microplate module

Condenser type optics focus the light through the excitation filters and then through the entrance slit to the excitation monochromator. A fiber bundle then guides the light from the excitation monochromator to the absorbance cuvette optics, which focuses the light through the cuvette. The absorbance cuvette measurement module is located right after the cuvette port. A silicon photo diode measures the light being transmitted through the sample. Before measurement of the cuvette, a reference measurement against air is performed with the cuvette port moved out of the light beam.

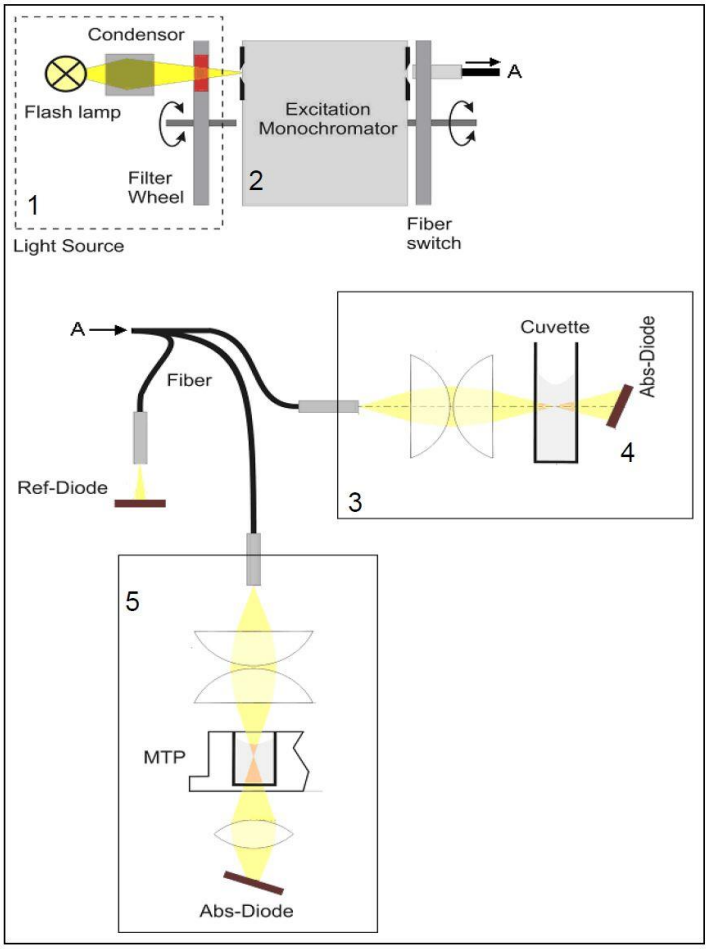


Figure 20: Optical System of the absorbance module of Infinite M configurations including the cuvette port. The figure also shows the light path of the absorbance microplate module (5).

For details of the light source (1) and the excitation monochromator (2), please refer to chapter 2.4.1 Fluorescence/A) Fluorescence Intensity (FI).

Note
The cuvette port is an option of the Infinite M configurations only. This option is not available for the Infinite F configurations. With the Infinite F configurations, cuvettes may be measured using a Tecan Cuvette Adapter placed on the plate transport.

Absorbance Optics Cuvette

A fiber bundle guides the light from the excitation monochromator system to the absorbance cuvette optics.

This optics consists of a pair of lenses focusing the light beam into the cuvette.

At the focal point, the spot diameter of the absorbance light beam is 1.9 mm.

Absorbance Detection Cuvette

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 being encountered with absorbance measurements below 4 OD. Measurement values above 4 OD are marked as **OVER** in the result sheet.

Cuvette types

The cuvette port is compatible with the following cuvettes:

Cuvette Type	Width x Depth	Maximum Height (including lid)	Filling Volume	Example
Standard cuvettes	12.5 x 12.5 mm	55 mm	2 ml	Hellma 110 QS, 10 mm*
Semi-macro cuvettes	12.5 x 12.5 mm	55 mm	1 ml	Hellma 108-QS, 10 mm*
Micro cuvettes	12.5 x 12.5 mm	55 mm	0.5 ml	Hellma 104.002 QS, 10 mm*
Ultra-micro cuvettes	12.5 x 12.5 mm	55 mm	100 µl	Hellma 105.202, 10 mm*

Cuvettes with a measurement window < 2 mm (diameter) cannot be used.

CAUTION

ALWAYS USE A VALID FILLING VOLUME. MAKE SURE THAT THE LIQUID LEVEL IN THE CUVETTE EXCEEDS 20 MM (HEIGHT). OTHERWISE, THE LIGHT PATH IN THE CUVETTE MIGHT NOT BE FILLED COMPLETELY WITH LIQUID WHICH CAN LEAD TO WRONG MEASUREMENT RESULTS.

CAUTION

THE CUVETTE PORT OF THE INFINITE M CONFIGURATIONS CANNOT BE USED FOR CUVETTES WITH A MEASUREMENT WINDOW < 2 MM (DIAMETER) AND A CENTER HEIGHT BELOW 15 MM.

*Hellma GmbH & Co. KG, Germany; www.hellma-worldwide.com

Inserting the Cuvette

The cuvette holder is attached securely to the cuvette carrier and moves the cuvette in and out. The cuvette carrier is an integral part of the instrument and cannot be removed.

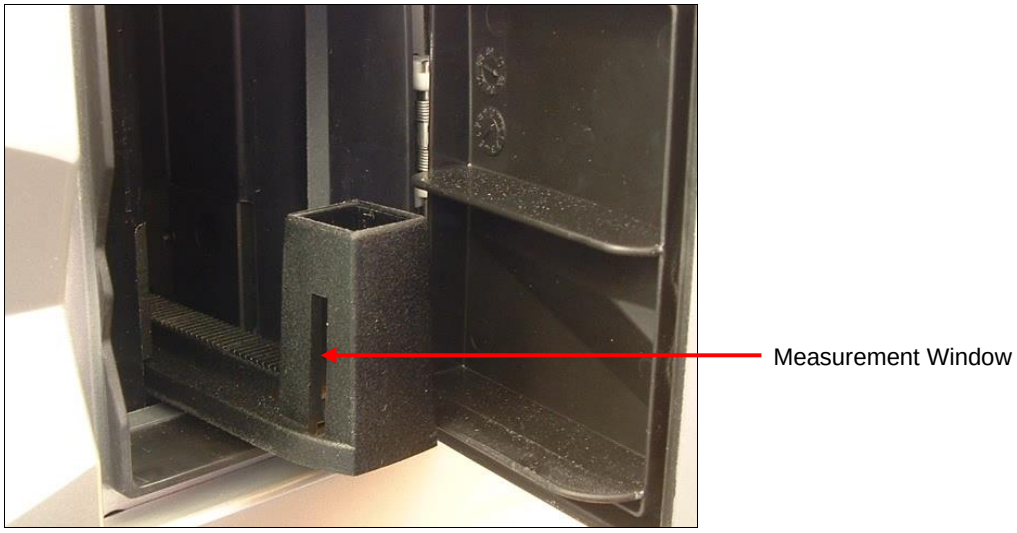


Figure 21: Cuvette Port Infinite M configurations

The cuvette must be inserted so that the measurement window of the cuvette corresponds to the measurement window of the cuvette holder:

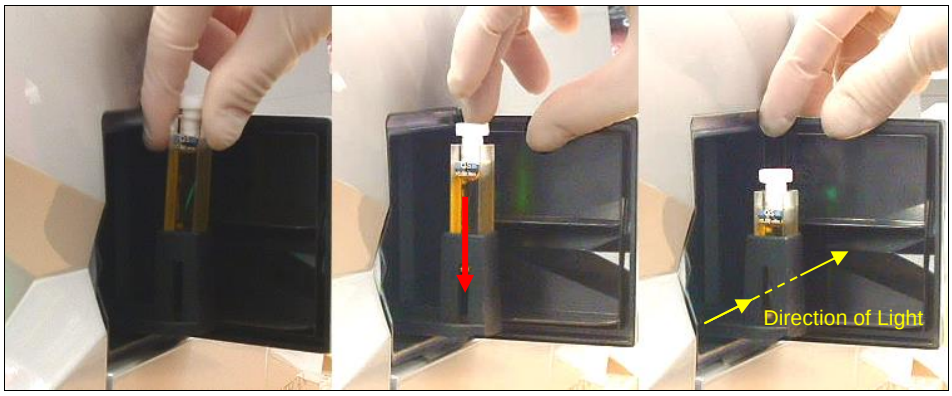


Figure 22: How to insert the cuvette into the cuvette holder

3. Installation

3.1 Unpacking and Inspection

The delivered packaging includes the following items:

- CABLE USB 2.0 A/B 1.8 M Black with housing receptacle ferrite
- Software storage media for Infinite F configurations/Infinite M configurations
- OOB Quality Report
- Transport lock (mounted)
- Instructions for Use
- Final test protocol

The Infinite F configurations packaging includes additionally the following items:

- Accessory Box
- Filter stop rings (8)
- Filter assembly tool
- Plastic tweezers
- Filter slide

The injector module packaging for 1 injector includes the following items:

- Bottle holder
- Beaker for priming
- 125 ml bottle brown
- Injector dummy (mounted)
- Waste tub
- 15 ml bottle

The second injector comes with the following items:

- Bottle holder
- Beaker for priming
- Waste tub
- 15 ml bottle

CAUTION

THE INFINITE READER HAS BEEN TESTED WITH THE SUPPLIED USB CABLE. IF ANOTHER USB CABLE IS USED, TECAN AUSTRIA CANNOT GUARANTEE THE CORRECT PERFORMANCE OF THE INSTRUMENT.

3.1.1 Unpacking Procedure

1. Visually inspect the container for damage before it is opened.
   Report any damage immediately.
2. 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. Ensure that the plate carrier cannot be accidentally hit when moved out. Ensure that the main switch and the main cable can be reached at all times and are in no way obstructed.
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 slip.
   Report any discrepancy immediately.
7. Check the instrument accessories against the packing list.
8. Save packing materials and transport locks (see next section) for further transportation purposes.

WARNING

THE INFINITE READER IS A PRECISION INSTRUMENT AND WEIGHS FULLY EQUIPPED APPROX. 16 KG.

CAUTION

THE MAXIMUM LOAD FOR THE INFINITE READER COVER IS 16 KG, HOWEVER THE LOAD MUST BE DISTRIBUTED EVENLY ACROSS THE ENTIRE SURFACE OF THE COVER.

CAUTION

THE MAXIMUM LOAD FOR THE PLATE TRANSPORT IS 100 G. OVERLOADING THE PLATE CARRIER CAN CAUSE INSTRUMENT DAMAGE WHICH MAY REQUIRE SERVICE.

CAUTION

ALLOW AT LEAST 10 CM DISTANCE BETWEEN THE BACK OF THE INSTRUMENT AND THE WALL OR ANY OTHER EQUIPMENT.

CAUTION

THE INSTRUMENT MUST BE PLACED IN A LOCATION AWAY FROM DIRECT SUNLIGHT. ILLUMINATION > 500 LUX CAN NEGATIVELY INFLUENCE LUMINESCENCE MEASUREMENTS.

3.2 Removal of the Transport Locks

CAUTION

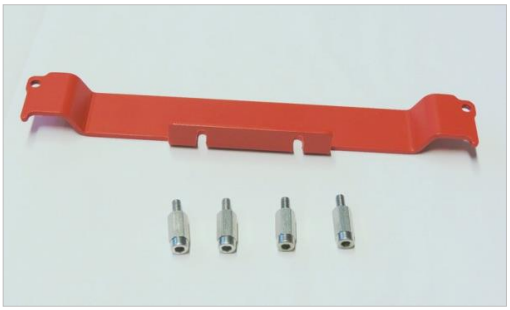
REMOVE THE TRANSPORT LOCK BEFORE OPERATING THE INSTRUMENT.

The instrument is delivered with the plate carrier locked into place, so that it cannot be damaged. Before the instrument can be used the transport lock must be removed using the following procedure:

1. Ensure that the instrument is disconnected from the main power supply.
2. Open the plate carrier compartment flap.
3. Remove the screws and pull the plate carrier out manually.

4. Remove the screws from the transport lock.

5. Remove the transport lock from the plate carrier.



6. The transport locks should be saved for further transportation purposes.

CAUTION

SAVE PACKING MATERIALS AND TRANSPORT LOCKS FOR FURTHER TRANSPORTATION PURPOSES. THE INFINITE READER MUST BE SHIPPED ONLY WITH THE ORIGINAL PACKAGING AND INSTALLED TRANSPORT LOCKS.

3.3 Transport and Storage

3.3.1 Transport

The Infinite reader must be shipped using the original packing and installed transport locks. Before shipping the instrument, it must be thoroughly disinfected (see 7.4 Instrument Disinfection).

3.3.2 Storage

Before storing the instrument the injectors must be rinsed using a wash procedure (see 4.10.1 Priming and Washing of the Infinite Reader). Select a location to store the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents, and acid vapors

Storage Specifications

Temperature	- 20 °C to + 60 °C	-4 °F to + 140 °F
Relative Humidity	< 80 % non-condensing	

3.4 Power Requirements

The instrument is auto sensing and it is therefore not necessary 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 100-120/220-240V.

If the voltage is not correct, please contact your distributor.

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 BE DAMAGED.

WARNING

IF THE INSTRUCTIONS GIVEN IN THIS DOCUMENT ARE NOT CORRECTLY PERFORMED, THE INSTRUMENT WILL EITHER BE DAMAGED OR THE PROCEDURE WILL NOT BE PERFORMED CORRECTLY AND THE SAFETY OF THE INSTRUMENT IS NOT GUARANTEED.

3.5 Switching the Instrument On

CAUTION

BEFORE THE INSTRUMENT IS SWITCHED ON FOR THE FIRST TIME AFTER INSTALLATION, IT SHOULD BE LEFT TO STAND FOR AT LEAST 3 HOURS, SO THERE IS NO POSSIBILITY OF CONDENSATION CAUSING A SHORT CIRCUIT.

1. Ensure the computer is switched OFF and the instrument's main power switch on the back panel of the instrument is in the OFF position.
2. Connect the computer to the instrument with the delivered USB interface cable.
3. Insert the power cable into the main power socket (with protective ground connection) on the back panel of the instrument.
4. All connected devices must be approved and listed as per IEC 60950-1 Information Technology Equipment – Safety or equivalent local standards.
5. Switch the instrument ON using the main power switch on the back panel of the instrument.

CAUTION

THE INFINITE READER HAS BEEN TESTED WITH THE SUPPLIED USB CABLE. IF ANOTHER USB CABLE IS USED, TECAN AUSTRIA CANNOT GUARANTEE THE CORRECT PERFORMANCE OF THE INSTRUMENT.

CAUTION

DO NOT REPLACE DETACHABLE MAIN POWER SUPPLY CORDS WITH INADEQUATELY RATED CORDS.

4. Operating the Instrument

4.1 Introduction

The Infinite reader is operated using a personal computer-based software control. **i-control** or **Magellan** software may be used as the user interface. For details see the corresponding software Instructions for Use. This short introduction is for a general understanding of instrument parameters and operation. Suggestions are made on how to optimize instrument parameters for your applications.

Every effort has been made to ensure that the instrument will work correctly even if the default parameters are not appropriate for a particular application - with an important exception:

CAUTION

WHEN PLACING A MICROPLATE INTO THE PLATE CARRIER, ALWAYS MAKE SURE THAT THE CORRECT PLATE DEFINITION FILE (PLATE HEIGHT) HAS BEEN SELECTED IN THE SOFTWARE BEFORE YOU DO ANYTHING ELSE.

MAXIMUM PLATE HEIGHT IS 23 MM (INCLUDING LID).

CAUTION

BEFORE STARTING MEASUREMENTS, MAKE SURE THAT THE MICROPLATE POSITION A1 IS INSERTED CORRECTLY. THE POSITION OF WELL A1 MUST BE ON THE UPPER LEFT SIDE.

CAUTION

IN CASE OF SIGNIFICANT SOILING OF THE PLATE TRANSPORT, THE SPRING MECHANISM MIGHT NOT WORK PROPERLY, AND CAN LEAD TO WRONG POSITIONING. PLEASE CONTACT YOUR LOCAL SERVICE CENTER.

CAUTION

WHEN OPERATING THE INFINITE READER ALWAYS WORK ACCORDING TO GLP GUIDELINES.

CAUTION

THE INFINITE READER HAS A FAN ON THE BACKSIDE OF THE INSTRUMENT THAT DRAWS IN AIR. THE AIR FILTER MUST BE CHECKED EVERY 4 WEEKS AND BE REPLACED WHEN DIRTY. THE AIR FILTER MUST BE REPLACED AFTER 6 MONTHS.

4.2 General Operating Features

The Infinite reader has some general behavior and options, which are independent from a particularly selected measurement technique.

4.2.1 Instrument Start Up

Before the instrument is switched ON, check if the USB interface cable is connected.

Instrument Power On

When switching ON the instrument no initialization steps are performed.

Connect to Instrument

When the software connects to the instrument, communication is established between the instrument and the user interface.

The following steps are performed:

- OS filter wheels are initialized (M configurations only).
- Luminescence filter wheel is initialized.
- Z-transport of luminescence optics is initialized.
- Plate transport is initialized.
  (The plate transport is not moved out automatically.)
- The current versions of firmware and software are displayed.
- The instrument is ready for use.

4.3 General Options

The following options may be taken independently from the particular measurement technique.

Note
To keep temperature on a constant level and provide uniformity across the plate, the plate must be placed in incubation position.

When the heating function is used during shaking, the temperature may vary slightly.

Temperature Control

Some assays require an exact operating temperature. The Infinite reader can set up a specific temperature within a specific range, provide uniformity across the plate, and keep the temperature constant above ambient. The main cooling fans stop ventilation.

Heating up 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.

Temperature range: 5 °C above ambient to 42 °C.

Kinetic Measurements

i-control allows a plate to be measured repeatedly in equidistant time intervals. Fluorescence signal may significantly decrease over a longer period of time, especially when using low volumes. Depending on the amount of evaporation, the meniscus will shift to a lower position giving rise to slightly out of focus conditions. Usually, wells in the corner evaporate faster, the next at the edges of the microplate. When measuring fluorescence, decrease in signal may also result from photo bleaching.

Microplate Shaking

The Infinite reader provides two shaking modes: linear and orbital. The shaking amplitude can be selected from 1 – 6 mm in steps of 0.5 mm. The frequency is a function of the amplitude. The shaking duration is selectable from 1 – 1000 s.

Multi-labeling

The **i-control** software provides a basic multi-labeling capability. Up to four sets of instrument parameters can be edited. The corresponding plate measurements will be executed in the selected order. For example, when using more than one fluorescent label, different filter combinations could be selected. A multi-labeling measurement can be set up by using a plate strip with/without a **part of the plate** strip and up to 10 measurement strips (absorbance fixed wavelength, absorbance scanning, fluorescence intensity, fluorescence intensity scanning, luminescence).

4.4 Defining Filter Slides (Infinite F configurations)

4.4.1 About Filters

Fluorescence Filters

The optical filters (bandpass style) in a filter slide are specially designed for fluorescence measurements. The spectral rejection 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.

Absorbance Filters

Bandpass filters, which are commonly used in microplate readers for absorbance measurements, usually have a bandwidth of 10 nm. Therefore, it is not recommended to use fluorescence filters for absorbance measurements because the bandwidth (FWHM) is usually larger than 10 nm. This could cause a bright value error or low OD values when measuring dyes with narrow peaks.

4.4.2 Filter Slide and Filter Orientation

Filter Slide

The Infinite F configurations filter slide consists of an excitation and an emission part. The filter slide enables the user to work with four independent excitation/emission filter pairs, which can be defined on positions 1 to 4. The information about the inserted filters is saved on the integrated microchip.

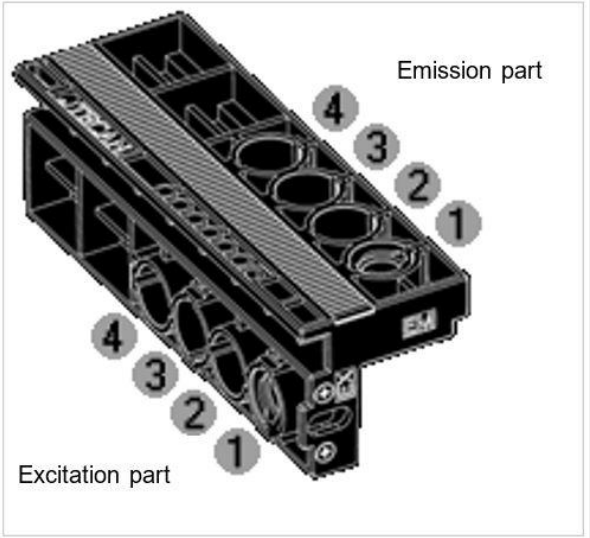


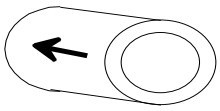
Figure 23: Infinite F configurations: Filter slide

Filter Types

CAUTION

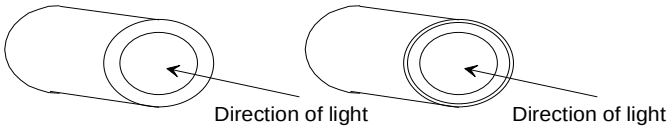
THERE ARE TWO TYPES OF FILTERS. IT IS IMPORTANT THAT LIGHT TRAVELS THROUGH BOTH TYPES OF FILTERS IN THE CORRECT DIRECTION. BEFORE INSERTING A NEW FILTER CAREFULLY CONSIDER THE FILTER AND THE DIRECTION OF LIGHT THROUGH THE FILTER SLIDE.

Filters with an arrow on the side:



Light must travel in the direction of the arrow.

Filters without an arrow on the side:



The end of the filter with the metal lip must face away from the light source.

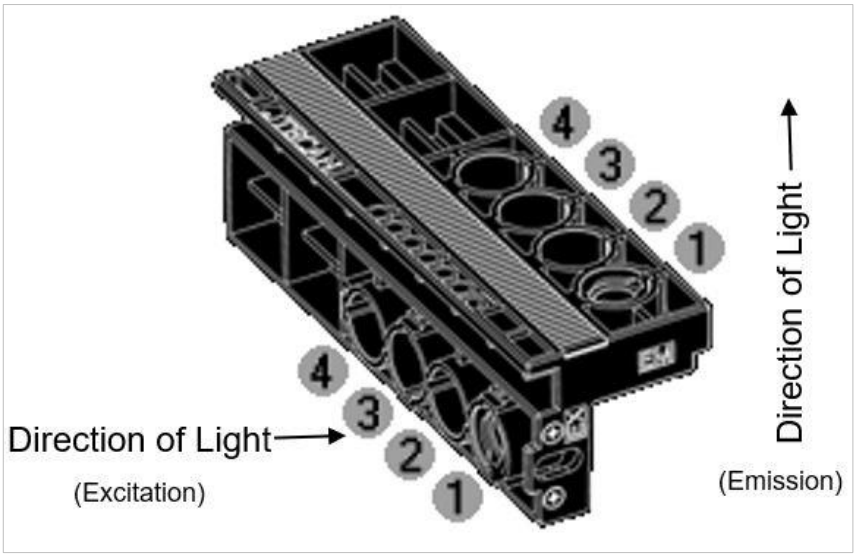


Figure 24: Infinite F configurations: Filter Slide - Direction of Light

Position of Polarization Filters

Note
Fluorescence polarization measurements on the Infinite F Plex require two identical excitation and emission filters placed together with the polarizers either on the positions 1 and 2 or 3 and 4.

The Infinite F Plex filter slide can be equipped with a maximum of two different fluorescence polarization filter pairs as each fluorescence polarization measurement requires two identical excitation and emission filters, which are placed together with the polarizers either on the position 1 and 2 or 3 and 4.

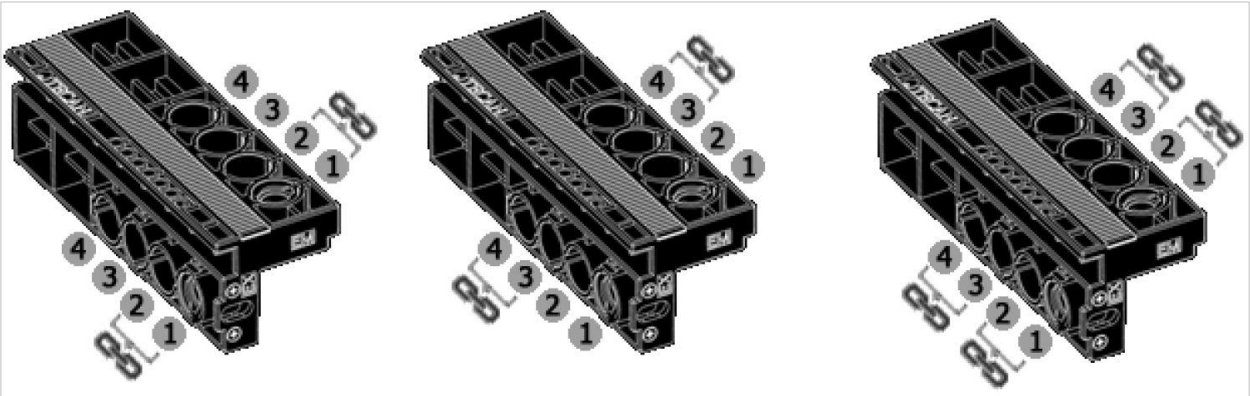


Figure 25: Infinite F Plex: Filter slide with the indicated positions for fluorescence polarization filters and polarizers.

4.4.3 Installing a Custom Filter

When installing a new filter use the filter assembly tool included in the accessories case. For installing the polarizers use the soft tweezers (plastic).

Removing a Filter

Align the filter assembly tool with the notch of the stop-ring. Turn the tool and remove the stop-ring by pulling it out of the filter slot.

Stop-ring

The filter will slide out of the filter slot when the filter carrier is turned over. Do not use the filter assembly tool to remove filters.

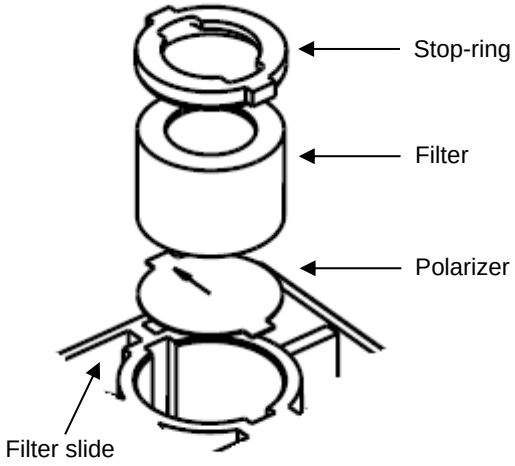
Mounting a Custom Filter

A new filter (and polarizer) must be inserted into the slide as shown below.

Note
Make sure that the filters are inserted correctly (see Filter Types). To ensure proper function, do not reuse the stop-rings more than 5 times.

CAUTION

TAKE CARE TO INSERT THE POLARIZERS AND THE FILTERS INTO THE FILTER SLIDE WHEN WORKING WITH FLUORESCENCE POLARIZATION.



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.

To install a custom filter, do the following:

If required, carefully insert a polarizer at the excitation and emission half of the filter slide using tweezers, taking care not to scratch it or get fingerprints on it.

Carefully insert the filter into the opening, taking care not to scratch or get fingerprints on the filter.

Place the stop-ring on the end of the filter assembly tool and turn it so it cannot slip off.

Filter assembly tool with stop-ring

Using the filter assembly tool, push the stop-ring into the filter slot and press firmly into place.

Rotate the tool until the notch in the stop-ring is aligned with the end of the filter assembly tool and remove the tool.

If there are unused openings remaining after the required filters have been inserted (e.g., the emission part of an absorbance filter), filter dummies should be mounted in the holes that are still open.

4.4.4 Defining the Filters

CAUTION

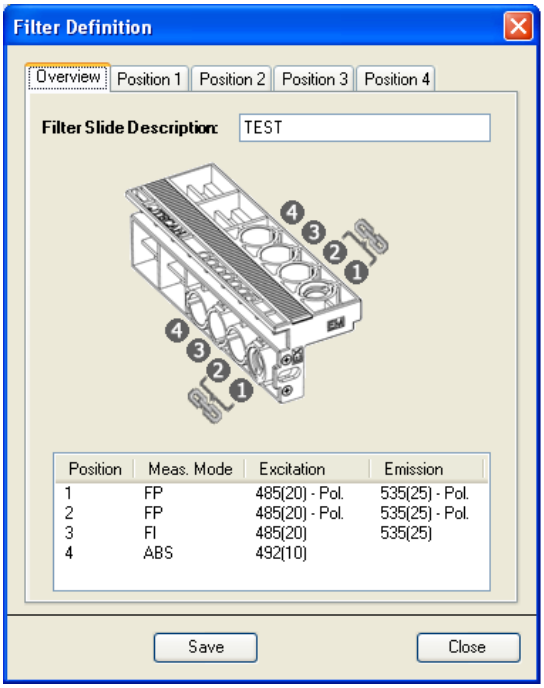
ANY CHANGES TO THE FILTERS IN THE FILTER SLIDE ARE TO BE CARRIED OUT BY TRAINED PERSONNEL! THE INSTRUMENT IS ABLE TO RECOGNIZE PREDEFINED FILTER SLIDES AND YOU SHOULD NOT ATTEMPT TO CHANGE THE FILTER VALUES. HOWEVER, IF THE FILTERS IN THE FILTER SLIDE HAVE BEEN CHANGED (BY A SERVICE ENGINEER) OR IF A NEW UNDEFINED CUSTOMIZED FILTER SLIDE IS TO BE USED, THE FILTER SLIDES NEED TO BE DEFINED.*

***DEPENDING ON THE FREQUENCY OF USE AND ENVIRONMENTAL CONDITIONS, OPTICAL FILTERS MAY DETERIORATE OVER TIME AND THEREFORE HAVE A LIMITED LIFETIME.**

Define a filter (pair) as follows:

- Select Filter Definitions from the Settings menu.

The following dialog box is displayed showing an overview tab and four filter definition tabs:



Overview: The overview provides the user with the current filter slide definition.

Filter Slide Description: Enter the filter slide description or the filter slide description will be generated automatically.

Note
No special characters (blank, ?, $, %, ., /, etc.) except _ are allowed for the filter slide description.

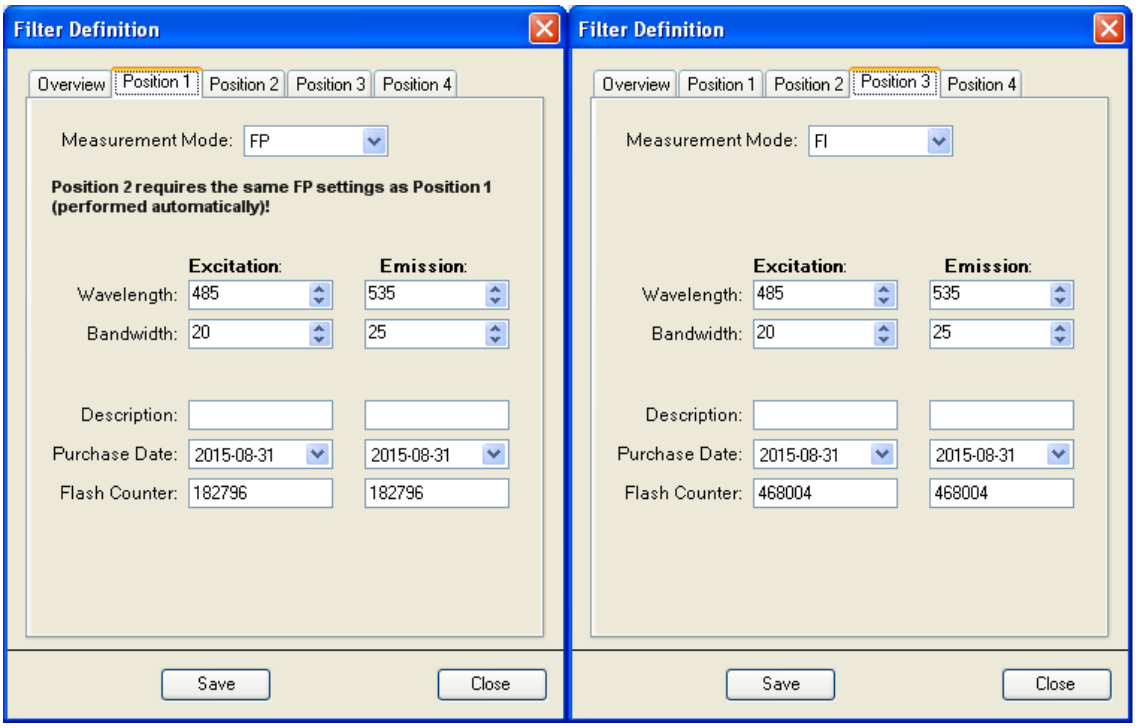
CAUTION

THE FILTER SLIDE DESCRIPTION IS PART OF THE G-FACTOR KEY VALUE. IF MANUALLY ENTERED, AVOID USING THE SAME DESCRIPTION FOR THE DIFFERENT FILTER SLIDES.

Position 1 –- 4: Filter definition editor for the filters (filter pairs) on positions 1, 2, 3 and 4.

Select the appropriate filter position and enter the new wavelength, bandwidth, and measurement mode for each new filter:

Measurement Mode: chose from the dropdown list **FI** for fluorescence intensity, **ABS** for absorbance, **FP** for fluorescence polarization and **Empty** for filter-free positions



Note
Fluorescence polarization mode on Position 1 requires the same filter settings on Position 2 and vice versa. Fluorescence polarization mode on Position 3 requires the same filter settings on Position 4 and vice versa. This is performed automatically.

CAUTION
MAKE SURE THAT THE FILTER SLIDE CONTAINS POLARIZERS TOGETHER WITH THE FILTERS DEFINED FOR FLUORESCENCE POLARIZATION.

Wavelength: Enter the filter wavelength within the following range:

(1) Fluorescence intensity mode: 230 to 850 nm (Excitation) and 280 to 850 nm (Emission)

(2) Fluorescence polarization: 300 to 850 nm (Excitation) and 330 to 850 nm (Emission)

(3) Absorbance mode: 230 to 1000 nm

Bandwidth: Enter the bandwidth (nm) of the filter

(4) Accept the new filter values by clicking **Save**. By closing the Filter Definition dialog, the system is ready to collect data with the new filters.

Description: This field can be used for individual user's remarks about the filter, e.g., filter name, application, etc.

Note
No special characters (blank, ?, $, %, ., /, etc.) except _ are allowed for the filter slide description.

Purchase Date: This option enables the user to enter the purchase or installation date of the filter

Flash Counter: The flash counter monitors the number of flashes through a filter. The flash counter number provides the user only with additional information about the filter in use. The flash counter number is saved together with other information about the filter on the filter slide microchip.

If you replace a filter, this information will be lost unless the last filter flash number is manually documented by the user.

For a brand-new filter, set the counter to 0. For a previously used filter, enter the last collected flash number if the number is available.

CAUTION

IT IS RECOMMENDED TO MANUALLY DOCUMENT THE LAST FLASH COUNTER NUMBER BEFORE REPLACING THE FILTER; OTHERWISE, THIS INFORMATION WILL BE LOST.

CAUTION

DO NOT INSERT FILTERS SLIDES IF THE INSTRUMENT IS NOT SWITCHED ON AND CONNECTED.

4.5 Optimizing Fluorescence Measurements

Fluorescence measurement results may be optimized by tuning instrument parameters on the one hand, and by selecting appropriate materials on the other hand.

4.5.1 Instrument Parameters

Gain Settings

The Infinite configurations fluorescence detection system uses an analog to digital (ADC: Analog Digital Converter) conversion of PMT signal. The gain setting controls the amplification of the PMT when converting fluorescence light into electrical current. The ADC needs a suitable input range of PMT current to provide a proper signal to noise ratio (S/N) on the one hand, and linearity on the other hand. Therefore, the gain should be tuned to make highest concentration microplate wells give highest possible readings. Then, readings of lower concentration microplate wells separate from background - as far as the background noise level allows for that.

Note
If any well of interest is assigned OVER (overflow), you may manually reduce the gain, or select an automatic gain option (see the software Instructions for Use).

PMT Properties

The gain for fluorescence intensity is selectable from 1 – 255. The performance of the PMT depends on the supply voltage. The Infinite reader PMTs are specified from 300 to 1250 V. The relationship between the gain settings of the Infinite reader and the voltage supply is described in Equation 1. The intended use of the Infinite reader PMT is therefore specified for gain settings from 60 to 255. Gain settings below 60 are possible, but the performance of the PMT is not specified for voltage supply < 300 V. Tecan therefore does not take responsibility for measurement results of Infinite reader when using gain settings below 60.

Equation 1:
$$U = \frac{Gain}{255} * 1250\,V$$

Where U is the voltage, Gain is the selected gain setting, 255 is the maximum possible gain and 1250 V is the maximum voltage supply of the PMT.

Example:

A gain of 100 corresponds to a voltage supply of 490 V:

Equation 2:
$$U = \frac{100}{255} * 1250 = 490\,V$$

4.5.2 Z-Optimization (FI Top measurements with Infinite M configurations only)

A useful feature of the **Infinite M** configurations is the z-optimization procedure. Z-Optimization is only available for FI Top measurements with the Infinite M configurations). For a particular assay, this procedure should be performed once to determine the optimum working distance between the sample in the plate and the fluorescence optics.

The z-position can be determined as follows:

(1) **Manual:**

When using the option **manual**, a numeric z-position value can be entered in the measurement strip. The default manual z-position is 20000 µm.

(2) **Calculated from well:**

When using the option **calculated from well**, the **Infinite M** configurations will automatically identify the z-position of maximum signal in the selected well for further measurements.

(3) **Same as** for multi-labeling measurements:

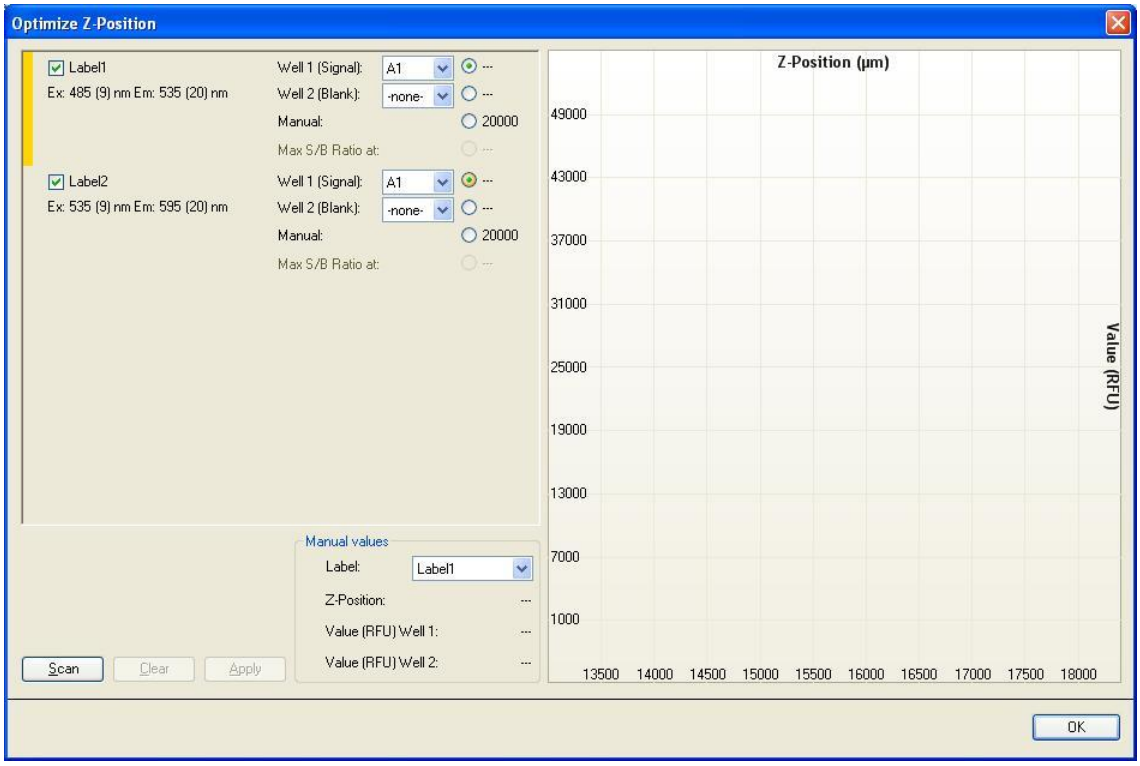
When using the option **same as**, the **Infinite M** configurations will automatically use the same z-position as for a previously defined label.

E.g., in a script with 2 FI Top labels named as Label 1 and Label 2 the z-position of Label 1 can also be used for Label 2 by selecting the option **Same as = Label 1**.

(4) **Instrument → Z-Position:**

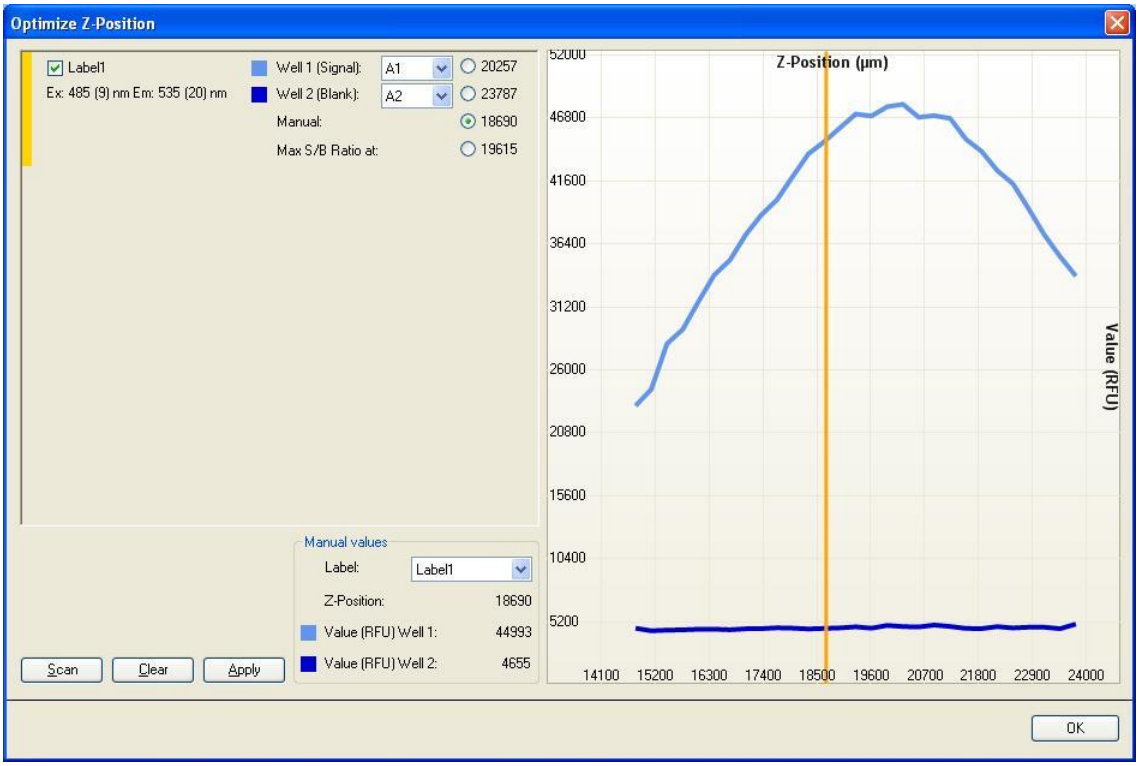
When using the **Z-position** function in the instrument menu, the user can determine the appropriate z-position from a graphical plot that shows the well(s) used for z-positioning. The selected value is applied for further measurements.

Select **Z-Position** from the **Instrument** menu:

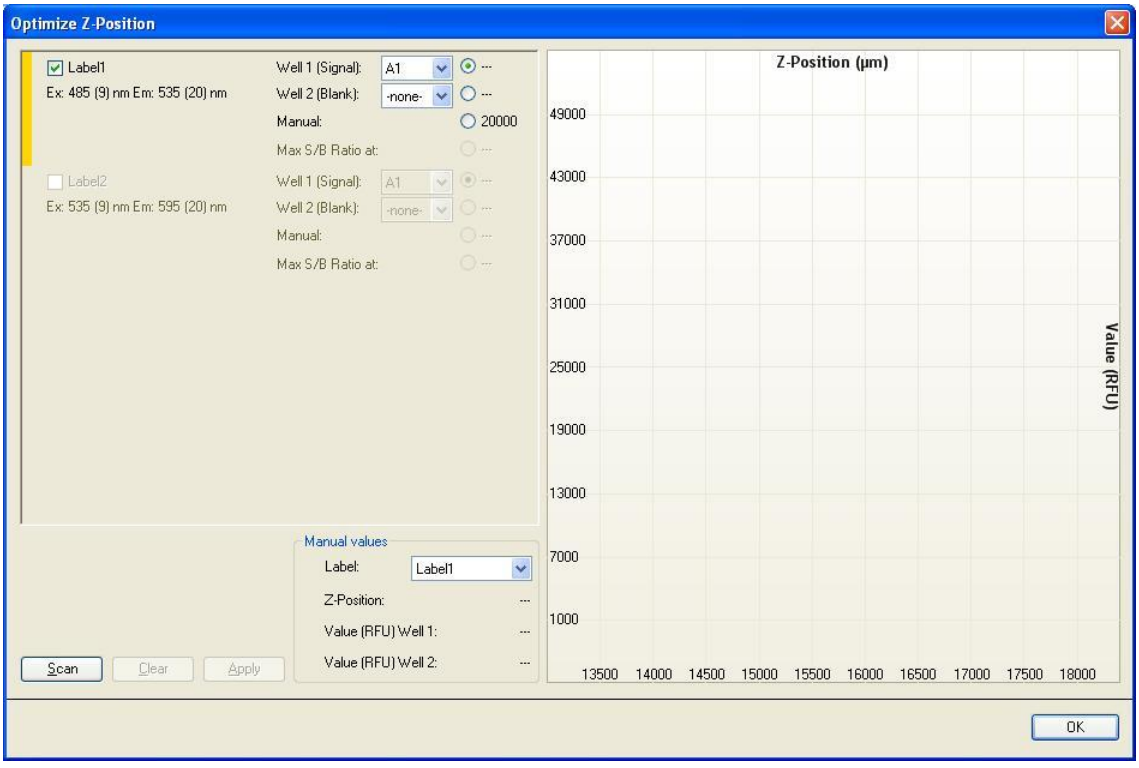


Select the label(s) for which the z-position optimization shall be performed. The optimal z-position can be simultaneously determined for up to 4 labels.

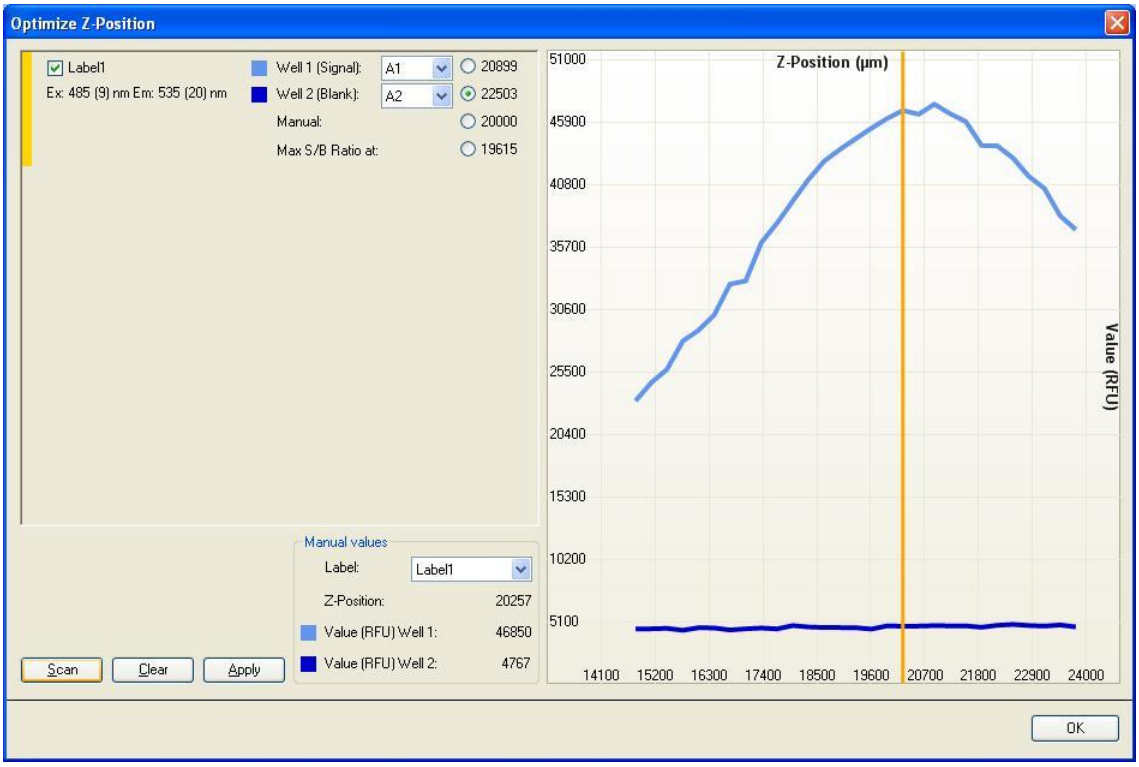
The label selection/number of labels depends on the measurement script previously defined in i-control. Additionally, if the z-position of one of the labels is defined as **Same as**, the label will be displayed but it cannot be selected for the z-optimization:



For each selected label, one or two wells of the defined plate range can be used for the z-position optimization. Select the well(s) and click **Scan** to start the z-optimization:

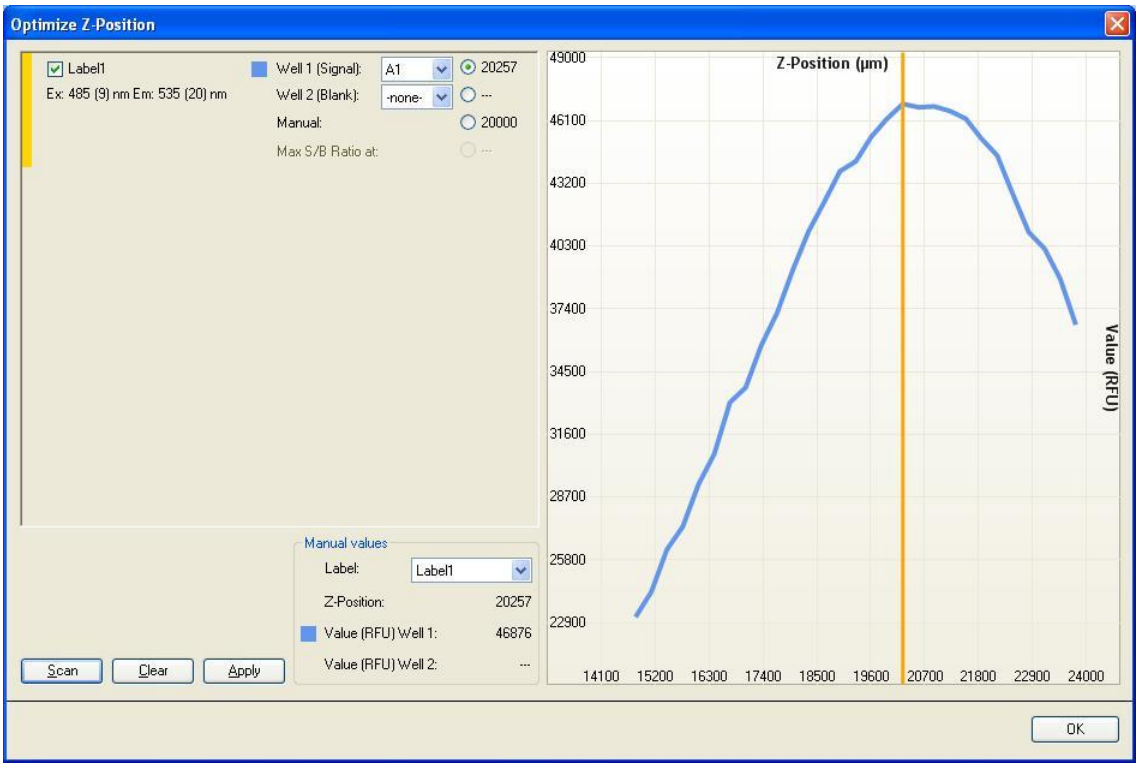


The z-positioning option **Max S/B Ratio** requires the measurement of two wells, one filled with a fluorophore of interest (signal) and one filled with buffer (blank). Both wells are scanned, and the resulting signal and blank curves are shown in the graph. The z-position may now be set to the maximum signal-to-blank (S/B) ratio:



Note
When the option Max S/B Ratio is used, the sample well is first measured with optimal gain and the very same gain value is then applied to the second measurement with the blank well. Therefore, both signal and blank curves are directly comparable.

The z-position for each selected label can be defined manually. In the graph window, the vertical yellow bar can be moved to the desired z-position.



Upon clicking **Apply**, the selected z-position will be automatically applied to the i-control script and used for the subsequent measurement.

Flash Settings

On the fly measurements with 1 flash (read) per well are possible for all plate types; however, measurement precision at low light levels depends on the reading time while fluorescence signal can be received.

Note
Increase the number of flashes (reads) per well until noise of BLANK wells does not further improve, or until measurement time per well becomes unacceptable.

For prompt fluorescence it does not help to increase the default integration time, because the detector will not receive more signal once the flash has vanished.

Timing Parameters for Time Resolved Fluorescence

For TRF, signal integration parameters need to be adjusted according to the label. The start of the signal Integration Time is delayed against the preceding flash by a Lag Time. TRF timing parameters may be established with the following procedure:

As a starting point you may take the Fluorescence Lifetime of the label for both Integration Time and Lag Time.

Coarse tuning: With Integration Time being fixed reduces the Lag Time to maximize Signal to Background (S/B).

Fine tuning: With Lag Time being fixed extends the Integration Time and check if S/B further improves.

Optional Fine-tuning: With either timing parameter being fixed you may vary the other one and check if S/B further improves.

Settle Time

Before measuring a well, a settle time may be set. Due to the stop and go motion of the plate carrier the meniscus of the dispensed liquid may still vibrate while signal is integrated. This can give rise to fluctuations of the measured values. The effect has been observed in wells of 96-well plates and larger wells. In particular, it is critical with absorbance measurements.

4.5.3 FI Ratio Mode

Ratio Mode

Up to 4 labels may be measured well-wise. This measurement mode is called **ratio mode**. Be aware that no **ratio** calculation is performed after this measurement. The Excel result sheet shows the raw data. Further calculations have to be performed by the user.

Filter Switch Time (Infinite F configurations)/ Wavelength Switch Time (Infinite M configurations)

The Infinite F configurations can switch between two filters within 250 ms in case that the selected labels are measured with the same gain. Otherwise, the switching time is 400 ms. In this case the high voltage level at the PMT needs to be changed. The high voltage applied to the PMT needs some time to stabilize.

The Infinite M configurations can switch between two wavelengths within 150 ms in case that the selected labels are measured with the same gain and no order sorting (OS) switching point is involved (see Table 1: for switching points). Otherwise, the switching time is 400 ms. In this case the high voltage level at the PMT needs to be changed. The high voltage applied to the PMT needs some time to stabilize. The OS filter wheel needs to be moved.

	Excitation Wavelength	Emission Wavelength
OSF Switching Point 1	316 nm	401 nm
OSF Switching Point 2	386 nm	621 nm
OSF Switching Point 3	561 nm	-

Table 1: OSF (Order Sorting Filter) Switching Points (Infinite M configurations)

Example:

Fura-2: This application involves a filter/wavelength switch between 340 and 380 nm on the excitation side. The emission is measured at about 510 nm. The excitation filter/wavelength switch does not include an OS switch; therefore, the switch is possible within 150 ms on an Infinite M configuration and 250 ms on an Infinite F configuration.

4.6 FP Measurements

4.6.1 Fluorescence Polarization

Fluorescence Polarization (FP, P) is defined by the following equation:

Equation 3:

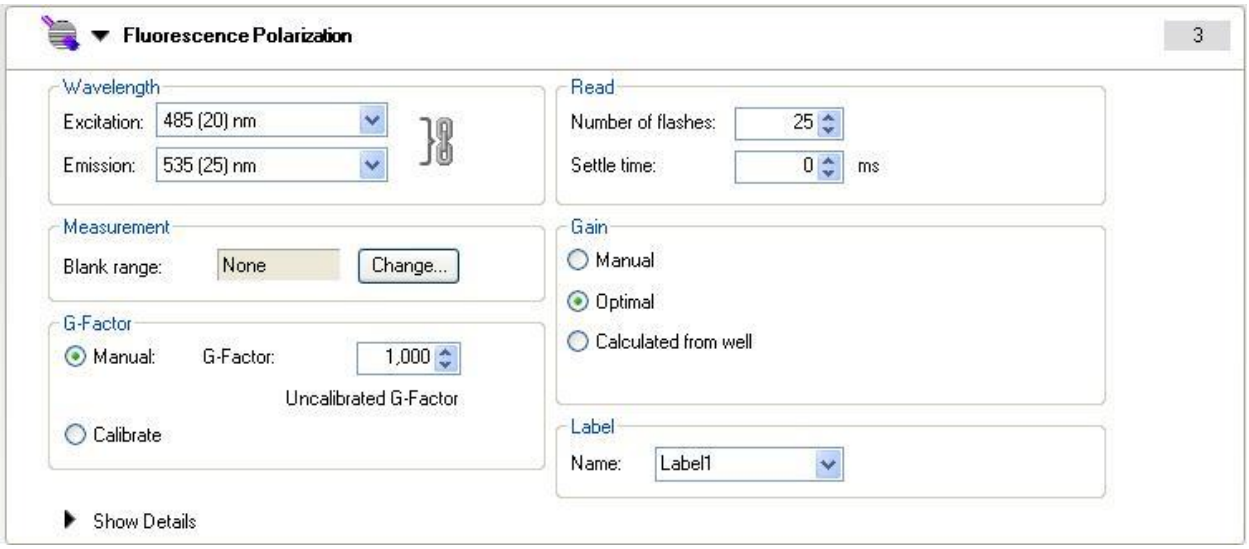
$$P = \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + I_{\perp})}$$

where $I_{||}$ and $I_{\perp}$ equal the emission intensity of the polarized light parallel and perpendicular to the plane of excitation, respectively. Polarization is a dimensionless unit, generally expressed in mP units.

To start an FP measurement, the program strip must contain a valid measurement **Blank range** and valid **G-Factor** settings.

4.6.2 Measurement Blank Range

Measurement blank reduction is performed automatically at each fluorescence polarization measurement; the mean value of the respective blank wells will be subtracted from each sample value (see 4.6.8 Calculation of Fluorescence Polarization Parameters).



In the **Measurement** group box, select the **Blank range** by clicking **Change** and then selecting the wells filled with the measurement (sample) blank.

4.6.3 G-Factor Settings

The given 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 and either the parallel or perpendicular intensity must be corrected by so called **G-Factor**. The G-factor compensates for differences in optical components between parallel and perpendicular measurement.

The G-Factor is the correction factor that can be determined for the wavelength of the fluorophore by measuring a sample with a known polarization value. A valid calibration of the instrument resulting in a G-factor is an important requirement for each fluorescence polarization measurement.

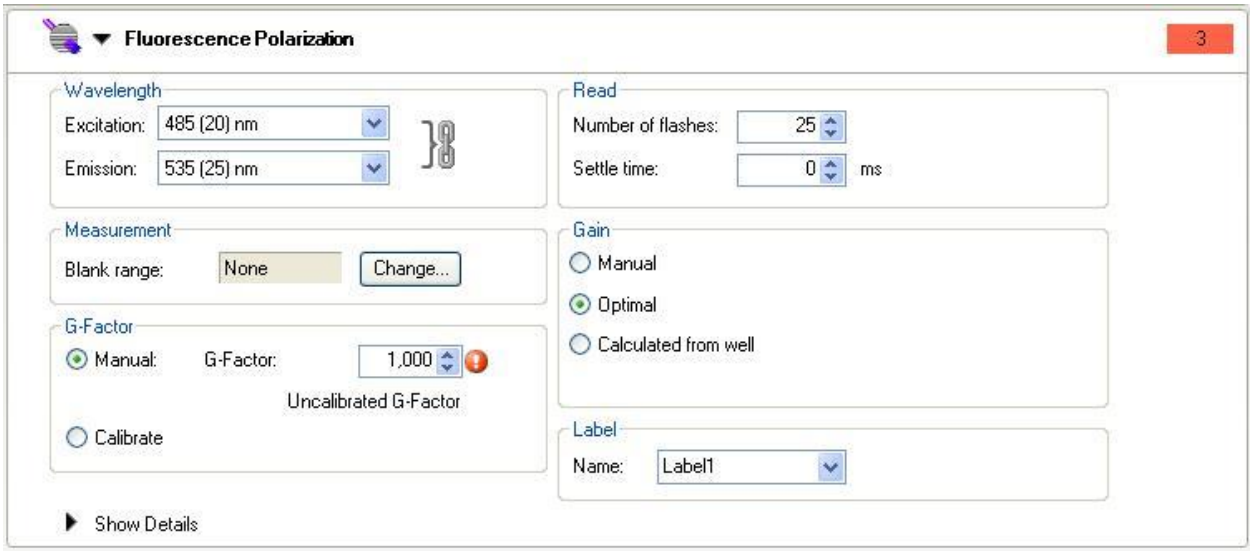
CAUTION

MAKE SURE THAT THE FILTER SLIDE CONTAINS POLARIZERS TOGETHER WITH THE FILTERS DEFINED FOR FLUORESCENCE POLARIZATION. MEASUREMENTS WITHOUT THE POLARIZERES WILL RESULT IN A FALSE G-FACTOR AND FALSE MEASUREMENT DATA.

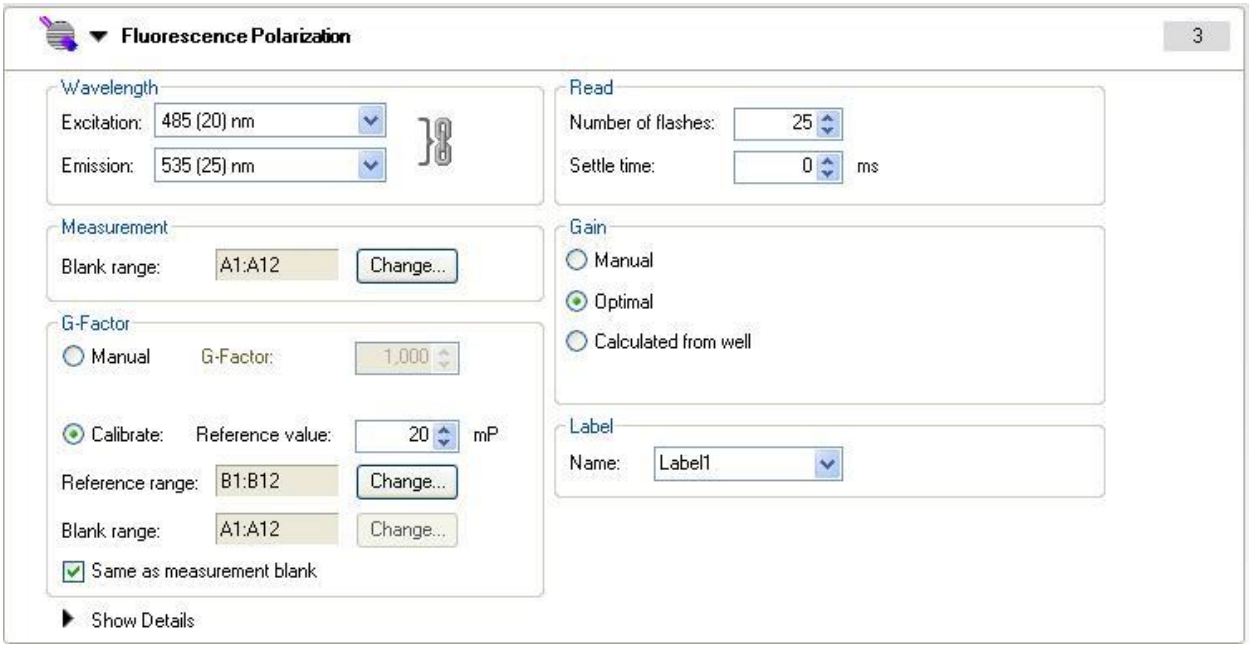
4.6.4 Measurement with an Uncalibrated G-Factor

If no calibrated G-factor is available, the default value of 1 will be displayed and marked as **Uncalibrated G-Factor**. To enable the measurement, confirm this value or select a new one by either clicking the up and down arrows or entering a value in the **G-Factor** field.

For the G-Factor calibration, see 4.6.5 Measurement with a Simultaneous G-Factor Calibration.



4.6.5 Measurement with a Simultaneous G-Factor Calibration



When **Calibrate** is selected, the G-factor is determined for the current measurement parameters and used for the following FP measurement. To perform the G-Factor calibration, please define:

Reference value: select a polarization value for the reference used, e.g., 20 mP for a 1 nM Fluorescein solution in 0.01 M NaOH.

Reference range: click **Change** and select the wells filled with the reference.

Blank range: click **Change** and select the wells filled with the reference blank. Select **Same as measurement blank** if the reference blank is the same as the measurement blank.

Note
By filling in more than one well with polarization references and reference blanks, the mean values will be calculated and therefore the calibration result will be more accurate.

G-Factor Storage

The calculated G-Factor is automatically stored on the computer's hard drive. Each G-Factor entry corresponds to the filter pair selection as well as the filter slide description. There is always only one G-Factor available for the respective filter pair combination and filter slide description, unless the same filter pair has been used with the different filter slides and thus stored with the different filter slide descriptions.

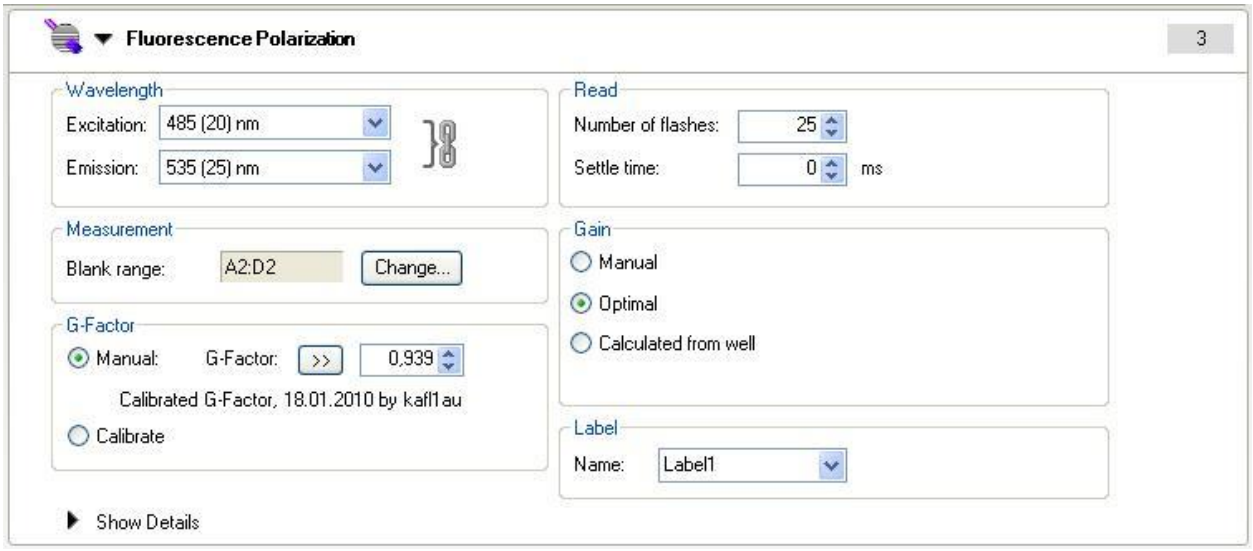
CAUTION

THE FILTER SLIDE DESCRIPTION IS PART OF THE G-FACTOR KEY VALUE. AVOID USING THE SAME FILTER SLIDE DESCRIPTION FOR DIFFERENT FILTER SLIDES AS THIS WILL AFFECT THE CORRECT G-FACTOR RECOGNITION.

4.6.6 Measurement with a Calibrated G-Factor

Note
Once calibrated, the G-factor is shown and can be used immediately if it matches the Ex/Em wavelength pair and the filter slide description.

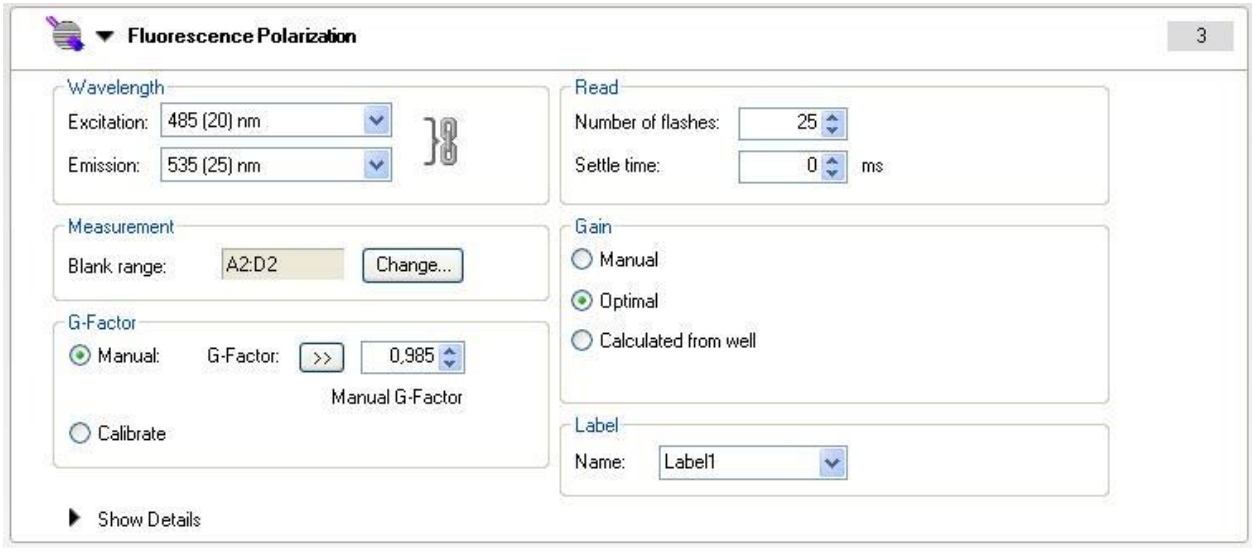
A calibrated G-factor will be displayed automatically or can be loaded by clicking the **>>** button only if it matches the selected fluorescence polarization filter pair and the filter slide description.



The calibrated G-Factor is marked as **Calibrated G-Factor** with date and signature.

4.6.7 Measurement with a Manual G-Factor

If the displayed G-Factor does not match the calibrated value (e.g., the G-Factor has been manually changed or loaded with a method), the corresponding value will be marked as **Manual G-Factor**.



The calibrated G-Factor can be restored by clicking the **>>** button on the left side of the displayed G-Factor.

Note
G-Factor adjustment via the >> button is only possible, if a calibrated G-Factor is available for the corresponding wavelength.

4.6.8 Calculation of Fluorescence Polarization Parameters

G-Factor:

$$G = \frac{(1+P_{ref})(\overline{RFU}^{\,cross}_{ref} - \overline{RFU}^{\,cross}_{buf})}{(1-P_{ref})(\overline{RFU}^{\,par}_{ref} - \overline{RFU}^{\,par}_{buf})}$$

P_{ref} ... *Polarization value of reference* [P]
$\overline{RFU}_{ref}$ ... *Averaged relative fluorescence units of reference*
$\overline{RFU}_{buf}$ ... *Averaged relative fluorescence units of buffer*

Blank Reduction:

The mean value of the respective blank wells is subtracted from each value.

$$\Delta RFU^{\,par} = \begin{cases} RFU^{\,par}_{ref} - \overline{RFU}^{\,par}_{buf} \\ RFU^{\,par}_{buf} - \overline{RFU}^{\,par}_{buf} \\ RFU^{\,par}_{smp} - \overline{RFU}^{\,par}_{blk} \\ RFU^{\,par}_{blk} - \overline{RFU}^{\,par}_{blk} \end{cases} \quad \textit{for each well}$$

$$\Delta RFU^{\,cross} = \begin{cases} RFU^{\,cross}_{ref} - \overline{RFU}^{\,cross}_{buf} \\ RFU^{\,cross}_{buf} - \overline{RFU}^{\,cross}_{buf} \\ RFU^{\,cross}_{smp} - \overline{RFU}^{\,cross}_{blk} \\ RFU^{\,cross}_{blk} - \overline{RFU}^{\,cross}_{blk} \end{cases} \quad \textit{for each well}$$

Intensities:

Parallel and perpendicular intensities are calculated using the following formulas:

$$I^{\,par} = G * \Delta RFU^{\,par}$$

$$I^{\,cross} = \Delta RFU^{\,cross}$$

Polarization:

$$P = \frac{I^{\,par} - I^{\,cross}}{I^{\,par} + I^{\,cross}}$$

Anisotropy:

$$A = \frac{I^{\,par} - I^{\,cross}}{I^{\,par} + 2 * I^{\,cross}}$$

Total Intensity:

$$I_{tot} = I^{\,par} + 2 * I^{\,cross}$$

4.7 Optimizing Absorbance Measurements

4.7.1 Measurement Parameters

Flash Settings

On the fly measurements with 1 flash (read) per well are possible for all plate types; however, measurement precision at low light levels depends on the reading time during which a fluorescence signal can be received.

Note
Increase the number of flashes (reads) per well until noise of BLANK wells does not further improve, or until measurement time per well becomes unacceptable.

Settle Time

A settle time before measuring a well may be set (critical for absorbance measurements). Due to the stop and go motion of the plate carrier the meniscus of the dispensed liquid may still vibrate while signal is integrated. This can give rise to fluctuations of the measured values. The effect has been observed in wells of 96-well plates and larger wells.

4.7.2 Absorbance Ratio Mode

Ratio Mode

Using the **Standard**-tab in i-control up to 4 labels may be measured well-wise. This measurement mode is called **ratio mode**. Be aware that no **ratio** calculation is performed after this measurement. The Excel result sheet shows the raw data. Further calculations have to be performed by the user.

Using the **Applications**-tab in i-control together with the NanoQuant Plate, the raw data for **Quantifying Nucleic Acids** and **Labeling Efficiency** are all automatically calculated for concentration or ratio-calculation by Excel software. The values can be used for further calculation if preferred.

Wavelength (Infinite M configurations)/Filter (Infinite F configurations) Switch Time

The Infinite F configurations can switch between two neighboring filters within 250 ms.

The Infinite M configurations can switch between two wavelengths in 150 ms. For conditions, see 4.5.3 FI Ratio Mode.

4.8 Multiple Reads per Well

The i-control software allows Multiple Reads per Well (MRW) to be performed in absorbance, fluorescence top and fluorescence bottom mode.

The Multiple Reads per Well functions can be activated on an absorbance or fluorescence intensity program strip by selecting the **Multiple Reads per Well** check box (see Figure 26 below).

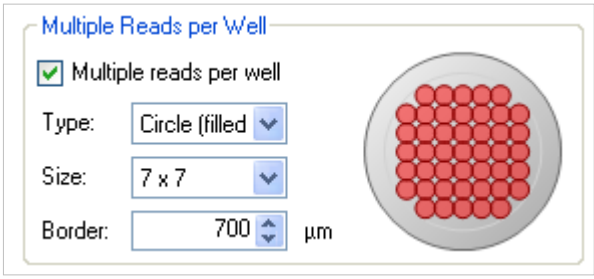


Figure 26: Multiple Reads per Well

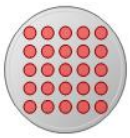
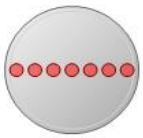
Note
The function Multiple Reads per Well is only available for the fixed wavelength reading modes absorbance, fluorescence intensity top and fluorescence intensity bottom. The function is not available for scan measurements.

4.8.1 MRW Type

The MRW types define the pattern how the measurement will be performed. The software allows seven different MRW types to be selected:

- Square
- Square (filled)
- Circle
- Circle (filled)
- X-line
- Y-line
- XY-line

Pattern examples:

Square: 	**Square (filled):** 
Circle: 	**Circle (filled):** 
X-line: 	**Y-line:** 
XY-line: 	

4.8.2 MRW Size

The MRW size determines the number of points to be measured in a well. Depending on the microplate type and instrument, Infinite F configurations or Infinite M configurations, the **size** is selectable from 1 x 1 to a maximum of 15 x 15 points. The diameter of the single measurement points corresponds to the theoretically calculated diameter of the light beam at the focal point (see Table 2).

Measurement Mode	**Infinite M** configurations	**Infinite F** configurations
Fluorescence Intensity Top	3 mm	2 mm
Fluorescence Intensity Bottom	2 mm	2 mm
Absorbance (microplate optics)	0.7 mm	0.5 mm

Table 2: Theoretically calculated beam diameter at the focal point.

The MRW type displayed in the software is therefore only a schematic overview of the measurement pattern. When measuring real samples, the pattern can vary, and the overlap of the single measurement points can be slightly different from the displayed pattern. It is therefore recommended to optimize the Multiple Reads per Well parameters for every new application.

4.8.3 MRW Border

In addition to **Size** and **Type**, a **Border** function allows the user to select a certain distance between light beam and the wall of the microplate well (distance in µm). As already stated in chapter 4.8.2 MRW Size, the software displays only a schematic overview of the measurement pattern. The border is calculated from the theoretical beam diameter of the instrument. However, when measuring liquid samples, the light beam diameter is influenced by the type and amount of liquid in a well.

In addition, the plate type (e.g., material of bottom of the microplate) also influences the characteristics of the light beam. Therefore, the theoretical border displayed in the software might not correspond to the actual border when measuring a real sample. It is therefore strongly recommended to optimize the **Multiple Reads per Well** parameters for every new application. Make sure that the selected border ensures sufficient distance between light beam and wall of the microplate well.

CAUTION

ALL ABSORBANCE AND FLUORESCENCE INTENSITY SPECIFICATIONS GIVEN IN THIS DOCUMENT ARE ONLY VALID FOR SINGLE POINT MEASUREMENTS (ONE MEASUREMENT POINT PER WELL). WHEN USING THE MULTIPLE READS PER WELL OPTION, THE SPECIFICATIONS ARE NOT VALID.

CAUTION

THE SOFTWARE DISPLAYS ONLY A SCHEMATIC VIEW OF THE MEASUREMENT PATTERN. THEREFORE, OPTIMIZE THE MULTIPLE READS PER WELL PARAMETERS FOR EVERY NEW APPLICATION. MAKE SURE THAT THE SELECTED BORDER IS SUFFICIENT TO AVOID AN OVERLAP BETWEEN THE LIGHT BEAM AND THE WELL WALL OF THE MICROPLATE.

CAUTION

A BORDER VALUE THAT IS TOO SMALL MAY CAUSE WRONG MEASUREMENT RESULTS DUE TO OVERLAP BETWEEN THE LIGHT BEAM AND THE WELL WALL OF THE MICROPLATE.

4.8.4 Result Display in MS Excel

The MS Excel result sheet generated by the i-control software displays a schematic graphical overview (**Multiple Reads per Well – Alignment**; see Figure 28) of the measurement points. A number is assigned to each measurement point. The results are presented in list form: number of measurement point versus result value (OD or RFU; see Figure 27: Alignment graphic (XY-Line, 3 x 3) for result of a fluorescence measurement). In addition, the standard deviation (**Stdev**) and the average value (**Mean**) of the measurement points/well are also displayed:

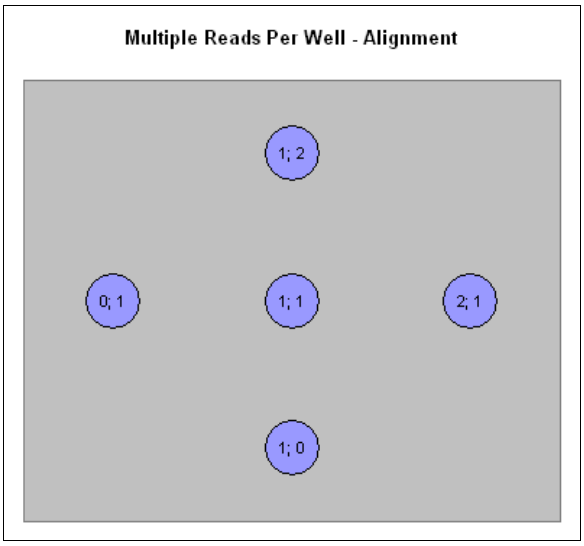


Figure 27: Alignment graphic (XY-Line, 3 x 3)

Well	Mean	StDev	1;2	2;1	1;1	0;1	1;0
A1	30	4	26	35	29	27	31
A2	28	3	28	31	23	28	30
A3	28	6	31	31	27	18	32
B1	33	5	29	35	30	41	30
B2	36	4	40	36	30	37	35
B3	32	8	30	41	22	29	39
C1	30	6	28	35	21	31	36
C2	35	5	30	36	31	37	41
C3	38	7	40	41	25	40	41

Figure 28: Example of MS Excel result list generated by i-control.

4.8.5 Miscellaneous Software Features of MRW

MRW is only available for the measurement modes **Absorbance**, **Fluorescence Intensity Top** and **Fluorescence Intensity Bottom**.

The MRW feature is not active when performing well-wise measurements.

Reference Wavelength (located on the absorbance strip) is not available in combination with **Multiple Reads per Well**.

4.9 Optimizing Luminescence Measurements

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT. SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

4.9.1 Integration Time

At very low light levels, a PMT does not yield a continuous output current, which is necessary for a reliable analog to digital conversion. Instead, it produces a sequence of pulses the average rate of which can be measured using a counter. The advantage of the photon counting technique at such low light levels is that pulse height selection criteria allow electronic noise to be discriminated.

At very low light levels the measured counts per second are proportional to the light intensity. Increase of measurement time per well yields more accurate values because of the irregular photon impact (photon statistics). The photonic noise (shot noise) cannot be reduced technically.

Note

The signal to noise (S/N) ratio can be optimized by increasing the integration time. Increasing the integration time by a factor of 10 results in an improvement of the S/N ratio by app. a factor of 3.

4.9.2 Light Level Attenuation

When using photon counting detection, optical attenuation of higher luminescence light levels (>10,000,000 counts per second) is necessary. In such a case, too many photons enter the luminescence detector at one time and cannot be distinguished as distinct exit pulses. Count rates would even fall behind values at lower light levels.

Therefore, values >10,000,000 counts per second (without attenuation) are displayed as **INVALID** in the results sheet.

The optical luminescence system of the **Infinite** reader can attenuate light levels by a fixed factor of either 1 (none) or 100 (2 OD). Correspondingly, the usable measurement range will be shifted to higher light levels (<1,000,000,000 counts per second).

4.10 Measurements with Injectors

4.10.1 Priming and Washing of the Infinite Reader

CAUTION

THE INJECTOR CARRIER MUST BE IN THE SERVICE POSITION FOR WASHING UND PRIMING.

PRIME AND WASH MUST NOT BE PERFORMED WHEN THE INJECTOR IS IN THE INSTRUMENT!

The initial filling step of the injector system (priming) as well as the cleaning step of the injector system (washing) must take place outside of the instrument.

For these procedures, the injector carrier is removed from instrument and put into the service position of the injector box.

Prime/Wash buttons for injectors A and B

*Figure 29: Injector-box with injector in **service position**; the injectors are removed from the carrier slot and inserted into the holder of the injector carrier system.*

For priming and washing 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 i-control software.

The prime volume depends on the tubing length. Two types of injector tubing are available: **long**: 105 cm, and **short**: 80 cm.

The minimal priming volume is 700 µl for an injector with short tubing and 850 µl for an injector with long tubing.

CAUTION

DO NOT TOUCH THE INJECTOR NEEDLES. THEY CAN BECOME EASILY BENT OR MISALIGNED, WHICH CAN CAUSE INJECTION PROBLEMS OR DAMAGE THE INSTRUMENT.

IF THE INJECTOR CARRIER IS NOT INSERTED CORRECTLY IN THE INJECTOR PORT, THE INJECTOR SENSOR DOES NOT DETECT THE INSERTED INJECTOR AND THEREFORE WASHING AND PRIMING IS ENABLED WHICH CAN DAMAGE THE INSTRUMENT. IN ADDITION TO THIS, THE ACTIONS DISPENSE AND INJECT WILL NOT BE POSSIBLE.

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.

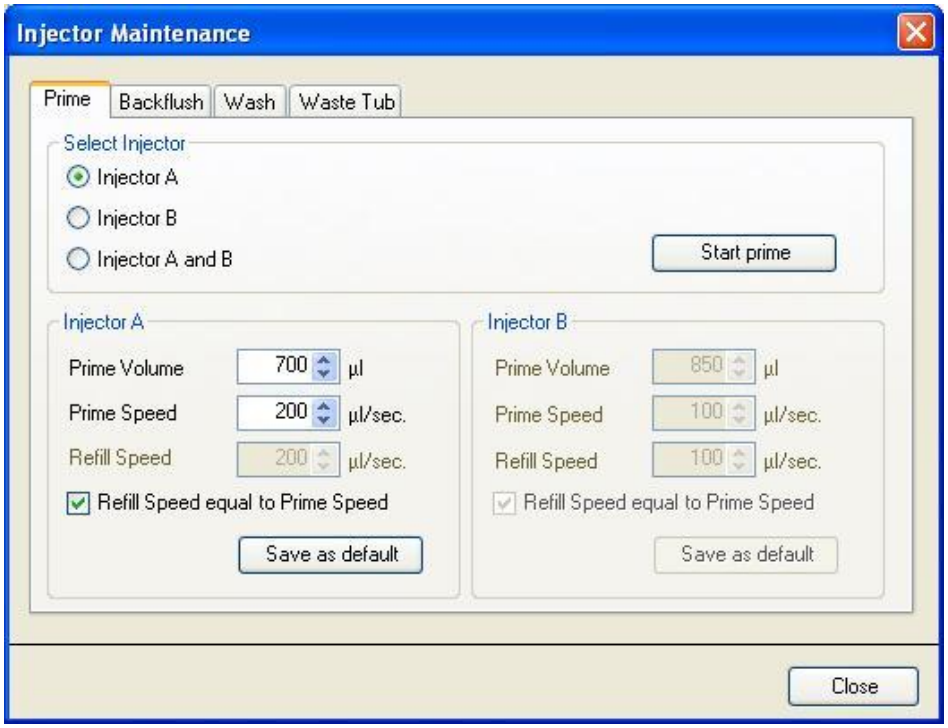
It is recommended to perform a washing step before priming.

Priming can be performed by using the i-control software or by using the hardware buttons on the injector box:

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 carrier slot and insert it into the service position of the injector box.
3. Put an empty container under the injector.

Priming Procedure (i-control):

1. Adjust parameters on the **Prime tab** of the **Injector Maintenance** dialog box in the **Settings menu**.
2. Start the priming procedure by clicking **Start prime** in the Injector Maintenance dialog box.
3. Visually inspect the syringes for air bubbles. Any bubbles should be removed after priming to ensure good injection performance.



4. Select one of the injectors **Injector A** or **Injector B** or **Injector A and B**.
5. Select the **Prime Volume** (700 -60000 µl – short tube)
   (850 -60000 µl – long tube)
6. Select the **Prime Speed** (100 - 300 µl/sec)
7. Select the **Refill Speed** (100 – 300 µl/sec.) or select **Refill Speed equal to Prime Speed**
8. Start prime by clicking the **Start prime** button.

9. Click the **Save as default** button to save the selected settings to the corresponding hardware button (A or B) on the injector box. When using the hardware buttons for priming, these settings will be applied.
10. Select **Close** to exit the dialog box

Priming Procedure (Hardware Button):

Priming can also be performed without using the software. Priming parameters can be stored on the injector by clicking **Save as Default** on the **Prime** tab of the **Injector Maintenance** dialog box of the i-control software (in the **Settings** menu, click **Injectors...** and the **Injector Maintenance** dialog box appears). Press the **Prime/Wash** button on the injector box to start the priming sequence using the default parameters, (see Figure 29: Injector-box with **injector in service position**, page 79). The injector must be connected **to** the instrument and the instrument must be switched on. Start the prime procedure by pressing the **Prime/Wash** button for less than 3 seconds.

Visually inspect the syringes for air bubbles. Any bubbles should be removed after priming to ensure good injection performance.

After a successful priming procedure, reinsert the injector into the instrument. Close the lid of the pump module completely before starting a measurement. The injectors are now ready to use.

When starting a measurement with the actions **injection** or **dispense**, 5 µl of liquid are dispensed into a disposable container on the plate carrier before starting **injection** or **dispense**. This initial dispense step makes sure that the injection/dispense conditions are equal for each well.

CAUTION
CLOSE THE LID OF THE PUMP MODULE (INJECTOR BOX) COMPLETELY BEFORE STARTING A MEASUREMENT.

Reagent Backflush

The dead volume of the injection system (injector needles, syringes, valves and tubing) is approximately 100 µl for each syringe after the backflush procedure has been performed. The function of backflush is to return any unused reagent to the reservoir bottles.

The injection speed can be adjusted via the software to allow for good mixing of reagents. The optimal injection speed depends on the assay parameters, such as viscosity of fluids, the plate format, and the measuring behavior of the liquids.

Reagent backflush allows reagents in the tubing system to be pumped back into storage bottles. This action can be performed optionally prior to washing the injector system to minimize the dead volume.

Before performing the **Backflush** procedure:

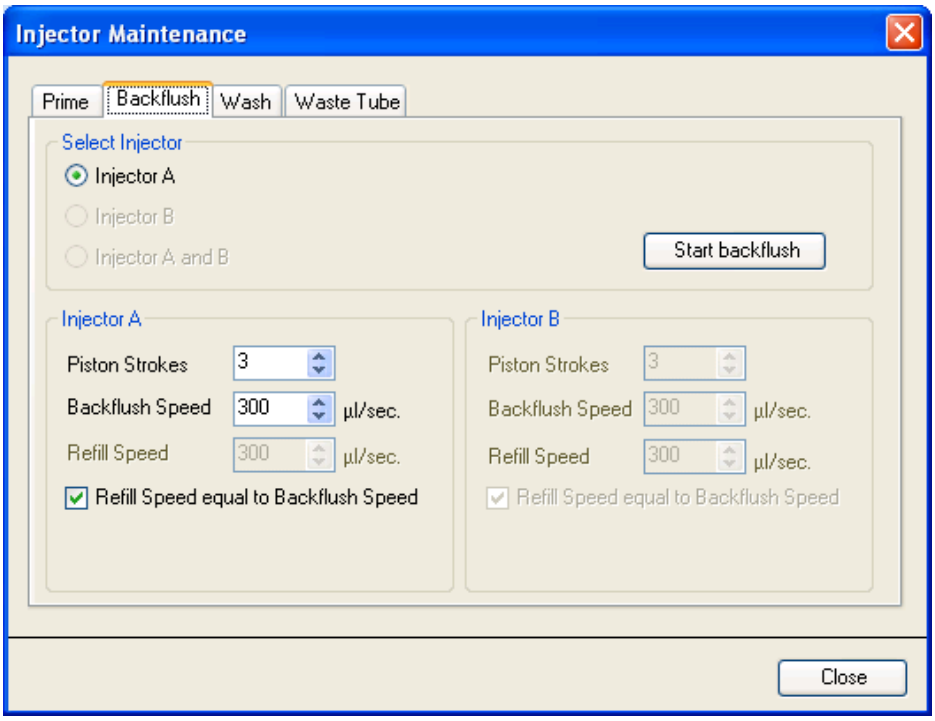
Remove the injector carrier from the instrument and insert the injector carrier into the service position of the injector box.

Insert the feeding tubing into the appropriate storage bottle.

Backflush Procedure (i-control):

Adjust parameters on the **Backflush** tab of the **Injector Maintenance** dialog box in the **Settings** menu.

Start the reagent backflush procedure by clicking **Start backflush**.



1. Select one of the injectors **Injector A** or **Injector B** or **Injector A and B** (only **primed** injectors are available for **backflush**).
2. Select the **Piston Strokes** (1 – 60; 1 stroke equals 1 ml)
3. Select the **Backflush Speed** (100 - 300 µl/sec)
4. Select the **Refill Speed** (100 – 300 µl/sec.) or select **Refill Speed equal to Backflush Speed**
5. Click **Start backflush** to start the reagent backflush procedure.
6. Click **Close** to exit the dialog box.

CAUTION

THE INJECTOR CARRIER MUST BE IN THE SERVICE POSITION FOR THE ACTION BACKFLUSH.

DO NOT PERFORM BACKFLUSH WHEN THE INJECTOR IS IN THE INSTRUMENT!

4.10.2 Washing

Before the instrument is switched off, it is recommended to perform a wash procedure to clean the injector system.

Washing can be performed by using the i-control software or by using the hardware buttons on the injector box.

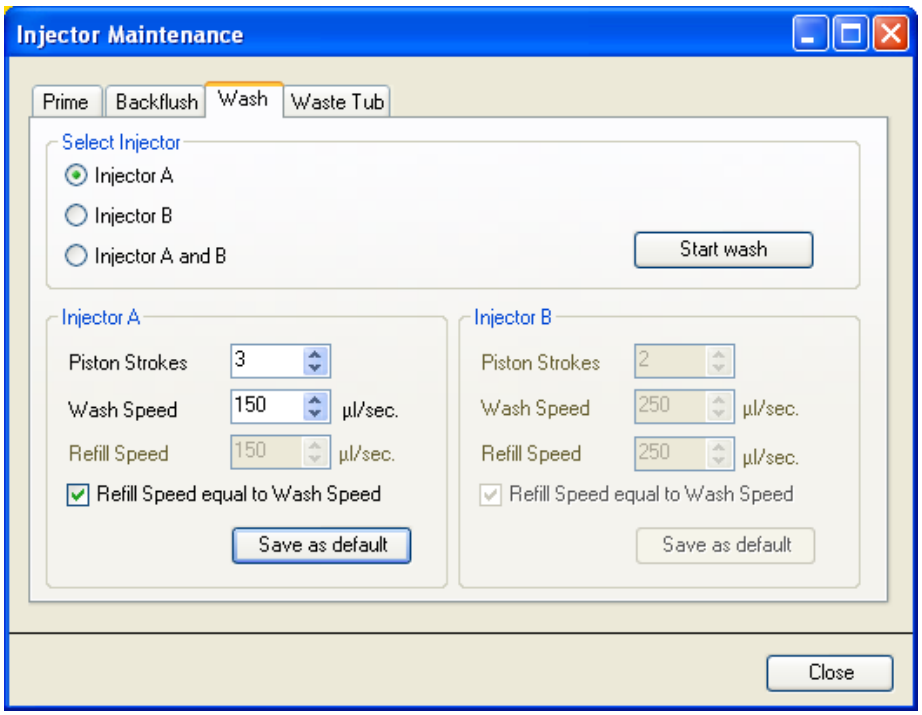
Before performing the washing procedure:

1. Fill the storage bottles with the appropriate wash reagents (distilled water, 70 % ethanol, etc…) and insert the feeding tubes. Make sure, that the tubes reach the bottom of the bottle.
2. Remove the injector from the carrier slot and insert it into the service position of the injector box.
3. Put an empty container under the injector.

Washing Procedure (i-control):

Adjust the parameters on the **Wash** tab of the **Injector Maintenance** dialog box in the **Settings** menu.

1. Start the washing procedure by clicking the **Start wash** botton.



2. Select one of the injectors **Injector A** or **Injector B** or **Injector A and B**.
3. Select the **Piston Strokes** (1 – 60; 1 stroke equals 1 ml)
4. Select the **Wash Speed** (100 - 300 µl/sec)
5. Select the **Refill Speed** (100 – 300 µl/sec.) or select **Refill Speed equal to Wash Speed**
6. Click **Start wash** to start the wash procedure.
7. Click **Close** to exit the dialog box.

Washing Procedure (Hardware Buttons):

Washing can also be performed without using the software. Washing parameters can be stored on the injector by clicking **Save as Default** on the **Wash** tab of the **Injector Maintenance** dialog box (in the **Settings** menu, click **Injectors...** and the **Injector Maintenance** dialog box appears) of the i-control software. Press the **Prime/Wash** button on the injector box to start the washing sequence using the default parameters. (See Figure 29: Injector-box with injector in **service position**, page 79). The injector must be connected **to** the instrument and the instrument must be switched on. Start the wash procedure by pressing and holding the Prime/Wash button for more than 3 seconds.

CAUTION

THE INJECTOR CARRIER MUST BE IN THE SERVICE POSITION FOR THE ACTION WASH.

DO NOT PERFORM WASHING WHEN THE INJECTOR IS IN THE INSTRUMENT!

CAUTION

BE SURE TO RUN A FINAL WASH PROCEDURE WITH DISTILLED WATER AND EMPTY THE INJECTOR SYSTEM. FOR GOOD CARE AND LIFETIME FILL THE INJECTOR SYSTEM WITH LIQUID (WATER) BEFORE TURNING OFF THE INSTRUMENT.

CAUTION

PLEASE SEE THE CORRESPONDING REAGENT KIT FOR ADVICE ON HOW TO REMOVE THE SUBSTRATE COMPLETELY FROM THE TUBING SYSTEM.

CAUTION

TAKE GOOD CARE OF THE INJECTORS, BECAUSE IF THEY ARE DAMAGED THE ACCURACY OF DISPENSING MAY BE AFFECTED. THIS CAN RESULT IN DAMAGE TO THE INSTRUMENT.

Note
Injector needles can be replaced by replacing the injector carrier together with the corresponding tubing.

CAUTION

THE BUTTON(S) ON THE INJECTOR BOX INCLUDE TWO FUNCTIONS:

- **PRESS THE BUTTON FOR LESS THAN 3 SECONDS TO START PRIME.**
- **PRESS THE BUTTON FOR MORE THAN 3 SECONDS TO START WASH.**

THE PARAMETERS HAVE TO BE SET IN THE I-CONTROL SOFTWARE.

Waste Tub

When starting a measurement with the actions **injection** or **dispense**, 5 µl of liquid are dispensed into a disposable container on the plate carrier before starting **injection** or **dispense**.

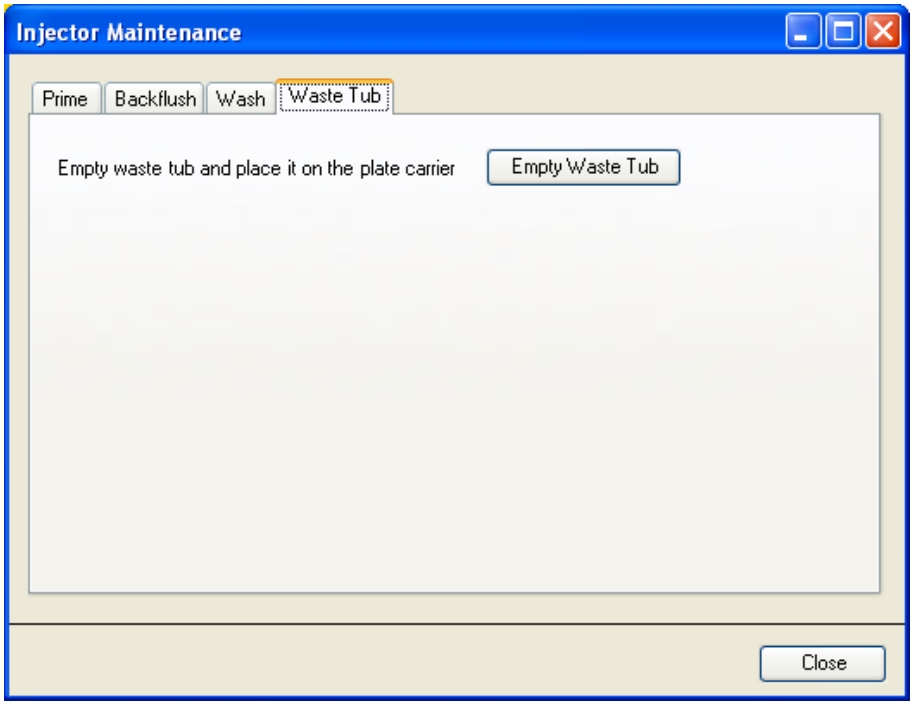
This initial dispense step makes sure that the injection/dispense conditions are equal for each well. This special dispense step depends on the selected refill mode selected on the injector or dispense strip (see chapter 4.10.4 Injector for details).

When using **standard** refill mode, the dispense step is performed after each refill. When using r**efill for every injection** the dispense step is only performed once when starting the measurement.

The disposable waste container (waste tub) must therefore be emptied from time to time. The maximum filling volume is 1.5 ml. An internal counter checks the dispensed liquid volumes; the software alerts the user when it is time to empty the waste tub.

Figure 30: Waste tub on plate carrier

Empty Waste Tub Procedure (i-control):



Click the **Empty Waste tub** button and the plate carrier will move out automatically. Remove the waste tub and empty the contents. After the waste tub has been emptied place it back on the plate carrier. The i-control software will alert you when the waste tub needs to be emptied again.

CAUTION

PLACE THE WASTE TUB ON THE PLATE TRANSPORT BEFORE STARTING A MEASUREMENT WITH THE ACTIONS INJECTION AND/OR DISPENSE.

CAUTION

IT IS RECOMMENDED TO EMPTY THE WASTE TUB BEFORE STARTING A MEASUREMENT AND TO EMPTY IT AT LEAST ONCE A DAY.

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESSES RUN ON THE INFINITE READER.

TREAT THE USED MICROPLATE, 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.

4.10.3 Before Starting a Measurement with Injectors

Before starting a measurement make sure that:

- The tubes are clean. If not please refer to chapter 4.10.1 Priming and Washing of the Infinite Reader for details how to clean the injector system.
- The injector tubes are correctly inserted into the storage bottles and fixed.
- The injector system is primed. It is not possible to start a measurement without priming the system.

When priming the system:

- Check the tubes for leaks, visually or using a nonhazardous fluid, before priming with valuable reagents.
- Check the tubes for kinks, visually or using a nonhazardous fluid, before priming with valuable reagents.
- Make sure that the injector needles are not twisted.
- If the tubes require replacement for any reason, after the tubes have been changed do not forget to perform washing and priming before starting a measurement.

4.10.4 Injector Modes (i-control)

When using the injector, two modes are available:

Dispense: The dispense mode allows liquid to be dispensed plate-wise into the selected wells

Injection: This mode must be used in combination with a measurement strip. The injection is performed in a well-wise mode.

Dispense Mode

The dispense settings can be adjusted via the software:

Dispense	**Select Injector:** Injector A and/or Injector B can be selected. **Speed:** The injection speed is selectable from 100 – 300 µl/sec for each injector. Select **Refill speed** from 100 – 300 µl/sec. for each injector or select **Refill Speed equal to Dispense Speed**. Select refill mode **Standard**, if refill should be performed when syringe is empty (multiple dispense steps are performed before refilling, refill occurs after dispensing approx. 800 µl). Select **Refill for every dispense** if refill should be performed for every dispense step.

Using the Dispense Strip

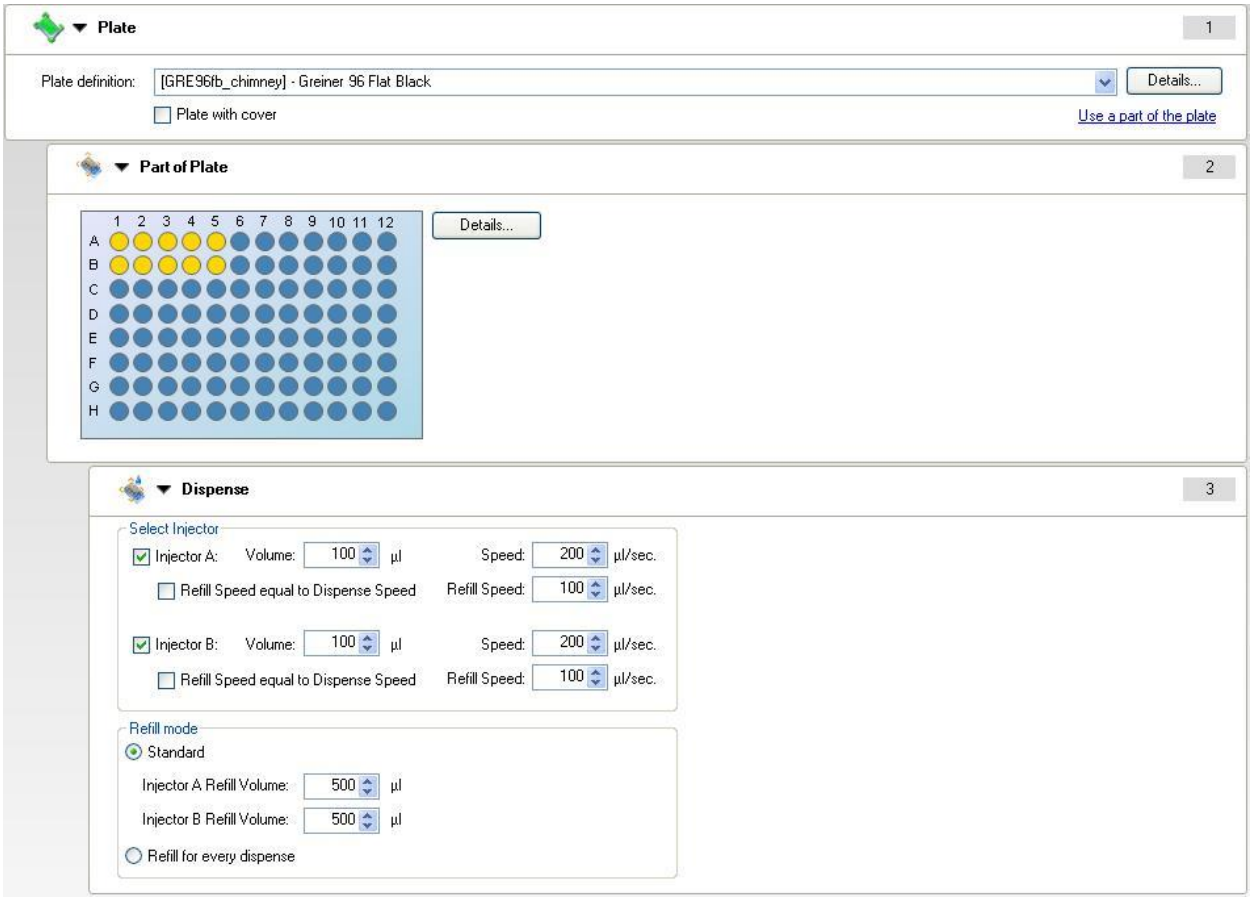
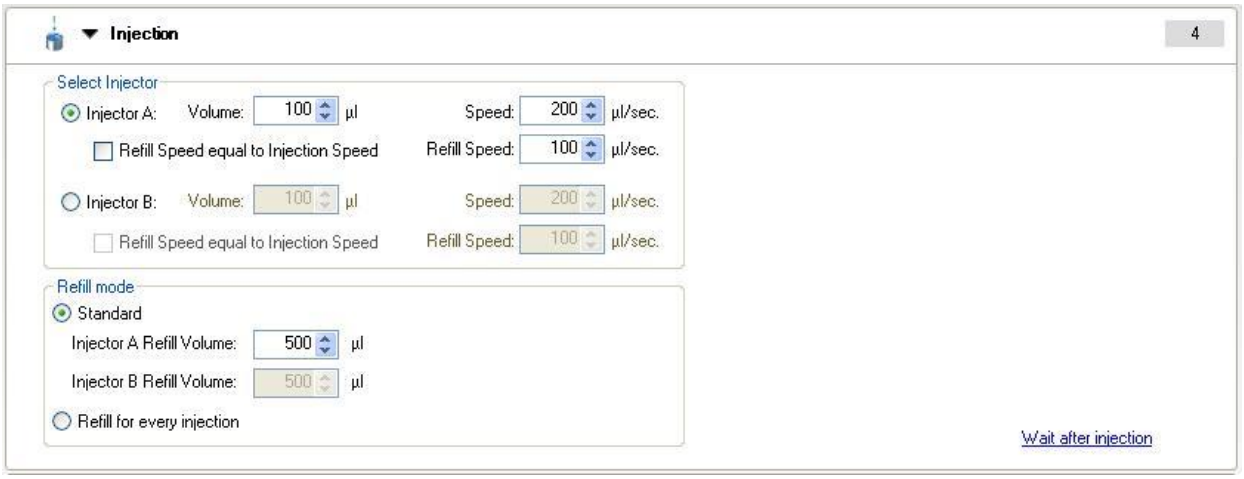


Plate	Select an appropriate plate type
Part of the plate	Optional; Select the wells to be dispensed
Dispense	Set up the dispense parameters. If both injectors are selected, all wells are first dispensed with injector A and then with injector B. The dispense strip does not require an additional measurement strip.
Dispense volume	The injection volume depends on the microplate type. The plate definition files include a so-called working volume. This working volume defines the maximum volume to be dispensed into the selected microplate. Therefore, always make sure that the selected plate definition file contains the correct setting for the working volume. The maximum dispense volume is 800 µl/dispense strip. If volumes greater than 800 µl are to be dispensed (e.g., into 6-well plates), more than one dispense strip must be used.

Injection Mode

The injection settings can be adjusted via the software:



Injection	Select Injector: Injector A or Injector B can be selected. It is not possible to select both injectors on one strip. If a measurement with two injectors is to be performed, two injector strips are necessary. **Speed:** The injection speed is selectable from 100 – 300 µl/sec for each injector. Select a **Refill speed** from 100 – 300 µl/sec. for each injector or check the **Refill Speed equal to Injection Speed** box. Select refill mode **Standard** if refill should be performed when syringe is empty (multiple injection steps are performed before refilling, refill occurs after dispensing approx. 800 µl). Select **Refill for every injection** if refill should be performed for every injection step.
Injection volume	The injection volume depends on the microplate type. The plate definition files include a so-called working volume. This working volume defines the maximum volume to be injected into the selected microplate. Therefore, always make sure that the selected plate definition file contains a correct setting for the working volume. The maximum injection volume is 800 µl/injection strip. If volumes greater than 800 µl are to be injected (e.g., into 6-well plates), more than one injection strip must be used.

Using the Injection Strip

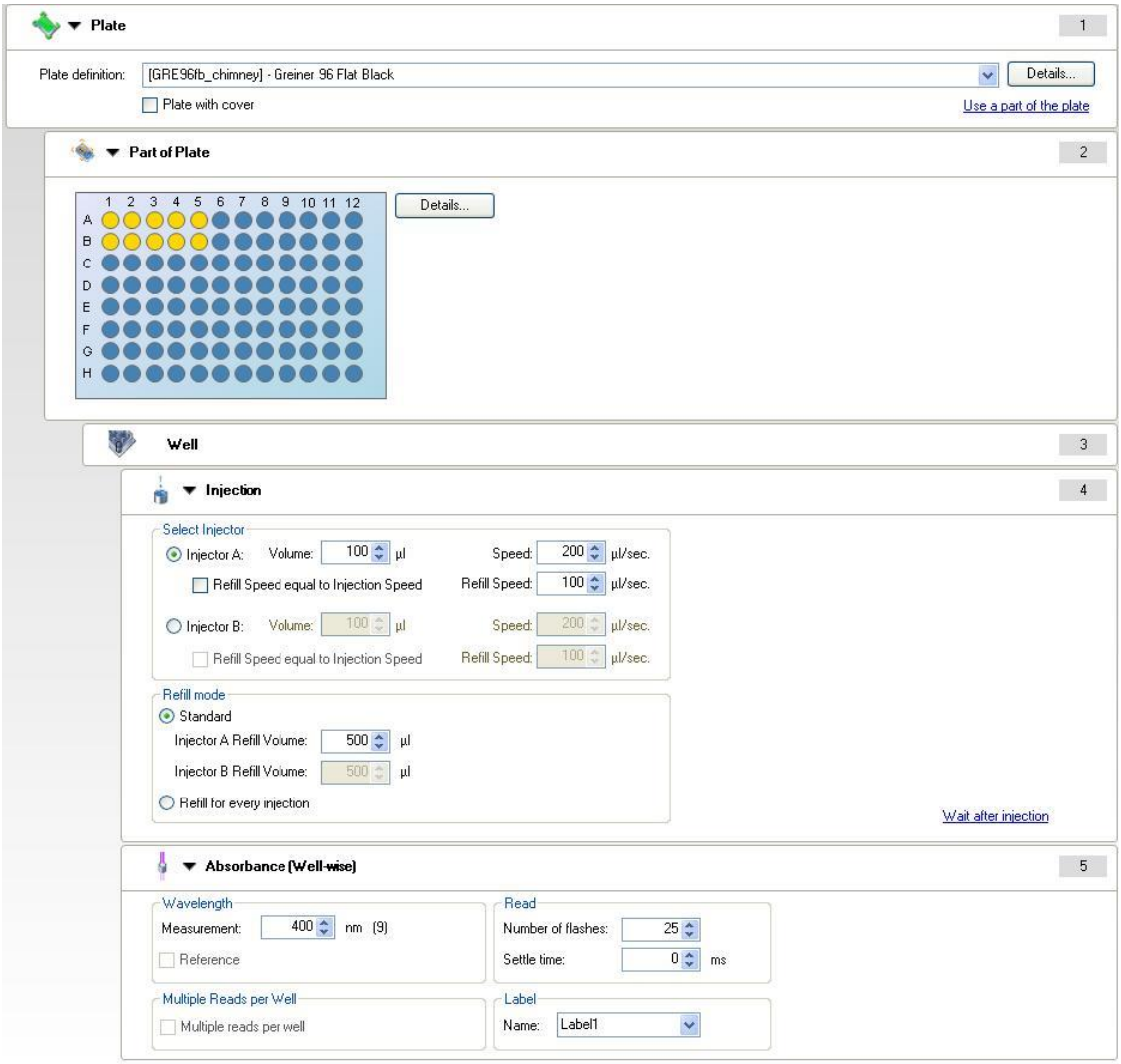
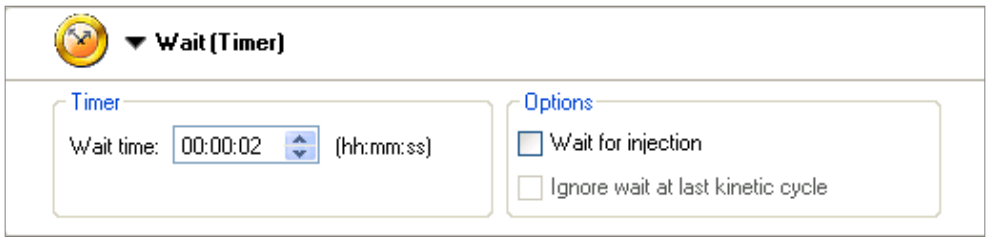


Plate	Select an appropriate plate type.
Part of the plate	Optional; Select the wells to be dispensed
Well	The well strip is mandatory. Injection is only possible with a **well** strip. This strip ensures that the following indented strips are performed well-wise.
Injection	Set up the injection parameters. Only one injector can be selected per strip. If both injectors are required or one injector will perform two injections, an additional injection strip must be inserted.
Measurement strip (Example Absorbance)	It is mandatory to use at least one measurement strip in combination with the injection strip. The position of the measurement strip(s) (before and/or after the injection strip) depends on the application and is therefore user selectable.

Note
Make sure that the corresponding Working Volume value in your plate definition file is higher than the volume used for injection.

Wait Strip

A **Wait timer** (delay or settle time) strip can be inserted into the procedure.



Wait time	Select a time in hh:mm:ss from 00:00:01 up to 23:59:59
Options	If **Wait for injection** is sele**cted, the wait time includes the injection time.** **If Wait for injection is** NOT selected, the wait time is added to the injection time.

4.11 Blanking Measurements

The software allows a so-called **Blanking** measurement. **Blanking** in the **Instrument** menu is only available when a measurement script containing a cuvette measurement is open. When **Blanking** is selected in the **Instrument** menu, an absorbance measurement with the cuvette port is activated according to the parameters (wavelength, flash number, settle time) of the active script. The user is requested to insert the blank cuvette (e.g., containing buffer solution) and to start the measurement. The blank data are then written into an Excel spreadsheet. The data are also stored in the software and can be applied to the following cuvette measurements performed with the same parameters. The blank data are automatically subtracted when the check box **Apply Blanking** is selected in the **Absorbance** or **Absorbance Scan** strip.

The blank data are stored in the software if no other blanking measurement is performed, or the software is closed. Be aware that the stored blanking data will be overwritten without a warning message if another blanking measurement is started. The stored blanking data will also be deleted without a warning message when closing the software.

CAUTION

BLANKING DATA WILL BE OVERWRITTEN WITHOUT A WARNING MESSAGE WHEN STARTING ANOTHER BLANKING MEASUREMENT. BLANKING DATA WILL BE DELETED WITHOUT A WARNING MESSAGE WHEN CLOSING I-CONTROL SOFTWARE.

4.12 Cuvette Measurements

4.12.1 Cuvette Strip

For performing cuvette measurements, a **Cuvette** strip is necessary

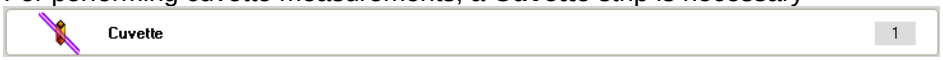


Figure 31: Cuvette strip

For a few applications it might be necessary to combine a microplate measurement with a cuvette measurement. The i-control software therefore allows the usage of one cuvette strip and one plate strip within one measurement script. The cuvette measurement must be positioned before the microplate measurement. To perform an accurate microplate measurement, the cuvette door must not be open. The software therefore does not allow the user to use a **Move cuvette OUT** strip before the microplate measurement (see also chapter 4.12.3 i-control Cuvette Examples).

4.12.2 Cuvette Movements

The cuvette can be moved in and out with the **cuvette in** and **cuvette out** buttons or by selecting **Cuvette in/Cuvette out** in the **Instrument/Movements** dialog box.

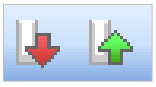


*Figure 32: Cuvette **out** and **in** button*

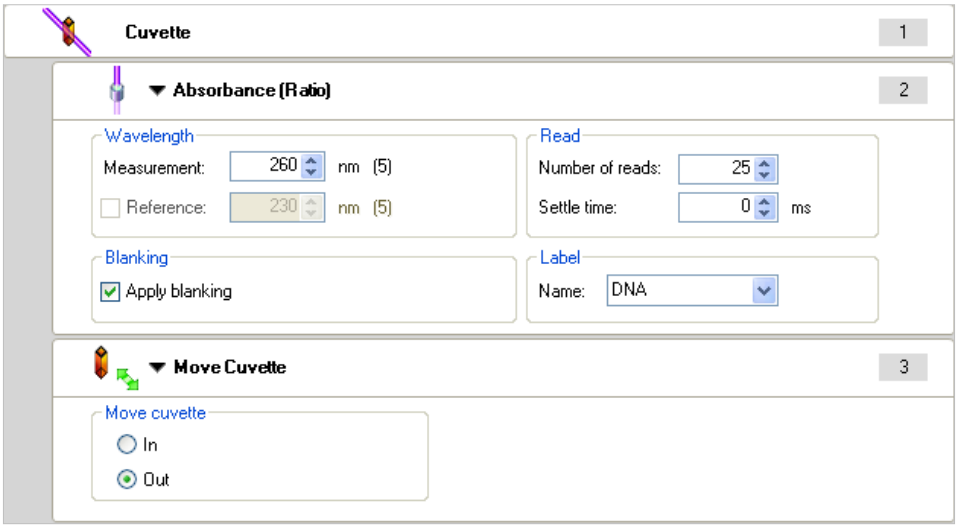
4.12.3 i-control Cuvette Examples

Example 1:

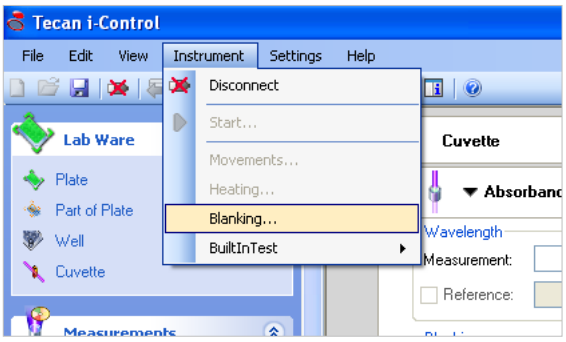
Example of how to use the**Blanking** measurement when measuring a DNA sample:

Prepare cuvette with sample buffer

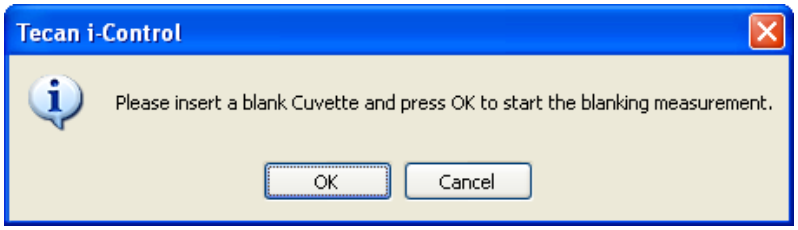
Set up the DNA measurement in the i-control software:



Select **Blanking** from the **Instrument** menu:

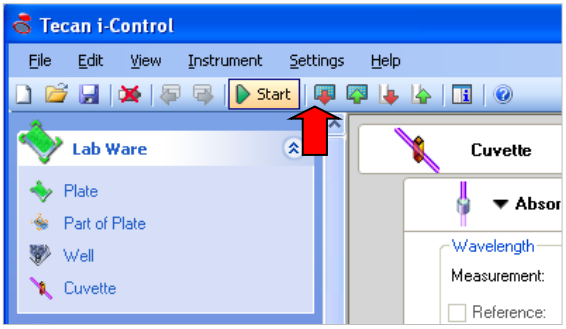


The instrument is initialized, and the cuvette holder moves out. The user is requested to insert the blank cuvette:



Insert the blank cuvette and click **OK** to start the blank measurement. The measured blank data are displayed in an Excel spreadsheet. The cuvette holder moves out.

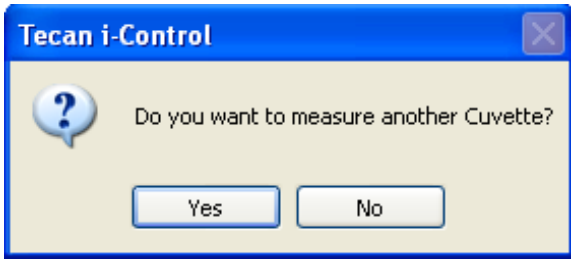
Remove and blank cuvette. Prepare sample cuvette and put it on the cuvette holder. Start the measurement by clicking **Start**:



The cuvette holder is moved in and the measurement is performed. The measured data (Value) as well as the blank data (Blank) and the blanked data (Diff) are displayed in an Excel spreadsheet:
Example for data display when measuring two cuvettes:

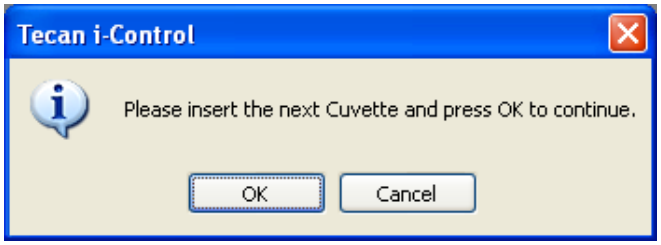
Info:	Blank data from: 20.03.2006 15:34:31				
	Value	Blank	Diff		
Cuv: 1	1.032	0.054	0.978		
Cuv: 2	1.409	0.054	1.355		

After finishing the measurement of the first cuvette (Cuv: 1) the following message is displayed:



Click **No** to finish the measurement.

Click **Yes** to continue:

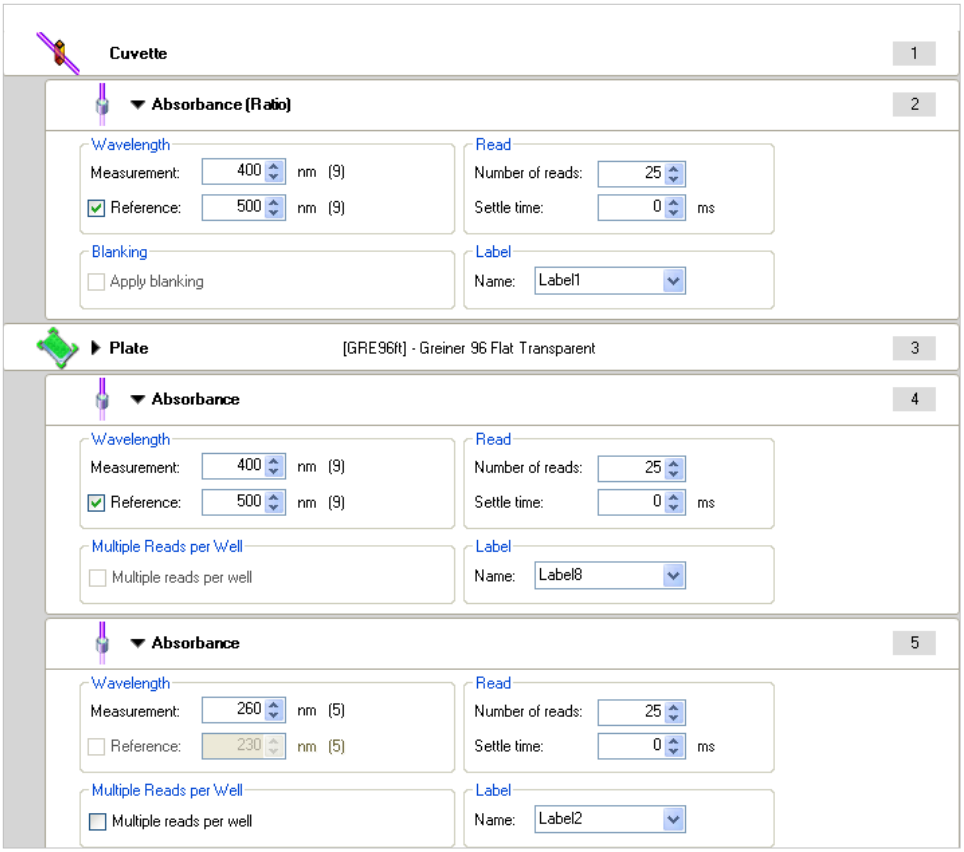


Insert the next sample cuvette and click **OK** to continue the measurement.

Example 2:

Combination of microplate and cuvette measurement:

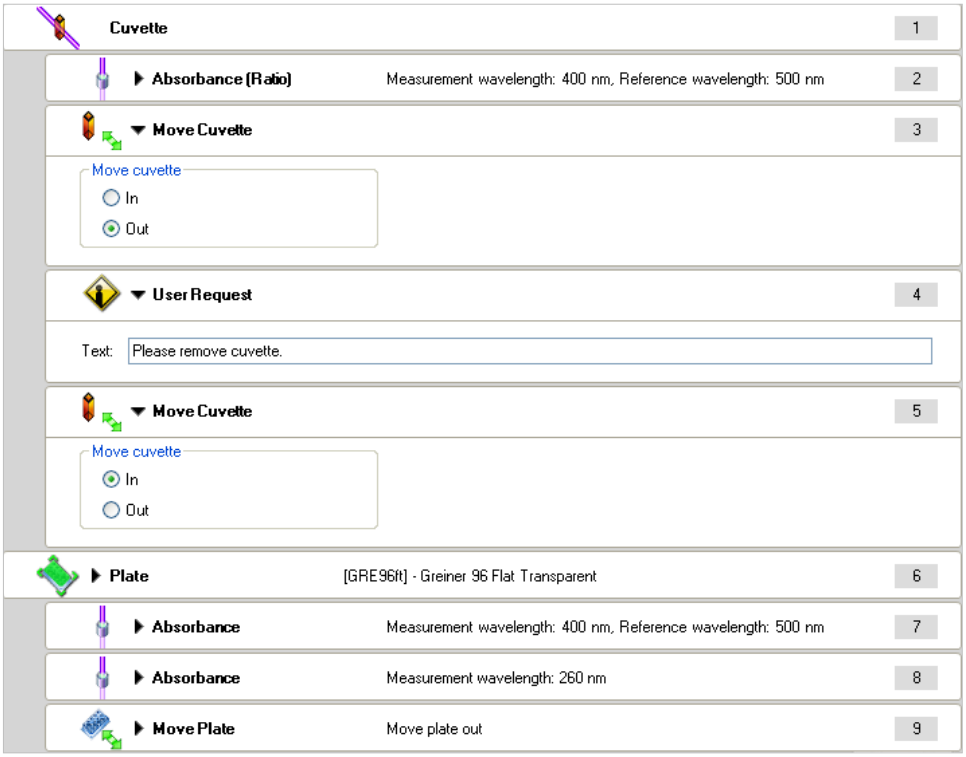
For some applications it might be necessary to compare data measured on a microplate with cuvette data. The following example shows how to set up this measurement in general:



Cuvette	Necessary for cuvette measurements.
Absorbance strip (cuvette)	Up to 4 absorbance fixed wavelength strips are allowed. Reference wavelength is only selectable when using one absorbance fixed wavelength strip. **Apply blanking** is disabled when a reference wavelength is selected. Select the appropriate measurement parameters (wavelength, number of flashes and settle time)
Plate	Necessary for microplate measurements. Select an appropriate plate type for the measurement.
Part of Plate (not shown)	Optional. Use the **part of plate** strip if only a part of the plate shall be measured.
Absorbance strip (microplate)	Up to 10 absorbance fixed wavelength strips are allowed. Reference wavelength is only allowed on the first absorbance strip. Reference wavelength is disabled on absorbance strips 2 to 10. Select the appropriate measurement parameters (wavelength, number of flashes and settle time) for your application.

Example 3:

Usage of **Move Cuvette OUT** strip when measuring a combination of microplate and cuvette:



Cuvette	Necessary for cuvette measurement
Absorbance strip (cuvette)	Up to 4 absorbance fixed wavelength strips are allowed. Reference wavelength is only selectable when using one absorbance fixed wavelength strip. **Apply blanking** is disabled when reference wavelength is selected. Select the appropriate measurement parameters (wavelength, number of flashes and settle time)
Move Cuvette (Out)	The cuvette holder is moved **out**.
User Request	The user request interrupts the measurement and therefore allows removing the cuvette from the cuvette port. When confirming the request, the measurement continues.
Move Cuvette (In)	The cuvette port is moved in.
Plate	Necessary for microplate measurements. Select an appropriate plate type for the measurement.
Part of Plate (not shown)	Optional. Use the **part of plate** strip if only a part of the plate will be measured.
Absorbance strip (microplate)	Up to 10 absorbance fixed wavelength strips are allowed. Reference wavelength is only allowed on the first absorbance strip. Reference wavelength is disabled on absorbance strips 2 to 10. Select the appropriate measurement parameters (wavelength, number of flashes and settle time) for your application.
Move Plate	Optional. To move the microplate automatically out of the instrument when finishing the measurement, select **Move plate OUT**.

4.13 i-control Examples

Example 1: Dual-Luciferase® Assay (Promega Corp.)

For assay details please refer to www.promega.com.

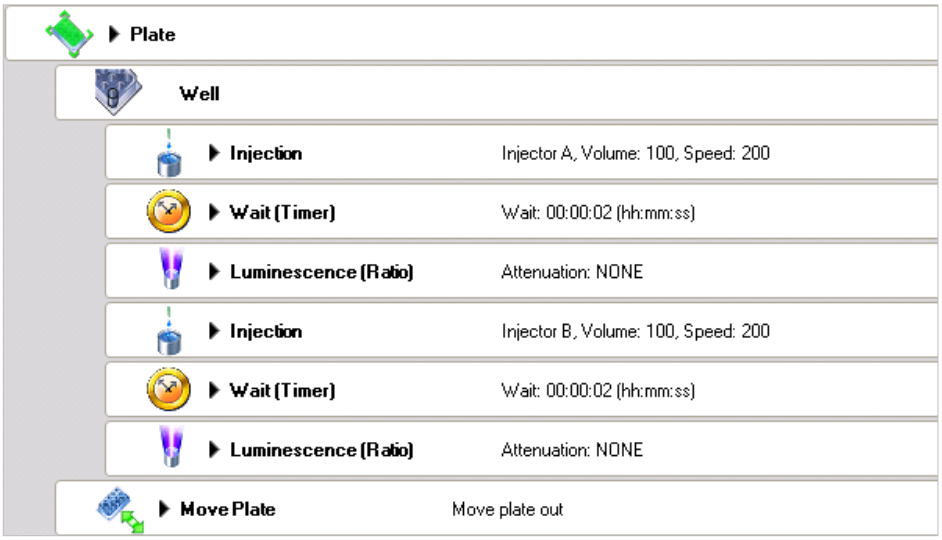


Plate	Select an appropriate plate type. For luminescence measurements, white microplates are recommended. For this example, a white 96-well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate is to be processed.
Well	Mandatory for measurements with **injection**
Injection (1)	Injector A injects 100 µl with speed 200 µl/sec., refill mode: **standard**
Wait (Timer)	2 s wait time
Luminescence (1)	Luminescence measurement with 10 s integration time, attenuation **none**
Injection (2)	Injector B injects 100 µl with speed 200 µl/sec., refill mode **standard**
Wait (Timer)	2 s wait time
Luminescence (2)	Luminescence measurement with 10 s integration time, attenuation **none**
Move Plate	Plate is moved out after finishing all wells

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT. SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

Example 2: Enliten® ATP Assay System Bioluminescence Detection Kit for ATP (Promega Corp.)

For assay details please refer to www.promega.com.

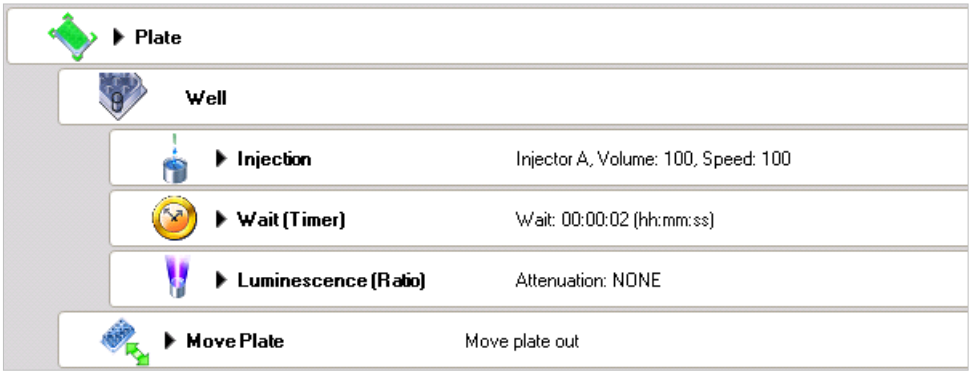


Plate	Select an appropriate plate type. For luminescence measurements, white microplates are recommended. For this example, a white 96 well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with **injection**
Injection	Injector A injects 100 µl with speed 100 µl/sec., refill mode: **standard**
Wait (Timer)	2 s wait time
Luminescence	Luminescence measurement with 10 s integration time, attenuation **none**
Move Plate	Plate is moved out after finishing all wells.

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT. SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

Example 3: Measurement of Ca^{2+} sensitive probes – Fura-2

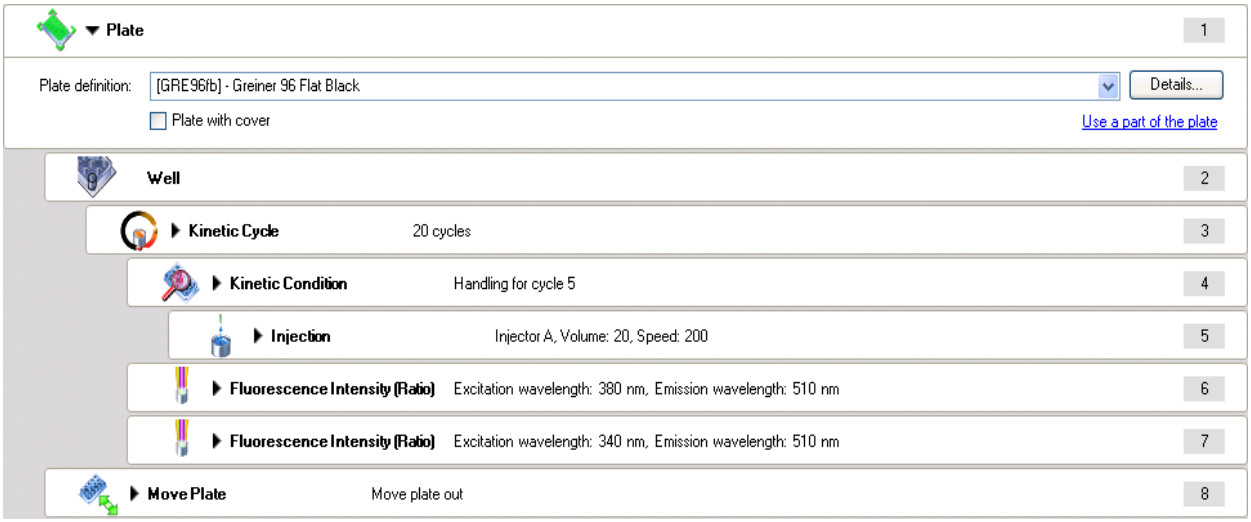


Plate	Select an appropriate plate type. For fluorescence measurements, black microplates are recommended. For this example, a black 96 well plate was selected.
Part of the plate	(Not shown), can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with **injection**
Kinetic Cycle	Select the number of necessary cycles
Kinetic condition	This strip allows actions to be performed once in a kinetic run at a certain cycle. The intended injection strip below it is only processed once at the selected cycle.
Injection	Injector A injects 20 µl with speed 200 µl/sec., refill mode: not selectable; injection is performed at cycle 5 (defined by kinetic condition strip)
Fluorescence Intensity (1)	Select the appropriate parameters for the first label: Excitation wavelength: 380 nm, Emission wavelength: 510 nm; number of flashes: 25; integration time: 40; gain: manual
Fluorescence intensity (2)	Select the appropriate parameters for the second label: Excitation wavelength: 340 nm, Emission wavelength: 510 nm; number of flashes: 25; integration time: 40; gain: manual
Move Plate	Plate is moved out after finishing all wells

Example 4: Measurement of Ca^{2+} sensitive probes – Indo-1

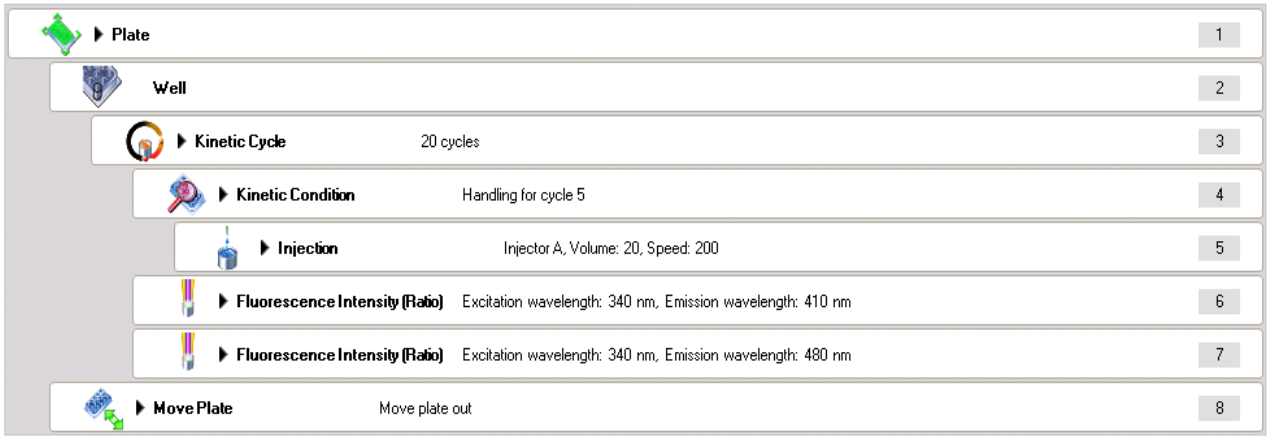


Plate	Select an appropriate plate type. For fluorescence measurements, black microplates are recommended. For this example, a black 96 well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with **injection**
Kinetic Cycle	Select the number of necessary cycles
Kinetic condition	This strip allows actions to be performed once in a kinetic run at a certain cycle. The intended injection strip below it is only processed once at the selected cycle.
Injection	Injector A injects 20 µl with speed 200 µl/sec., refill mode: not selectable; injection is performed at cycle 5 (defined by kinetic condition strip)
Fluorescence Intensity (1)	Select the appropriate parameters for the first label: Excitation wavelength: 340 nm, Emission wavelength: 410 nm; number of flashes: 25; integration time: 40; gain: manual
Fluorescence intensity (2)	Select the appropriate parameters for the second label: Excitation wavelength: 340 nm, Emission wavelength: 480 nm; number of flashes: 25; integration time: 40; gain: manual
Move Plate	Plate is moved out after finishing all wells.

4.14 Finishing a Measurement Session

4.14.1 Disconnecting the Instrument

When disconnecting, communication between the instrument and the computer is terminated.

Note
Remove the microplate and/or cuvette before disconnecting the instrument from the computer.

4.14.2 Instrument Shut Down

Upon shut down, the instrument activity is stopped immediately. Normally, you should disconnect before shutting down. In the rare case of an unexpected hardware error, immediate instrument shut down will reduce the risk of possible damage.

5. Instrument Features

5.1 Introduction

Note
All specifications are subject to change without prior notification.

The following types of measurement are provided with the fully equipped Infinite reader:

Measurement Type	Description
Fluorescence Intensity Top/Bottom	See 5.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)
Fluorescence Time Resolved	See 5.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)
Fluorescence Polarization	See 5.4 Fluorescence Polarization (FP) - Infinite F Plex only
Absorbance	See 5.5 Absorbance
Glow Type Luminescence	See 5.6 Glow Type Luminescence
Dual Color Luminescence	See 5.8 Dual Color Luminescence (e.g., BRET)
Flash Type Luminescence	See 5.7 Flash Type Luminescence

All standard microplates from 6 to 384-wells that conform to the following standards can be measured in any of the above measurement types:

- ANSI/SBS 1-2004;
- ANSI/SBS 2-2004;
- ANSI/SBS 3-2004 and
- ANSI/SBS 4-2004.

The instrument can perform kinetic measurements.

Reading may be restricted to one part of the microplate.

5.2 Instrument Specifications

The table below lists the technical specifications of the instrument:

Parameters	Characteristics
General	
Measurement	Software controlled
Interface	USB
Filter Handling	
Infinite F configurations	External filter exchange
Infinite M configurations	Monochromator-based wavelength selection – no filters necessary
Microplates to be measured	From 6-well to 384-well plates (SBS standard formats)
Plate Definition	Via scanning software
Temperature Control	From 5 °C above ambient up to 42 °C
Plate Shaking	Linear and orbital shaking, amplitude selectable from 1 – 6 mm in 0.5 mm steps
Light Source	High energy xenon flash lamp, lifetime: 10^8 flashes
Optics	Fused Silica Lenses
Detectors	
Fluorescence	Spectrally enhanced PMT: red-sensitive PMT
Luminescence	Low dark count photomultiplier tube photon-counting electronics
Absorbance	Silicon photodiode
Power Supply	Auto-sensing: 100 – 120 V / 220 – 240 V, 50-60 Hz
Power Consumption	150 VA

Parameters	Characteristics	
Physical		
Outer Dimensions		
Basic instrument	Width: 425 mm Height: 253 mm Depth: 457 mm	16.73 inches 9.96 inches 17.99 inches
Pump Module	Width: 250 mm Height: 155 mm Depth: 156 mm	9.84 inches 6.10 inches 6.14 inches
Weight		
Infinite F configurations	14.0 kg	
Infinite M configurations	15.8 kg	
Pump Module	3.4 kg	
Environmental		
Ambient Temperature		
Operation	+ 15 °C to + 30 °C	(+ 59 °F to + 86 °F)
Non-operation	- 20 °C to + 60 °C	(- 4 °F to + 140 °F)
Relative Humidity		
Operation	< 80 % non-condensing	
Over-voltage Category	II	
Usage	General Laboratory Instrument	
Noise Level	< 60 dBA	
Pollution Degree	2	
Method of Disposal	Electronic waste (infectious waste)	

5.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)

Parameters	Characteristics
Wavelength Range - Infinite M configurations:	Excitation: 230 - 850 nm Emission: 280 - 850 nm
	selectable in 1 nm steps
Wavelength Range - Infinite F configurations:	Excitation and Emission: 230 - 850 nm
Standard Filter:	Not applicable – instruments are equipped with user-defined filters

Gain Setting	Values	Measurement Range
Manual	1 - 255	0 - 60,000 RFU
Optimal	automatic	0 - 60,000 RFU
Calculated from Well	automatic	0 - 60,000 RFU

TRF Parameters	Characteristics
Integration Time	10 - 2000 µs
Lag Time	0 - 2000 µs

5.3.1 Definition of the Detection Limit

The detection limit is the fluorophore concentration where the background-subtracted signal equals 3 times the standard deviation of the background noise.

When selecting 1 flash per well, the plate carrier does not stop at the measurement position. Using more flashes per well may improve the detection limit, but the total measurement time will be longer.

5.3.2 Fluorescein (Fluorescence Intensity) Top

Plate Type (number of wells)	96
Dispensed Volume [µl]	200
Flashes (Reads) per Well	25
Fluorescein Detection Limit [pM]	< 20 pM
Uniformity at 25 nM Fluorescein	< 3 % CV
Reproducibility at 25 nM Fluorescein	< 2 %

5.3.3 Fluorescein (Fluorescence Intensity) Bottom

Plate Type (number of wells)	96
Dispensed Volume [µl]	200
Flashes (Reads) per Well	25
Fluorescein Detection Limit [pM]	100 pM
Uniformity at 25 nM Fluorescein	< 3 % CV
Reproducibility at 25 nM Fluorescein	< 2 %

5.3.4 Europium (Time Resolved Fluorescence)

Plate Type (number of wells)	96
Dispensed Volume [µl]	200
Flashes (Reads) per Well	25
Europium Detection Limit (Infinite F configurations)	< 150 fM
Europium Detection Limit (Infinite M configurations)	< 5 pM (Typical value)

5.4 Fluorescence Polarization (FP) - Infinite F Plex only

Note
The option Fluorescence Polarization is only available for the Infinite F Plex. This module cannot be installed on other Infinite configurations.

Parameters	Characteristics
Wavelength Range	Excitation: 300 – 850 nm
	Emission: 330 – 850 nm
Standard Filter	Configuration of Default filter slide: **Exc 485 (20) nm** Position on Exc filter slide: Exc1: 485 (20) - parallel Exc2: 485 (20) - perpendicular **Em 535 (25) nm** Position on Em filter slide: Em1: 535 (25) - parallel Em2: 535 (25) - parallel

Gain Setting	Values	Measurement Range
Manual	1 - 255	0 - 60,000 RFU
Optimal	automatic	0 - 60,000 RFU
Calculated from Well	automatic	0 - 60,000 RFU

FP Parameters	Characteristics
Integration Time	20 - 2000 µs
Lag Time	0 - 2000 µs
FP Precision (Infinite F configurations only)	< 5 mP @ 1nM Fluorescein

5.5 Absorbance

Parameters	Instrument Type	Characteristics
Wavelength Range	Infinite F configurations	230 – 1000 nm
	Infinite M configurations	230 – 1000 nm no filter necessary, selection in 1 nm steps possible
Measurement Range	Both	0 – 4 OD

The following specifications are valid for the wavelength range from 300 – 700 nm for Infinite configurations.

Plate type (number of wells)	96
Accuracy 0 – 2 OD	< ± (1 % + 10 mOD)
Accuracy 2 – 3 OD	< ± 2.5 %
Baseline Flatness	± 10 mOD (1 sigma)
Infinite M configurations	
Wavelength Accuracy	≤ ± 1.5 nm λ > 315 nm; ≤ ± 0.8 nm λ ≤ 315 nm
Infinite F configurations	
Wavelength Accuracy	Dependent on filters used

The specifications are valid for measurements performed with 25 flashes (reads) per well.

5.6 Glow Type Luminescence

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT.
SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	380 – 600 nm
Linear Dynamic Range	6 orders of magnitude
Integration Time/well	100 – 20000 ms
Cross Talk % (black plate)	< 0.01 %
Measurement range	> 6 orders of magnitude 8 orders of magnitude (extended dynamic range)
Attenuation of Light	100 (OD2 attenuation filter), 1 (no attenuation)

5.6.1 ATP Glow Luminescence

Plate Type (number of wells)	96
Total Dispensed Volume[µl]	200
Integration Time/well [ms]	1000
ATP Detection Limit	3 fmol/well

CAUTION

SPECIFICATIONS ARE ONLY VALID WHEN THE INSTRUMENT IS PLACED IN A LOCATION WITH ILLUMINATION < 500 LUX.

5.7 Flash Type Luminescence

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT. SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	380 – 600 nm
Measurement Range	> 6 orders of magnitude 8 orders of magnitude (extended dynamic range)
Integration Time/well	100 – 20000 ms
Cross talk % (black plate)	< 0.01 %
Attenuation of Light	100 (OD2 attenuation filter), 1 (no attenuation)
ATP Detection Limit	< 80 amol/well

CAUTION

SPECIFICATIONS ARE ONLY VALID WHEN THE INSTRUMENT IS PLACED IN A LOCATION WITH ILLUMINATION < 500 LUX.

5.8 Dual Color Luminescence (e.g., BRET)

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT. SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

Parameters	Characteristics
Built-in wavelength:	See 2.5.4 Absorbance System (Infinite F configurations)
Integration time:	100 - 20000 ms. Different integration times are possible for each wavelength.
Plate type:	96 and 384-well microplates
Dynamic range	6 decades

5.9 "On the Fly" Measurements

On the Fly measurements are the fastest measurements possible using the Infinite reader. These measurements are performed using only one flash (number of flashes).

96-well plates (FI, TRF, Absorbance) Measurement time: < 20 s

384-well plates (FI, TRF, Absorbance) Measurement time: < 30 s

(Plate-in/out movement not included).

5.10 Cuvette Features (Infinite M configurations only)

Note
The option Cuvette is only available for the Infinite M configuration. This module cannot be installed in an Infinite F configuration.

The cuvette option allows for performing absorbance measurement in fixed wavelength and scan mode.

Parameters	Characteristics
Wavelength Range	230 – 1000 nm (no filter necessary, selection in 1 nm steps possible)
Measurement Range	0 – 4 OD

5.10.1 Cuvette Specifications

The following specifications are valid for the wavelength range from 300-700 nm, number of flashes 25:

Cuvette	Hellma 110-QS, 10 mm
Accuracy 0 – 2 OD	< ± (1 % + 18 mOD)
Accuracy 2 – 3 OD	< ± 2.5 %
Reproducibility 0 – 2 OD	< ± (1 % + 10 mOD)
Reproducibility 2 – 3 OD	< ± 2.5 %
Linearity 0 – 2 OD	R^2 > 0.998
Baseline Flatness	± 10 mOD (1 sigma)

CAUTION
THE CUVETTE PORT OF AN INFINITE M CONFIGURATION CANNOT BE USED FOR CUVETTES WITH A MEASUREMENT WINDOW < 2 MM (DIAMETER) AND A CENTER HEIGHT BELOW 15 MM.

5.11 Injector Specifications

Parameters	Characteristics
Accuracy	< 10% for injection volume of 10 µl
	< 2% for injection volume of 100 µl
	< 0.7% for injection volume of 450 µl
Precision	< 10% for injection volume of 10 µl
	< 2% for injection volume of 100 µl
	< 0.7% for injection volume of 450 µl

5.11.1 Injector Reagent Compatibility

The injector system of the Infinite F configurations and Infinite M configurations consists of the following materials:

- Teflon (PTFE): Tubing, valve plug, seal
- KelF: Valve body
- SC05: Injector needles

Please refer to the following list for reagent compatibility. Rating **A** indicates a good compatibility with the injector system. Chemicals with a rating **D** must not be used with the Infinite injectors. They will severely damage the injector system.

A Rated Chemicals	D Rated Chemicals
Acetic Acid < 60%	Butyl Amine
Acetonytrile	Carbon Tetrachloride (dry)
Chloroform	Diethyl Ether
Dimethyl Formamide	Ethanolamine
Ethanol	Ethylene Diamine
Hexane	Furfural
Methanol (Methyl Alcohol)	Hydrofluoric Acid
Sulfuric Acid, diluted (Concentration ≤ 1 N)	Monoethanolamine
Tetrahydrofuran	Potassium Hydroxide (Caustic Potash)
Water, Deionized	Potassium Hypochlorite
Water, Distilled	Sodium Hydroxide
Water, Fresh	Sodium Hypochlorite
	Concentrated Sulfuric Acid

CAUTION

THE INFORMATION IN THIS TABLE HAS BEEN SUPPLIED TO TECAN AUSTRIA BY OTHER REPUTABLE SOURCES AND IS TO BE USED ONLY AS A GUIDE IN SELECTING EQUIPMENT FOR APPROPRIATE CHEMICAL COMPATIBILITY. BEFORE PERMANENT INSTALLATION, TEST THE EQUIPMENT WITH THE CHEMICALS AND UNDER THE SPECIFIC CONDITIONS OF YOUR APPLICATION.

WARNING

VARIATIONS IN CHEMICAL BEHAVIOUR DURING HANDLING DUE TO FACTORS SUCH AS TEMPERATURE, PRESSURE AND TEMPERATURE, PRESSURE, AND CONCENTRATION CAN CAUSE EQUIPMENT TO FAIL, EVEN THOUGH IT PASSED AN INITIAL TEST. SERIOUS INJURY MAY RESULT. USE SUITABLE GUARDS AND/OR PERSONAL PROTECTION WHEN HANDLING CHEMICALS.

5.12 Measurement Accessories

5.12.1 Recommended Filters (Infinite F configurations only)

Please ask your local Tecan dealer for a recommended filter set. Filters designed for a different type of instrument will not necessarily perform well with the Infinite F configurations.

Note
If the excitation and the emission maximum of a fluorescent species are close together, they should not be directly translated into center wavelengths for fluorescence filters.

To provide acceptable background, usually, the upper cutoff for excitation wavelengths on the one hand, and the lower cutoff for emission wavelengths on the other hand need to be separated. This compromise depends on the blocking properties of the filters. For many fluorescent molecules the signal can be improved by the filter bandwidth away from the other center wavelength.

5.12.2 Recommended Types of Microplates

CAUTION

TO AVOID INSTRUMENT DAMAGE AND SAMPLE SPILL, MAKE SURE THAT THE TYPE OF MICROPLATE THAT IS TO BE USED FOR THE MEASUREMENT CORRESPONDS TO THE SELECTED PLATE DEFINITION (PDFX) FILE.

Generally, for high fluorescence sensitivity, black microplates are recommended. For low concentrations of TRF labels, white microplates seem superior. You may check if white plates are superior with UV excitation wavelengths.

We do not recommend using volumes less than a third of the maximum volume. When using lower volumes, check the availability of a suitable plate type.

To ensure good performance for Fluorescence Bottom Reading, we recommend using black plates with transparent bottom.

All standard microplates from 6 to 384 wells (maximum plate height 23 mm including lid) that conform to the following standards can be measured:

- ANSI/SBS 1-2004,
- ANSI/SBS 2-2004;
- ANSI/SBS 3-2004 and
- ANSI/SBS 4-2004.

When installing the operating software (i-control or Magellan), pre-defined plate definition files are installed. Please refer to the following list for the corresponding ordering numbers of the microplates. Please order microplates at your local microplate supplier.

Manufacturer / Pdfx-Name	Cat.-No.		Drawing-No.:
Greiner			
GRE6ft	657 160	657 185	**AC-9909**
GRE12ft	665 180	665 102	**AC-9910**
GRE24ft	662 160	662 102	**AC-9911**
GRE48ft	677 180	677 102	**AC-9912**
GRE96ft	655 101	655 161	**AC-9701**
GRE96fb_chimney	655 079 655 086	655 077 655 076	**AC-65507x**
GRE96fw_chimney	655 073 655 083	655 074 655 075	**AC-65507x**
GRE96ut	650 101 650 161 650 160	650 180 650 185	**AC-6501xx**
GRE96vt	651 101 651 161	651 160 651 180	**AC-6511xx**
GRE384fb	781 079 781 086 781 077	781 076 781 094 781 095	**AC-0205**
GRE384ft	781 061 781 101 781 162 781 185	781 186 781 165 781 182	**AC-0205**
GRE384fw	781 073 781 080 781 074	781 075 781 097 781 096	**AC-0205**
GRE384sb	784 209		**AC-8808**
GRE384st	784 201		**AC-8808**
GRE384sw	784 207		**AC-8808**
GRE96ft_half area	675 161 675 101	675 801	**AC-675801**
GRE96fw_half area	675 074 675 075	675 094 675 095	**AC-675801**
GRE96fb_half area	675 077 675 076	675 097 675 096	**AC-675801**
Corning			
COS6ft	3506	3516	**DWG00673**
COS12ft	3512	3513	**DWG00674**
COS24ft	3524 3526	3527	**DWG01261**
COS48ft	3548		**DWG00676**

Manufacturer / Pdfx-Name	Cat.-No.		Drawing-No.:
COS96fb	3916 3915	3925	**DWG00120**
COS96ft	3370	3628	**DWG00120**
COS96fw	3362 3912	3922	**DWG00120**
COS96rt	3360 3367 3788	3795 3358	**DWG01123**
COS96ft_half area	3690 3695	3697	**DWG00122**
COS384fb	3708 3709	3710	**DWG00679**
COS384ft	3680 3700	3701 3702	**DWG00679**
COS384fw	3703 3704	3705	**DWG00679**
COR96fb clear bottom	3631		**DWG00678**
COR96fw clear bottom	3632		**DWG00678**
COR96fb half area	3694		**DWG00123**
COR96fw half area	3693		**DWG00123**
COR96fb half area clear bottom	3880		**DWG01471**
COR96fw half area clear bottom	3883		**DWG01471**
COR96fc UV transparent	3635		**DWG00678**
COR96fc half area UV transparent	3679		**DWG00678**
COR384fb clear bottom	3711		**DWG00682**
COR384fw clear bottom	3706		**DWG00682**
COR384fc UV transparent	3675		**DWG01479**
Nunclon			
NUN96ft	439 454 442 404 475 094	269 620 269 787	**MTP-0001**
NUN384ft	242 765 242 757 164 688	464 718 265 196	**MTP-0002**
NUN384fb	264 556 164 564	460 518	**MTP-0002**

Manufacturer / Pdfx-Name	Cat.-No.		Drawing-No.:
NUN384fw	264 572 164 610	460 372	**MTP-0002**
NUN96ut	143 761 163 320 262 170	262 162 475 434 449 824	**MTP-0003**
NUN96fb_LumiNunc FluoroNunc	137 101 137 103 237 105 237 107	237 108 437 111 437 112	**MTP-0004**
NUN96fw_LumiNunc FluoroNunc	136 101 136 102 236 105 236 107	236 108 436 110 436 111	**MTP-0004**
BD Falcon			
BD24_FluoroBlok	351155 351156	351157 351158	**MTP-0005**
BD96_FluoroBlok	351161 351162	351163 351164	**MTP-0006**
Tecan			
TEC96fb: Tecan 96 flat black	30122298		—
TEC384fb: Tecan 384 flat black	30122299		—
TEC96fw: Tecan 96 flat white	30122300		—
TEC384fw: Tecan 384 flat white	30122301		—
TEC24ft_cell: Tecan 24 cell culture flat transparent	30122302		—
TEC48ft_cell: Tecan 48 cell culture flat transparent	30122303		—
TEC96ft_cell: Tecan 96 cell culture flat transparent	30122304		—
TEC384ft_cell: Tecan 384 cell culture flat transparent	30122305		—
TEC96fb_cell_clear: Tecan 96 cell culture flat black, clear bottom	30122306		—

Manufacturer / Pdfx-Name	Cat.-No.	Drawing-No.:
TEC384fb_cell_clear: Tecan 384 cell culture flat black, clear bottom	30122307	—
NanoQuantPlate	—	**MTP-0007**
PerkinElmer		
PE96fw_OptiPlate	6005290	http://www.perkinelmer.com/Catalog/Product/ID/6005290
PE96fw_ProxiPlate	6006290	http://www.perkinelmer.com/Catalog/Product/ID/6006290
PE384fg_AlphaPlate	6008350	TechnicalDataSheet_DimensionsOfProxiplate-384Plus
PE384fg_ProxiPlate	6008270	
PE384fw_ProxiPlate	6008280	
PE384fw_OptiPlate	6008290	TechnicalDrawing2: Dimensions apply to 384 well OptiPlates

Table 1: Plate definition files and the corresponding catalog numbers

5.12.3 Luminescence Detection

CAUTION

SWITCH ON THE INSTRUMENT AT LEAST 15 MINUTES BEFORE STARTING A LUMINESCENCE MEASUREMENT. SOME COMPONENTS NEED TO WARM UP TO GUARANTEE STABLE CONDITIONS FOR THE MEASUREMENT.

The Infinite reader luminescence detection system utilizes the single photon counting measurement technique. This is based on a dedicated luminescence PMT with appropriate measurement circuitry. This technique is very robust against noise. It is preferred for measurement of very low light levels.

For best performance it is recommended to use white plates for luminescence measurements. For details see 4.9 Optimizing Luminescence Measurements.

Note

Results of luminescence measurements are always displayed in counts per second (cps).

CAUTION

THE INSTRUMENT MUST BE PLACED IN A LOCATION AWAY FROM DIRECT SUNLIGHT. ILLUMINATION > 500 LUX CAN NEGATIVELY INFLUENCE LUMINESCENCE MEASUREMENTS.

6. Quality Control

6.1 Periodic Quality Control Tests

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

The tests described in the following sections 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 instrument parameters; 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 of the appropriate settings (filters, flashes, delays, etc.).

CAUTION

BEFORE STARTING MEASUREMENTS, MAKE SURE THAT THE MICROPLATE POSITION A1 IS INSERTED CORRECTLY. THE POSITION OF WELL A1 MUST BE ON THE UPPER LEFT SIDE.

CAUTION

THIS SECTION PROVIDES 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.

6.2 Specifications - Passed/Failed Criteria

Note
All specifications are subject to change without prior notification.

The following table gives an overview of the passed/failed criteria for the specification test of the Infinite reader.

Specification	**Passed/Failed Criteria**
Fluorescence Top Sensitivity	< 20 pM Fluorescein
Fluorescence Top Uniformity	< 3 % CV
Fluorescence Top Precision	< 2 % CV
Fluorescence Bottom Sensitivity	100 pM
Fluorescence Bottom Uniformity	< 3 % CV
Fluorescence Bottom Precision	< 2 % CV
Time Resolved Fluorescence Sensitivity (Infinite F configurations only)	< 150 fM (with 510 dichroic) < 3 pM (with 50% mirror)
Time Resolved Fluorescence Precision (Infinite F configurations only)	< 2 % CV
FP Precision (Infinite F configurations only)	< 5 mP
Luminescence Sensitivity Glow Type	< 3 fmol/well
Luminescence Sensitivity Flash Type	< 80 amol/well
Absorbance Accuracy	0 – 2 OD: ≤ ± 1 % + 10 mOD 2 – 3 OD: ≤ ± 2.5 %
Absorbance Baseline Flatness (1 sigma)	< ± 10 mOD
Absorbance Wavelength Accuracy	≤ ± 1.5 nm λ > 315 nm; ≤ ± 0.8 nm λ ≤ 315 nm
Specification Cuvette (Infinite M configurations only)	**Passed/Failed Criteria**
Absorbance Accuracy	0 – 2 OD: ≤ ± 1 % + 10 mOD 2 – 3 OD: ≤ ± 2.5 %
Absorbance Baseline Flatness (1 sigma)	< ± 10 mOD

6.3 Specifications - Test Instructions

6.3.1 Fluorescence Top

For the Infinite reader with the option **Fluorescence Top**, the following tests can be performed to prove the specifications:

- Sensitivity
- Uniformity
- Precision

These test instructions are valid for the Infinite reader:

- Infinite F configurations
- Infinite M configurations
- Spectrally enhanced version

Sensitivity:

Perform the following measurement to determine the detection limit for Fluorescein:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Top	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	485 (20) nm 485 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	535 (25) nm 535 nm
Number of flashes	25	
Integration Time	40	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fb	

Plate Layout:

Pipette 200 µl of 1 nM Fluorescein or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

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

Material/Reagents:

1 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)

0.01 M NaOH (=Blank) (NaOH pellets, Merck article no. 6495 or Sigma S8045)

1 Greiner 96-well plate black

200 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

$$\text{DetectionLimit} = \frac{\text{Concentration}_F}{(\text{mean}_F - \text{mean}_B)} * 3 * \text{Stdev}_B$$

Concentration_F	Concentration of the fluorophore in pM units
mean_F	Average RFU value of wells filled with fluorophore
mean_B	Average RFU value of wells filled with blank
stdev_B	Standard deviation of RFU values of wells filled with blank

The result of the formula **Detection Limit** determines the sensitivity in pM units.

Uniformity:

Perform the following measurement to determine the **Uniformity**:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Top	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	485 (20) nm 485 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	535 (25) nm 535 nm
Number of flashes	25	
Integration Time	40	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fb	

Plate Layout:

Pipette 200 µl of 1 nM Fluorescein or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

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

Material/Reagents:

1 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)

0.01 M NaOH (=Blank) (NaOH pellets, Merck article no. 6495 or Sigma S8045)

1 Greiner 96-well plate black

200 µl Pipette + tips

Calculation of Uniformity:

$$\text{Uniformity}\,(\%) = \frac{\text{stdev}_F * 100}{\text{mean}_F}$$

mean_F	Average RFU value of wells filled with fluorophore
stdev_F	Standard deviation of RFU values of wells filled with fluorophore

The result of the formula determines the uniformity in % CV.

Precision:

Perform the following measurement to determine the precision/reproducibility:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Top	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	485 (20) nm 485 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	535 (25) nm 535 nm
Number of flashes	25	
Integration Time	40	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fb	
Part of the Plate	A1	
Kinetic	20 Cycles	
Interval Time	Minimal	

Plate Layout:

Pipette 200 µl of 1 nM Fluorescein or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

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

Material/Reagents:

1 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)
0.01 M NaOH (=Blank) (NaOH pellets, Merck article no. 6495 or Sigma S8045)
1 Greiner 96-well plate black
200 µl Pipette + tips

Calculation of Precision:

$$Precision(CV\%) = \frac{stdev_{wellA1} * 100}{mean_{wellA1}}$$

$mean_{wellA1}$	Average RFU value of well A1 over the 20 kinetic
$stdev_{wellA1}$	Standard deviation of RFU values of Well A1 over the 20 cycles

The result of the formula determines the Precision in % CV.

6.3.2 Fluorescence Bottom

For the Infinite reader with the option **Fluorescence Bottom** the following tests may be performed to prove the specifications:

- Sensitivity
- Uniformity
- Precision/Repeatability

These test instructions are valid for the Infinite reader:

- Infinite F configurations
- Infinite M configurations
- Spectrally enhanced version

Sensitivity:

Perform the following measurement to determine the detection limit for Fluorescein:

Measurement Parameters:

Parameter	**Setting**	
Reading Mode	Fluorescence Bottom	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	485 (20) nm 485 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	535 (25) nm 535 nm
Number of flashes	25	
Integration Time	40	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fb	

Plate Layout

Pipette 200 µl of 25 nM fluorescein or the blank solution (0.01 M NaOH) into the appropriate wells according to the plate layout:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank
B												
C												
D												
E												
F												
G												
H												

Material/Reagents:

25 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)
0.01 M NaOH (=Blank) (NaOH pellets, Merck article no. 6495 or Sigma S8045)
1 Greiner 96-well plate µClear, black with transparent bottom
200 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

See 6.3.1 Fluorescence Top: Sensitivity.

Uniformity:

Perform the following measurement to determine the Uniformity:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Bottom	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	485 (20) nm 485 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	535 (25) nm 535 nm
Number of flashes	25	
Integration Time	40	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fb	

Plate Layout:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank
B												
C												
D												
E												
F												
G												
H												

Filling volume: 200 µl

Material/Reagents:

25 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)
0.01 M NaOH (=Blank) (NaOH pellets, Merck article no. 6495 or Sigma S8045)
1 Greiner 96-well plate µClear, black with transparent bottom
200 µl Pipette + tips

Calculation of Uniformity:

See 6.3.1 Fluorescence Top: Uniformity.

Precision:

Perform the following measurement to determine the precision/reproducibility:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Bottom	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	485 (20) nm 485 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	535 (25) nm 535 nm
Number of flashes	25	
Integration Time	40	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fb	
Part of the Plate	A1	
Kinetic	20 Cycles	
Interval Time	Minimal	

Plate Layout:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank	25 nM Fluorescein	Blank
B												
C												
D												
E												
F												
G												
H												

Filling volume: 200 µl

Material/Reagents:

25 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)

0.01 M NaOH (=Blank) (NaOH pellets, Merck article no. 6495 or Sigma S8045)

1 Greiner 96-well plate µClear, black with transparent bottom

200 µl Pipette + tips

Calculation of Precision:

See 6.3.1 Fluorescence Top: Precision.

6.3.3 Time Resolved Fluorescence

For the Infinite reader with the option **Fluorescence Top**, the following tests may be performed to prove the specifications:

- Sensitivity
- Precision/Repeatability

These test instructions are valid for the Infinite F configurations.

Sensitivity:

Perform the following measurement to determine the sensitivity:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Top	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	340 (35) nm 340 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	612 (10) nm 617 nm
Number of flashes	25	
Integration Time	400	
Lag Time	100	
Settle Time	0	
Gain	Optimal	
Plate Type	GRE96fw	

Plate Layout:

Pipette 200 µl of 1 nM Europium solution or the blank solution (enhancement solution) into the appropriate wells according to the plate layout:

<>	1	2	3	4	5	...
A	1 nM Europium	Blank	Blank	Blank	Blank	
B						
C						
D						
E						
F						
G						
H						

Material/Reagents:

1 nM Europium (B119-100, HVD Live Sciences)

Enhancement Solution (=Blank) (1244-105, HVD Live Sciences)

1 Greiner 96-well plate white

200 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

See 6.3.1 Fluorescence Top: Sensitivity.

Precision:

Perform the following measurement to determine the precision/reproducibility:

Measurement Parameters:

Parameter	Setting	
Reading Mode	Fluorescence Top	
Ex Wavelength	Infinite F configurations: Infinite M configurations:	340 (35) nm 340 nm
Em Wavelength	Infinite F configurations: Infinite M configurations:	612 (10) nm 617 nm
Number of flashes	25	
Integration Time	400	
Lag Time	100	
Time between Move and Flash	0	
Gain	Optimal	
Plate Type	GRE96fw	
Part of the plate	A1	
Kinetic	20 Cycles	
Interval Time	Minimal	

Plate Layout:

See 6.3.3 Time Resolved Fluorescence Precision.

Calculation of Precision:

See 6.3.1 Fluorescence Top: Precision.

6.3.4 Fluorescence Polarization (Infinite F Plex only)

For the Infinite F Plex with the option **Fluorescence Polarization**, the following tests may be performed to prove the specifications:

- Precision/Repeatability

These test instructions are valid for the Infinite F Plex only:

- Spectrally enhanced version

Precision:

Perform the following measurement to determine the precision:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Polarization
Ex Wavelength	485 (20) nm
Em Wavelength	535 (25) nm
Number of flashes	25
Integration Time	40
Lag Time	0
Time between Move and Read	0
Gain	Optimal
Plate Type	GRE96fb
Reference from/to	A1 – D1
Reference blank from/to	A2 – D2
Reference value	20 mP
Measurement blank from/to	same as reference blank

Plate Layout:

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

Filling volume: 200 µl/well

Material/Reagents:

1 nM Fluorescein (in 0.01 M NaOH) (Fluorescein sodium salt, Sigma F6377)

0.01 M NaOH (=Blank) (NaOH pellets, Merck Article No. 6495 or Sigma S8045)

1 Greiner 96-well plate, black, flat bottom

200 µl Pipette + tips

Calculation of Precision:

The precision is calculated from the wells filled with fluorescein. The precision corresponds to one-time standard deviation of the mP values of the fluorescein wells.

6.3.5 Glow Luminescence

For the Infinite reader with the option **Luminescence** the following tests may be performed to prove the sensitivity specifications:

Sensitivity:

Perform the following measurement to determine the sensitivity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Luminescence
Integration Time	1000 ms
Settle Time	0
Plate Type	GRE96fw
Part of the Plate	A1 – D10

Plate Layout:

Pipette 200 µl of the ATP reagents into the appropriate wells according to the plate layout:

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

ATP	$2*10^{-8}$ M ATP (final concentration in well)
B	Blank (ATP reagent: Tris-EDTA=1:5)
Bx	Blank (wells used for cross-talk calculation)

Material/Reagents:

BioThema ATP Kit (ATP-Kit SL 144-041, BioThema AB)

1 Greiner 96-well plate white

200 µl Pipette + tips

Calculation of the Sensitivity (Detection Limit):

$$DetectionLimit(fmol/well) = \frac{2 \cdot 10^{-8} * 3 * Stdev_B}{mean_{ATP} - mean_B} * 0.0002 * \frac{1}{1e^{-15}}$$

$2*10^{-8}$	Concentration of ATP standard [M]
$Stdev_B$	Standard deviation of Blank
$mean_{ATP}$	Average of wells filled with ATP standard
$mean_B$	Average of Blank wells
0.0002	Conversion into mol/well
$1/1e^{-15}$	Conversion into fmol/well

The result of the formula determines the detection limit in fmol/well.

6.3.6 Absorbance Accuracy

Use MultiCheck Plate – For details please refer to the MultiCheck Instructions for Use.

6.3.7 Absorbance Wavelength Accuracy

The wavelength accuracy defines the deviation of the set measurement wavelengths from the nominal wavelength. This test is only valid for the Infinite M configurations.

Measurement Parameters:

Parameter	Setting
Reading Mode	Absorbance Scan
Measurement Wavelength from/to	300 – 850 nm
Step Size	1 nm
Number of flashes	25
Settle Time	0
Plate Type	MultiCheck plate

Material/Reagents:

MultiCheck plate

Calculation of Wavelength Accuracy:

Please refer to the data sheet in the instructions for use for your MultiCheck plate.

$$Wavelength\ \ Accuracy = Max_t - Max_m$$

Max_t	theoretical maximum
Max_m	measured maximum

6.3.8 Absorbance Baseline Flatness (Infinite M configurations)

Perform the following measurement to determine the baseline-flatness:

Measurement Parameters:

Parameter	Setting
Reading Mode	Absorbance
Measurement Wavelength from/to	300 - 700 nm
Number of flashes	25
Settle Time	0
Plate Type	GRE96ft
Part of the Plate	A1

Plate Layout:

No plate is necessary for measurement – the plate carrier must be empty for this measurement.

Material/Reagents:

No material or reagents necessary for this test.

Calculation of Baseline Flatness:

Calculate the standard deviation.

6.3.9 Absorbance Baseline Flatness (Infinite F configurations)

Perform the following measurements to determine the baseline-flatness with the available filter:

Measurement Parameters:

Parameter	Setting
Reading Mode	Absorbance
Measurement Wavelength	340 nm 405 nm 492 nm 590 nm 620 nm 700 nm
Number of flashes	25
Settle Time	0
Plate Type	GRE96ft
Part of the Plate	A1
Kinetic Cycles	20, Minimal Interval Time

Plate Layout:

No plate is necessary for measurement – the plate carrier must be empty for this measurement.

Material/Reagents:

No material or reagents necessary for this test.

Calculation of Baseline Flatness:

Calculate the standard deviation over 20 cycles for each wavelength.

6.3.10 Absorbance Cuvette (Infinite M configurations only)

For the Infinite M configurations with the option **Cuvette** the following test may be performed to prove the accuracy specification:

Accuracy

Perform the following measurement to determine the absorbance accuracy:

Measurement Parameters:

Parameter	Setting
Reading Mode	Absorbance
Measurement Wavelength	1) 440 nm 2) 635 nm
Number of flashes	25
Time between Move and Read	0
Cuvette Type	Calibrated cuvette, e.g., Starna RM-N1N35N + a D3 Cuvette

Material:

Starna® reference material RM-N1N35N + D3 cuvette

(for more information, please refer to www.starna.co.uk)

Calculation of Accuracy:

Calculate the deviation of the measured value from the reference value supplied with the calibrated cuvette.

7. Cleaning and Maintenance

7.1 Introduction

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.

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

This section contains the following procedures:

- Liquid Spills
- Instrument Disinfection
- Disinfection Certificate
- Instrument and Material Disposal

WARNING

ALL PARTS OF THE INSTRUMENT THAT COME INTO CONTACT WITH POTENTIALLY INFECTIOUS 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 CLEANING PROCEDURES AND ALSO WHEN MAKING ADJUSTMENTS TO THE INSTRUMENT.

7.2 Liquid Spills

1. Switch OFF the instrument.
2. Wipe up the spill immediately with absorbent material.
3. Dispose of contaminated material appropriately.
4. Clean the instrument surfaces with a mild detergent.
5. For biohazardous spills clean with B33 (Orochemie, Germany).
6. 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 MUST BE TREATED AS POTENTIALLY INFECTIOUS AND THE DISPOSAL MUST BE PERFORMED ACCORDING TO THE INFORMATION GIVEN IN CHAPTER 7.4.4 DISPOSAL.

IF THE SPILL OCCURS IN THE INSTRUMENT, A SERVICE TECHNICIAN IS REQUIRED.

WARNING

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.

7.3 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, WASH THE WHOLE INJECTOR SYSTEM THOROUGHLY BETWEEN DIFFERENT APPLICATIONS USING THE INJECTOR.

7.3.1 Daily Maintenance

If not otherwise stated by the manufacturer of the kit to be used, the following tasks must be performed at least daily:

- Inspect the pump(s) and tubing for leaks.
- Flush the whole system thoroughly with distilled or deionized water after each use and when the pump is not in use. Failure to do so can result in crystallization of reagents. These crystals can damage the syringe seal and valve plug resulting in leakage.

CAUTION

DO NOT ALLOW THE PUMP(S) TO RUN DRY FOR MORE THAN A FEW CYCLES.

7.3.2 Weekly/Periodical Maintenance

The injector system (tubing, syringes, and injector needles) must be cleaned weekly to remove precipitates and eliminate bacterial growth:

Follow these steps to clean the pump/injector system with 70 % EtOH (ethanol):

1. Depending on the user's application flush thoroughly the system with buffer or distilled water before washing with 70 % EtOH.
2. Prime the pump with 70 % EtOH with syringes fully lowered for 30 minutes.
3. After the 30-minute period, cycle all the fluid from the syringe and tubing into a waste container.
4. Wash the pump/injector system with 70 % EtOH
5. Wash the pump/injector system with distilled or deionized water
6. Prime the pump/injector system with distilled water. Leave the fluid pathway filled for storage.
7. Clean the end of the injector needles 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.

7.4 Instrument Disinfection

All parts of the instrument that come into contact with the patient samples, positive control samples or hazardous material must be treated as potentially infectious areas.

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 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.

Before the instrument is returned to the distributor for servicing, it must be disinfected, and a disinfection certificate completed. If a disinfection certificate is not supplied, the instrument may not be accepted by the servicing center, or it may be held by the customs authorities.

7.4.1 Disinfection Solutions

The instrument should be disinfected using the following solution:

- B33 (Orochemie, Germany)

7.4.2 Disinfection Procedure

WARNING

THE DISINFECTION PROCEDURE SHOULD BE PERFORMED IN A WELL-VENTILATED ROOM BY AUTHORIZED TRAINED PERSONNEL WEARING DISPOSABLE POWDER-FREE GLOVES, PROTECTIVE GLASSES AND PROTECTIVE CLOTHING.

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

1. Disconnect the instrument from the main power supply.
2. Disconnect the instrument from any accessories that are used.
3. Carefully wipe all outside surfaces of the instrument with a wad of cotton wool soaked in the disinfecting solution.
4. Make certain, that the same disinfection procedure is performed with the plate carrier.
5. Repeat the disinfection procedure on any accessories, which are also being moved for returned.
6. After the disinfection procedure has been performed, make certain that the disinfection certificate is completed.

7. Complete a safety certificate and attach it to the outside of the box so that it is clearly visible.

See 7.4.3 Safety Certificate for an example of the safety certificate, which must be completed before the instrument is returned to the service center for service or repair.

7.4.3 Safety Certificate

To ensure the safety and health of personnel, our customers are kindly asked to complete two copies of the **Safety Certificate** (which was delivered with the instrument) 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 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 7.4.2 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 should 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.

Your local Tecan customer support can send you a new copy of the Safety Certificate, if required.

7.4.4 Disposal

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

This gives instructions on how to lawfully dispose of waste material accumulating in connection with the instrument.

CAUTION

OBSERVE ALL FEDERAL, STATE AND LOCAL ENVIRONMENTAL REGULATIONS.

ATTENTION

DIRECTIVE 2002/96/EC ON WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE)

NEGATIVE ENVIRONMENTAL IMPACTS ASSOCIATED WITH THE TREATMENT OF ELECTRICAL AND ELECTRONIC EQUIPMENT WASTE

- **DO NOT TREAT ELECTRICAL AND ELECTRONIC EQUIPMENT AS UNSORTED MUNICIPAL WASTE.**
- **COLLECT WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT SEPARATELY.**

7.4.5 Disposal of Packing Material

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

Returning Packing Material

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

7.4.6 Disposal of Operating Material

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF PROCESSES RUN ON THE INSTRUMENT.

TREAT THE USED MICROPLATE, 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.

7.4.7 Disposal of the Instrument

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

CAUTION

ALWAYS DISINFECT THE INSTRUMENT BEFORE DISPOSAL.

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 (I.E. CLEAN AND DISINFECT).**

8. Troubleshooting

Error #	Error Text	Description
1	Command is not valid	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
2	Parameter out of range	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
3	Wrong number of parameters	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
4	Invalid parameter	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
5	Invalid Parameter at pos	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
6	[prefix] is missing	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
7	RS485 Timeout at module [module descr]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
8	Invalid module number [Nr]	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
9	Binary Transfer command: [cmd] at module [n]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
10	Error at command [cmd] at module [n],	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
11	LID open	Plate transport or filter slide lid were open during a measurement, or the instrument was used in very bright environment (<< 500 LUX). Please check if the lid closes completely or if the environment was too bright.
12	LUMI FIBER broken	Hardware Failure Luminescence Module. Please report this error to your local Tecan customer support office.
13	Z Motor out of Safety-Range	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
14	Filter is not defined	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
15	X drive init error	Hardware Failure Plate Transport Module. Please report this error to your local Tecan customer support office.
16	Y drive init error	Hardware Failure Plate Transport Module. Please report this error to your local Tecan customer support office.
17	z drive init error	Hardware Failure z-drive Module. Please report this error to your local Tecan customer support office.
18	Injector A not available	Hardware Failure Injector A. Please report this error to your local Tecan customer support office.
19	Injector B not available	Hardware Failure Injector B. Please report this error to your local Tecan customer support office.
20	Injector Init Error:	Hardware failure Injector Module. Please report this error to your local Tecan customer support office.
21	Invalid Command: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
22	Invalid Operand: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
23	Invalid Command Sequence: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
24	N/A	N/A
25	Injector not init.: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
26	Plunger Overload:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
27	Valve Overload:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
28	Plunger Move not allowed:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
29	Command Overflow	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
30	Prepare: [s]: Gain:[g], Counts: [cts]	Unspecific Hardware failure. Please report this error to your local Tecan customer support office.
31	[ERR] at module [mod] (cmd:[cmd])	Unspecific Hardware failure. Please report this error to your local Tecan customer support office.
32	MTP is in Out-Position	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
33	[val] ... not set at (Ratiolabel [n])	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
34	Injectors are not enabled	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
35	Invalid Parameter Length (max: [n] char allowed)	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
36	Checksum Error	Communication Error on USB interface. Please report this error to your local customer support office.
37	Init Error at module [mod#]	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.
38	Instrument Initialization Error	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.
39	Injector A Communication Timeout	Communication Error on Injector Interface. Please report this error to your local customer support office.
40	Injector B Communication Timeout	Communication Error on Injector Interface. Please report this error to your local customer support office.
41	Prime Wash Error	Injectors still priming or washing. Please wait until prime or wash process is finished.
42	Instrument is locked	Instrument is locked after a serious hardware problem. For unlocking a reboot is necessary. Please report this error to your local customer support office.
43	Prepare: [channel]: Wavelength:[lambda] Gain:[g], Counts: [cts]	Unspecific Hardware failure. Please report this error to your local Tecan customer support office.
44	Steploss Error	Actuator failure. Please report this error to your local Tecan customer support office.
45	Sync Scan: Number of EX-Steps does not match EM-Steps	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
46	Handshake timeout at module	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.
47	Motor Timeout	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.
48	[Value] is not in defined a Range	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.
49	Sensor is broken	Sensor Failure. Please report this error to your local Tecan customer support office.

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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/ 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, Untersbergstr. 1A, A-5082 Grödig, Austria
T +43 62 46 89 330, F +43 62 46 72 770, office.austria@tecan.com, www.tecan.com

Declaration of Conformity

We, TECAN Austria GmbH herewith declare under our sole responsibility that the product identified as:

Product Type: **Microplate Absorbance Reader**

Model Designation: ***INFINITE 200 PRO***

Article Number(s): **30050303**

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.

These *Instructions for Use* and the included *Declaration of Conformity* are valid for all INFINITE 200 PRO instruments with the article numbers listed above. The model designation varies depending on the specific model with different article number.