TECAN.

Instructions for Use

Evaluation Plugin

SparkControl

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

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We would appreciate any comments on this publication.

Manufacturer
Tecan Austria GmbH
Untersbergstr. 1A
A-5082 Grödig, Austria
T +43 62 46 89 330
E-mail: office.austria@tecan.com
www.tecan.com

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Area of Application

See chapter 1 Introduction.

About the Instructions for Use

Original Instructions. As addition to the SparkControl manual, this document describes how to install and use the Evaluation plugin.

Remarks on Analysis and Processing Plugins

The use and functionality of further Analysis and Processing Plugins as part of the SparkControl software are described in additional instructions to this document.

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NOTE: Gives helpful information.



CAUTION: Indicates the 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.

Table of Contents

1 Introduction 5

2 Plugin Installation 6

2.1 Uninstall/Repair Installation 6
2.2 Licensing 6
2.3 Directory 7

3 Calculation 8

3.1 Calculation Strip 8
3.2 How to Write a Formula 9
3.2.1 Plate Identifiers 9
3.2.2 Formula Variables 11
3.2.3 Writing a Formula 11
3.3 Calculations 13
3.3.1 Formula Operators 13
3.3.2 Formula Functions 13
3.3.3 Predefined Formulas 14
3.3.4 Error Handling 14
3.4 Training Point 15

4 Standard Curve 17

4.1 Standard Curve Strip 17
4.2 Plate Layout 18
4.3 Standard Curve Analysis Types 18
4.3.1 Point to Point 18
4.3.2 Linear Regression 18
4.3.3 Four Parameters Marquardt 19
4.4 Analysis results 20
4.4.1 Concentrations and Standard Curve 20
4.4.2 Error Handling 20
4.5 Training Point 21

5 Dose-Response Curve 23

5.1 Dose-Response Curve Strip 23
5.2 Plate Layout 24
5.3 Analysis Results 24
5.3.1 Dose-Response Curve 24
5.3.2 Error Handling 24
5.4 Training Point 25

6 ResultAnalyzer 28

6.1 Measurement 28
6.2 Edit Method 28

Tecan Customer Support 29

1 Introduction

The Evaluation plugin provides functionality for evaluation of data generated by measurements with the Spark multimode reader.

The plugin provides functionality for defining basic mathematical calculations, standard curves and dose-response curves. It supports the evaluation of imaging as well as non-imaging data. In this way, it fits perfectly into multiple workflows related to assay development, assay quality, measuring the concentration of unknown analytes (quantitative assays) and evaluation of drug potency. By enabling multi-label calculations, mathematical steps such as ratio calculation (e.g. normalization of detected light signal by cell count) can be easily done. In addition, the system offers predefined calculations that enables quick and simple use of some commonly used formula.

All strips can be used in endpoint and kinetic measurements with all detection modes except scanning (i.e. spectral recording in absorbance, fluorescence and luminescence) and multiple reads per well in absorbance and fluorescence intensity measurements.

The Evaluation plugin is not supported in stacker measurements.

2 Plugin Installation

NOTE: You must have administrative rights to install the software.

NOTE: Install the software before plugging the instrument into the computer.

NOTE: Make sure that SparkControl and ResultAnalyzer are installed on the computer.

NOTE: Before upgrading the software, make sure that the instrument, the camera, and all accessories are unplugged from the computer.

CAUTION: Always finish all open kinetic runs before uninstalling or upgrading the software, otherwise open kinetic data will be lost.

The plugin software is installed using the following procedure:

1. Insert the installation USB stick.
2. Open the Windows Explorer and browse to folder **Software/<article number>Plugin.Evaluation Vx.y** on the installation stick. Select **Plugin.Evaluation <version>_setup.exe** and run it as an administrator to start the installation procedure.
3. The software will be installed to C:\Program Files\Tecan. The installation destination can be changed optionally.
4. Select **Install** to start the software installation.

2.1 Uninstall/Repair Installation

If for any reason the current version of the plugin software needs to be reinstalled, proceed as follows:

1. Insert the installation USB stick.
2. Open the Windows Explorer and browse to folder **Software** on the installation stick.
3. Select **Plugin.Evaluation <version>_setup.exe** and run it as an administrator to start the installation procedure.
   - Select **Uninstall** to uninstall the current software version, or
   - Select **Repair** to repair the installation and restore the original program files.

NOTE: When uninstalling the Evaluation plugin, the MATLAB Runtime component is not uninstalled. It has to be uninstalled manually by the user.

2.2 Licensing

Licensing of the Evaluation plugin is performed with the **Licensing Client Module**. The Licensing Client Module is accessible via Help/Activate License in the menu bar of SparkControl.

CAUTION: If a plugin requiring a license has not been registered, the corresponding strip(s) will not be visible in SparkControl and ResultAnalyzer.

2.3 Directory

For every executed measurement, SparkControl generates a workspace folder. Each workspace folder may contain several subfolders, the contents of which, however, depend on the measurement performed:

Database	A database file with raw data is created for every measurement.
Export	**xlsx:** Excel compatible result files are generated for every measurement and, depending on the Data handling settings, automatically saved in the **xlsx** folder. **pdf:** For fluorescence imaging measurements and Tecan apps (e.g. Cell counting, NanoQuant), a pdf report is created and saved in the **pdf** folder. **Images:** If images are exported from ResultAnalyzer, they are saved in the **Images** folder. **csv:** Includes object list reports resulting from 2D and 3D imaging measurements as csv files. **Video:** Includes time-lapse videos generated via ResultAnalyzer. **PlateView:** Includes files with images displayed in plate view if exported after the measurement. **StandardCurve:** Includes generated standard curve(s) as html file **DoseResponseCurve:** Includes generated dose-response curve(s) as html file
Images	Contains acquired original images of measurements performed with the Cell Module and the Cell Imager module.
Methods	Contains method(s) after they have been exported from the corresponding workspace via ResultAnalyzer.
Analysis Plugins for Imaging	Contains plugin related data in corresponding subfolders: **Databases** **Images** (generated object lists only!)
Evaluation Plugin	Contains plugin related database with analysis results.
ResultAnalyzer	Contains ResultAnalyzer database and is created after a workspace has been opened in ResultAnalyzer.

3 Calculation

3.1 Calculation Strip

The Calculation strip enables the SparkControl user to set up mathematical calculations with single or multiple input data sets. The input data may result from a measurement (raw data, e.g. OD or RFU values) or previously conducted analysis steps (calculated data, e.g. blank reduction, imaging analysis data, calculated concentration values).

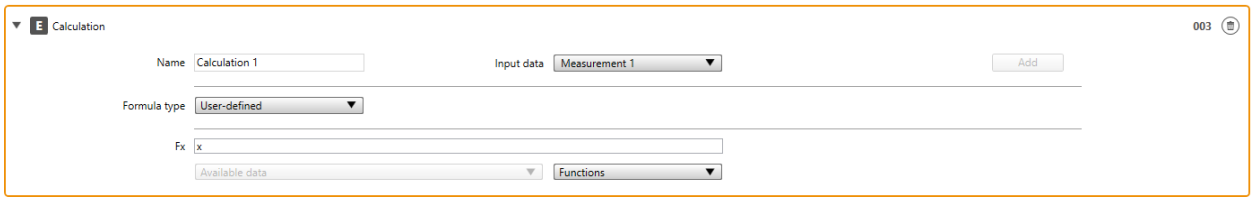


Figure 1: Calculation strip

The **Calculation** strip includes the following elements:

Name	Define a label name
Input data	Define the input data to be processed. For calculations with multiple input data (e.g. ratio calculation based on two fluorescence intensity measurements), select **Add** to insert the additional data set and **Remove** to delete it. If the selected input data (analysis label) have different sublabels (e.g. image analysis with analysis strip Counting), the sublabel has to be selected in addition.
Formula type	Select **User-defined** for a flexible, manual definition of a formula. Use **Predefined** to work with one of the predefined formulas, see chapter 3.3.3 Predefined Formulas.
Fx	Define a formula. See chapter 3.2 How to Write a Formula.
Available data	Provides a list of available data sets and operators that can be used to define a formula. See chapter 3.2 How to Write a Formula.
Functions	Lists all mathematical functions for the definition of formulas.
Alias	Assign alias designations for the selected input data and use them in formula definition.

NOTE: The Calculation strip allows computation of numeric values from all detection modes except scanning (absorbance, fluorescence and luminescence scan) and measurements with multiple reads per well.

NOTE: Special characters such as ., ', ( ), [ ], +, /, *, -, and <> are not allowed to be used in the label name of a Calculation strip.

3.2 How to Write a Formula

A formula requires valid input data and a valid formula structure. It may include plate identifiers, operators and functions. Understanding the use of plate identifiers and the formula syntax is crucial for a successful formula creation. The next chapters give insight into this process.

3.2.1 Plate Identifiers

The plate layout represents the composition of the plate for a given experimental setup and refers to the liquid in the corresponding well. This may include blanks, samples, standards, positive and negative control, etc. Their position on the plate is labeled by plate identifiers.

SparkControl provides various plate identifiers. Here are some frequently used ones:

Identifier	Description
BL	Blank
SM	Sample
ST	Standard (for generation of a standard curve)
CMx	Compound (x = A-K; for generation of a dose-response curve)
CMR	Reference Compound (for generation of a dose-response curve)
PC	Positive control
NC	Negative control

For the list of all supported identifiers, please see the SparkControl manual.

In the Plate Layout screen, every single well included the following information:

1st line: a unique identifier ID number

2nd line: information about the replicates

3rd line: values for defined dilution factors or concentrations

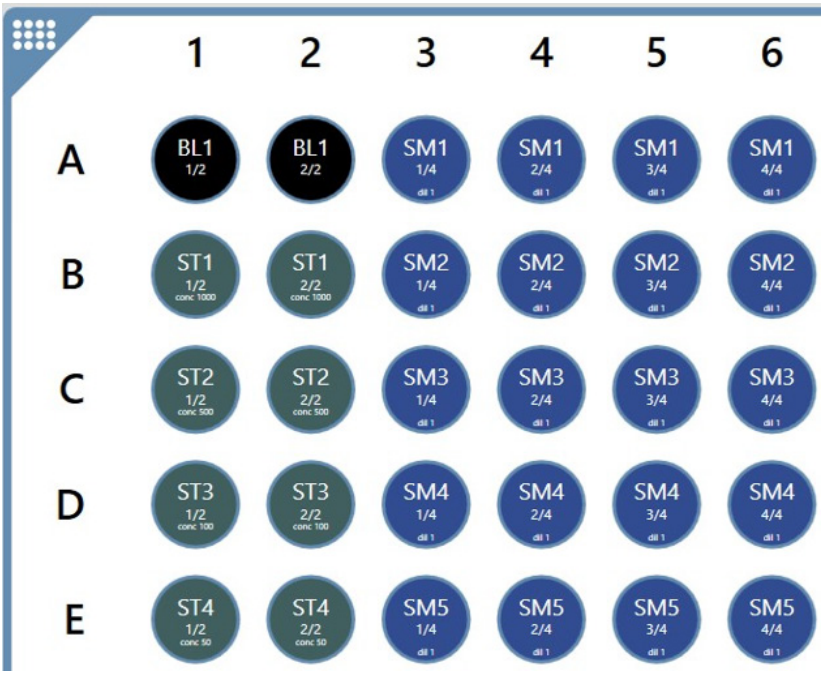


Figure 2: An excerpt from a defined plate layout

For identifiers such as BL, NC and PC ('controls'), the ID number is always 1. If there is more than one well assigned to this corresponding identifier type, they all are considered as replicates.

For identifiers such as ST, SM, CMx, the ID number correlates with the number of samples, standards, etc., defined. Accordingly, a group of 48 independent samples (i.e. samples, which are not replicates), would result in a plate layout with 48 wells with the well assignment from SM1 to SM48. However, a group of 48 samples defined as replicates will generate a plate layout with 48 wells, all assigned as SM1.

Examples of labeled wells

	BL1	1st line: Blank (mean value of all wells marked as BL1)
	NC1	1st line: Negative Control (mean value of all wells marked as NC1)
	SM1	1st line: Sample 1
	ST4	1st line: Standard 4
	1/4	2nd line: replicate 1 out of a total of 4 replicates
	4/4	2nd line: replicate 4 out of a total of 4 replicates
	dil 2	3rd line: a sample with the dilution factor 2
	conc 10	3rd line: a standard with the concentration value 10

For additional information about defining the plate layout, see the SparkControl manual.

All assigned identifiers can be used as variables in the formulas.

3.2.2 Formula Variables

All previously defined abbreviations for the identifiers contained within the analysis plate can be employed as variables in calculations. The formula syntax uses the following definitions:

PC1, SM1, ST1, BL1…Average values

PC1_1, SM1_1, ST1_1, ST4_1, BL1_1, BL1_2…Individual values, individual replicates

Example:

NC1 + 0.1…adds 0.1 to the average value of NC1

In addition to the identifiers, a formula can also work with the symbol x.

NOTE: The symbol x refers to the current value within a well.
For example: x-BL1…subtracts the empty value (blank) from the current value.

3.2.3 Writing a Formula

To include a calculation into a method select the **Calculation** strip in the **Analysis** tab and add it to the workflow pane. To enter a formula, execute the following steps:

1) Define the calculation **Name** or use the default one.

2) Select the **Input data**:

Available input data depend on the defined measurement and analysis setup: single or multiple measurement values (i.e. corresponding label names), image analysis data, the results from pre-calculations (e.g. fluorescence polarization values), and all already defined calculations. Select all input data that will be used in the formula definition via the **Add** button. If the selected label contains multiple sublabels (e.g. results from the image analysis strip Counting), they have to be selected as well.

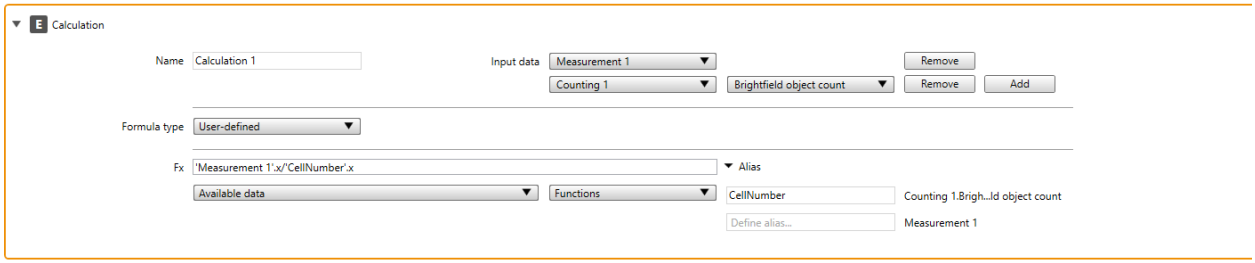


Figure 3: Calculation strip with multiple Input data selected

3) Select the **Formula type** (User-defined or Predefined).

4) Optionally, define **Alias**, an alternative name for the selected input data to be used in the formula definition to avoid the use of long input data.

5) For formula type **Predefined**, select one of the available formulas. For their description, see chapter 3.3.3 Predefined Formulas.

6) For formula type **User-defined**, enter a formula in the text box **Fx**.

Formulas can be entered by typing or by selecting the needed available data and functions from the list boxes **Available data** and **Functions**. Consider the next syntax rules:

Single-label calculations (calculation with one set of input data)

Fx	Example	Description
Variable	Fx = BL1	Returns the average blank values
	Fx = BL1_1	Returns the value of the first blank replicate
	Fx = x	Returns the value of the current well
	Fx = x-BL1	Subtracts the average of blank values from the current value
	Fx = SM1	Returns the average value of replicates for SM1
Variable with math. operator or function	Fx = NC1+0.1	Adds 0.1 to the average value of the negative control
	Fx = AVG(ST1)	Calculates the average value for all replicates of ST1
	Fx = PC1-SM2_1	Subtracts the first replicate value of the second sample from the average value of the positive control

When defining the calculations based on the single input data, the reference to the input data is not mandatory and can be left out for the sake of simplicity as shown in the table above. If the input data are included, they have to be enclosed in **apostrophes** followed by a **point** and a formula **variable**.

Here some examples with the single input data ABS492:

Formula with reference to input data	Simplified formula
Fx = 'ABS492'.x	Fx = x
Fx = AVG('ABS492'.ST1)	Fx = AVG(ST1) or Fx = ST1
Fx = 'ABS492'.PC1-'ABS492'.BL1	Fx = PC1-BL1

Multi-label calculations (calculation with multiple sets of input data)

When defining the calculations based on the multiple input data, defining the input data is mandatory. Write a formula by starting with the input data set within **apostrophes** followed by a **point** and a formula **variable**.

Fx	Description
'Measurement 1'.x/'Measurement 2'.x	Ratio calculation with input data Measurement 1 and Measurement 2
'Measurement 1'.x-'Measurement 2'.x	Difference calculation with input data Measurement 1 and Measurement 2
('FI320'.x-'FI320'.BL1)/('FI360'.x-'FI360'.BL1)	Ratio calculation with blank reduction using values from the input data FI320 and FI360, respectively

When writing the formula with the help of the **Available data**, select from this list the appropriate data. The data set will appear in the formula text box within apostrophes. To complete the definition, enter a point followed by the variable (e.g. identifier name). Make use of the integrated Microsoft IntelliSense help.

The use of **Alias** may be especially helpful in multi-label calculations to reduce the length of the input data and thus achieve better transparency in a defined formula. Here an example:

Fx = ('Counting 1.Blue object count'.x/'Counting 2.Blue object count'.x)*100

With ‘Blue_1’ and ‘Blue_2’ as alias for ‘Counting 1.Blue object count’ and ‘Counting 2.Blue object count’, respectively, the formula is simplified to

Fx = ('Blue_1'.x/'Blue_2'.x)*100



NOTE: An integrated formula checker verifies the plausibility of the formula. A warning appears in case the created formula is not executable.



NOTE: The Evaluation plugin uses Microsoft IntelliSense help to support quick and correct writing of formulas.



NOTE: Defined formulas are calculated for every applicable well. If average data is selected as Input data, then calculations will be assigned to the first replicate of an identifier only.



NOTE: In kinetic measurements, calculations like Fx = x-BL1 will be executed for every measured cycle.



NOTE: All values are calculated in double precision, although they are displayed with only 5 significant digits.



NOTE: Measurements may contain a maximum of 10 Calculation strips.

For more information on formula operators and functions, see the next chapters.

3.3 Calculations

3.3.1 Formula Operators

Operator	Description
+	Plus
-	Minus
*	Multiplication
/	Division

3.3.2 Formula Functions

AVG(argument)

This function calculates the average value of argument. The argument must be an identifier.

Example: *AVG(SM1)*

Averages all replicates of the first sample.

SD(argument)

This function calculates the standard deviation of argument. The argument must be an identifier.

Example: *SD(NC1)*

The standard deviation of the negative control will be formed.

CV%(argument)

This function calculates the coefficient of variation CV in % of argument. The argument must be an identifier.

Example: *CV%(ST2)*

CV% for all replicates of the second standard.

NOTE: A formula function calculates the corresponding value for the selected identifier defined as replicate. If e.g. an average value over all samples is to be defined, define it manually as Fx = (SM1+SM2+SMn)/n.

3.3.3 Predefined Formulas

Average

This formula calculates the average value of all replicates for every identifier on the plate.

Standard deviation

This formula calculates the standard deviation value of all replicates for every identifier on the plate.

$$S = \sqrt{\frac{\sum(x_i - x)^2}{n-1}}$$

x: Sample average
x_i: Individual population value
n: Total number of samples

For n = 1, the standard deviation will be 0.

Z'

This formula calculates Z', a statistical parameter for the quality of an assay:

$$\text{Z'-factor} = 1 - \frac{3(\sigma_p + \sigma_n)}{|\mu_p - \mu_n|}$$

p = positive control, n = negative control, σ = mean, μ = standard deviation

NOTE: The use of the correct identifier for the calculation of Z' is not limited to the identifiers PC (positive control) and NC (negative control). Use optionally any identifier on the plate representing the positive and negative control, respectively (e.g. PC, NC, SM, ST, CM).

3.3.4 Error Handling

If a calculation cannot be executed (masked well, input data not found, identifier not found, calculation error, division by 0), the wells are marked as follows:

Special character	Description
( )	Parentheses indicate a masked value.
<MIN	The calculated concentration is below the lowest defined standard concentration.
>MAX	The calculated concentration is above the highest defined standard concentration.
NoCalc	Input data/identifier is not found; calculation is not possible.
CalcError	Calculation error (e.g. division by 0).

3.4 Training Point



NOTE: In the exercises and related solutions below, assumptions were made regarding the plate layout, measurement setup and analysis. These assumptions can be subjected to individual changes. In the same way, the presented analysis approach may address one but not always the only possible way to achieve the final goal.

Exercise 1:

Fluorescence intensity is measured at Ex/Em = 485/560 nm for a plate including samples and blanks:

No. of samples = 4 (2 replicates/sample)

No. of blank wells = 2 (defined as replicates)

Subtract the average blank value from the average value of each sample.

Solution

Measurement tab:

Step 1:

In the Plate strip, define the measurement area. Click **Plate Layout** and select 8 wells to be assigned as sample. In the **Well assignment** box, select the identifier **SM** with No. of replicates = 2 and filling direction according to your scheme. To assign the selected identifier to the wells, double click the identifier **SM** or select **Fill selection**. The assigned identifiers are now part of the plate layout and displayed as SM1, SM2, SM3 and SM4, with each sample present as duplicate (indicated by 1/2 and 2/2 in the second line of the well assignment).

Repeat the well assignment for the blank wells, resulting in BL1 on your plate layout.

Step 2:

Add a Fluorescence Intensity strip to the measurement workflow pane. Use the excitation and emission wavelength value of 485 nm and 560 nm, respectively.

Analysis tab:

Step 3:

Add Calculation strip to the workflow pane and name it as ‘AVG value’. Select the formula type **Predefined** with the predefined formula **Average**. This will calculate the average values of all replicates for every identifier on the plate (SM1, SM2, SM3, SM4 and BL1)



NOTE: The predefined formula **Average** calculates the average value of all replicates for every identifier on the plate. The formula function **AVG** calculates the average value of a single identifier only, e.g. Fx = AVG(SM1).

Step 4:

Add a new Calculation strip and call it ‘Blank reduction’. Select ‘AVG value’ as input data. To define the formula, work with the formula type **User-defined** with

Fx = x-BL1

The result is the subtraction of the average blank value from the average value of each sample.

Exercise 2:

The experimental set up consists of a kinetic fluorescence imaging measurement incl. image analysis Counting and detecting the luminescence signal for a plate filled with HeLa cells. Correct the measured cell count by the blank value. Use the blank-corrected count values for the normalization of the luminescence signal.

Solution

Measurement tab:

Step 1:

In the Plate strip, define the measurement area. Click **Plate Layout** and select at least one well to be assigned as blank. In the **Well assignment** box, select the identifier **BL**. To assign the selected identifier to the well(s), double click the identifier **BL** or select **Fill selection**. The assigned identifier is now part of the plate layout and displayed as BL1.

Step 2:

Add measurement strips as follows:

Kinetic Loop

> Well

>> 2D Imaging (Name = Imaging, Brightfield channel selected)

>> Luminescence (Name = Luminescence)

Analysis tab:

Step 3:

Add analysis strip Counting (Name = Analysis 1).

Step 4:

Continue by selecting and defining the first Calculation strip (Name = Cell count blanked, Input data = Analysis 1 (main label) and Brightfield count (sublabel). Select the formula type **User-defined** with

Fx = x-BL1

The result is the subtraction of the average blank value from the value of every well per cycle.

Step 5:

Add the second Calculation strip and call it ‘Normalization’. Select ‘Cell count blanked’ and ‘Luminescence’ as input data. To define the formula, work with the formula type **User-defined** incl.

Fx = 'Luminescence'.x/'Cell count blanked'.x

The result is the ratio between labels calculated per well and every cycle.

4 Standard Curve

4.1 Standard Curve Strip

A standard curve is a common graphical element in quantitative analysis. It represents the relationship between input data (e.g. absorbance, fluorescence signal, processed data) plotted on the y-axis and the corresponding concentration values marked on the x-axis. The standard curve is created with the help of standards with known concentration values.

The standards curve is used to quantify the concentration of an unknown substance (e.g., protein, nucleic acid) in a sample measured with the same instrument settings and parameters as the standards. Its measured value (input data) is taken and correlated to the corresponding concentration at the point of intersection with the standard curve.

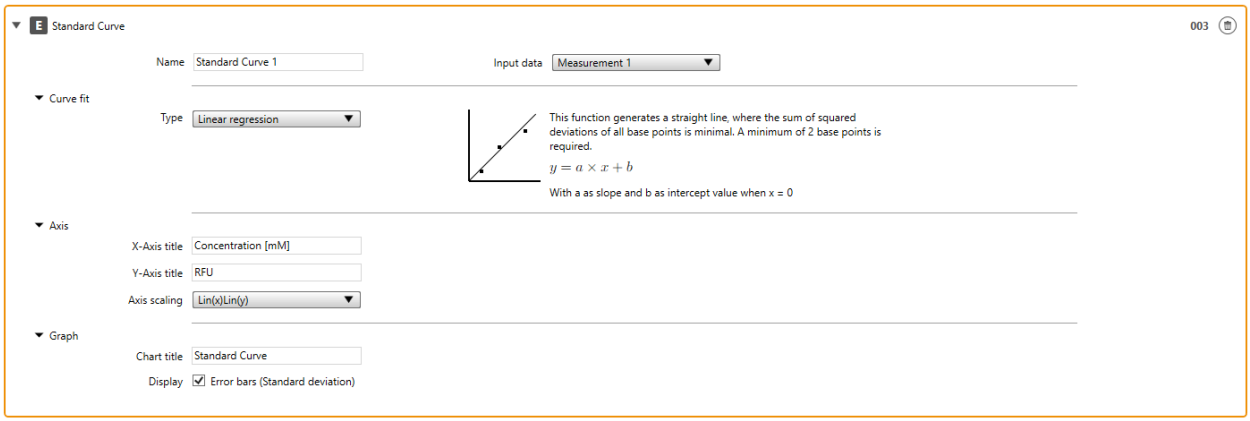


Figure 4: Standard Curve strip

The **Standard Curve** strip includes the following elements:

Name	Define a label name.
Input data	Select the Input data to be used for the standard curve. Select measurement data or any available analysis results.
Curve fit	
Type	Select interpolation algorithm to be employed when calculating the standard curve
Axis	
X-Axis title	Enter an axis label.
Y-Axis title	Enter an axis label.
Axis scaling	Select scaling of the axis (does not affect the calculation): • Lin(x)Lin(y): x and y axis are scaled linear • Lin(x)Log(y): x axis is scaled linear, y axis is scaled logarithmically • Log(x)Lin(y): x axis is scaled logarithmically; y axis is scaled linear • Log(x)Log(y): x and y axis are scaled logarithmically
Graph	
Chart title	Enter a label for the graph.
Display	If standards are defined as replicates, select **Error bars** to view a bar showing the range plus/minus standard deviation for each base point.

NOTE: Include units in the x-axis and y-axis title in order to be displayed on the generated standard curve.

4.2 Plate Layout

In an experimental setup with a standard curve, a typical plate layout contains wells identified as standards (ST) and samples (SM). The standards with known concentrations are used to generate a standard curve, which is used to determine the unknown concentration values of the samples afterwards. To enter the concentrations for the standards and, optionally, the dilution factors for the samples, open the table **Concentrations**, **dilution values** in the Plate Layout screen:

Select identifier	Select ST to add the corresponding concentration values. Select SM to add the corresponding dilution factors. All wells, which match the selected well type identifier, are listed.
Concentration/ Dilution	This table displays all wells in the Identifier column, which match the selected identifier (as from the Select identifier list). The corresponding dilutions, concentrations values are inserted and listed in the Concentration/Dilution column. If dilution factors are defined, they are considered by the calculation of the unknown sample concentration. E.g. entering a dilution factor of 2 means that the sample has been diluted by half. The calculated concentration will therefore be multiplied by 2.
Unit text field	The concentration unit can be determined.



NOTE: Concentration and dilution values have to be inserted manually. Save time by copying and pasting the values from an existing Excel table.

4.3 Standard Curve Analysis Types

4.3.1 Point to Point

Using this process, the adjacent base points will be joined by means of a straight line.

A minimum of 2 base points is required for this calculation.

There are no parameters for use as variables in the transformations.

4.3.2 Linear Regression

A straight line will be formed, where the sum of the squared deviations of all base points is a minimum.

A minimum of 2 base points is required for this calculation.

$y = ax + b$

In the Standard Curve view, the following additional information is displayed:

Formula with coefficient values a (slope) and b (intercept)

Coefficient of determination R^2

Extrapolation is not supported by the software.

4.3.3 Four Parameters Marquardt

The four-parameter approximation needs to fulfill the same base point requirements as LogitLog.

This method produces an S-shaped curve, which asymptotically reaches an upper and lower limit. A minimum of four base points is required for this calculation.

$$y = d + \frac{a - d}{1 + (\frac{x}{c})^b}$$

The parameters a, b, c, d are optimized by the Levenberg-Marquardt algorithm, an iterative technique that finds a local minimum of a function that is expressed as the sum of squares of nonlinear functions.

The algorithm stops if an accuracy of 1E-7 (FLT_EPSILON) is reached (success) or the maximum number of 30,000 iterations is exceeded (failure) before the given accuracy is reached.

Extrapolation of the curve is not supported by the software.

In the Standard Curve dialog box, the following additional information is displayed:

- Coefficient values a, b, c and d
- Coefficient of determination R^2

The parameters a, b, c, d, have appealing geometric interpretations.

a = response at a minimum dose (x-value); minimum asymptote

d = response at an infinitely high dose; maximum asymptote

c = is the point of symmetry on the curve (IC50/EC50); and below this point the curve is a mirror image

b = is a measure of the steepness of the curve at the inflection point (the Hill's slope)

Notice that the curve is always decreasing (if a>d) or increasing (if a<d).

NOTE: The Four Parameters Marquardt fit cannot handle zero concentration value, because $(x/c)^b$ is undefined or infinite at zero or negative values.

4.4 Analysis results

4.4.1 Concentrations and Standard Curve

The Evaluation plugin calculates and displays:

- Concentration of individual replicate
- Standard curve graph
- Standard curve parameters

4.4.2 Error Handling

If any of the values in the calculated concentrations lie outside of the range stipulated by the standards, the entry **>MAX** and **<MIN**, respectively, will be displayed within the plate's wells respectively.

If a concentration cannot be calculated, the wells are marked **NoCalc** (input data not found) or **CalcError** (calculation error).



NOTE: If the calculated concentration for the lowest standard falls below the lowest defined concentration, the well will be marked as **<MIN**.

If the calculated concentration for the highest standard falls above the highest defined concentration, the well will be marked as **>MAX**.

4.5 Training Point

Exercise 3:

Perform colorimetric quantification of total protein in a sample by measuring absorbance at the user-defined wavelength. Prepare a 96-well plate with triplicate wells defined as blank (A1-A3), standards (B1-F3) and samples (G1-H3, A4-H12). Use blank-corrected data as input data for the standard curve. Calculate an average concentration value for every sample.

Solution

Measurement tab:

Step 1:

In the Plate strip, define the measurement area A1-H12. Click **Plate Layout**.

Select A1-A3. In the **Well assignment** box, select the identifier **BL**. To assign the selected identifier to the wells, double click the identifier **BL** or select **Fill selection**. The assigned identifier is now part of the plate layout and displayed as BL1 in triplicate wells.

Assign the identifier **ST** with No. of replicates = 3 to the wells B1-F3. The standards ST1, ST2, ST3, ST4 and ST5 are displayed as triplicates (indicated by 1/3, 2/3 and 3/3 in the second line of the well assignment).

Finaly, select the rest of the measurement area and perform the well assignment for the sample wells. Use the identifier SM with No. of replicates = 3. This will create triplicates for SM1, SM2, ... SM25 and SM26.

Step 2:

In the Plate Layout screen, go to **Concentration/Dilution values**.

Select the identifier **ST** and define the following concentrations [µg/ml]:

ST1 = 120

ST2 = 350

ST3 = 500

ST4 = 750

ST5 = 1000

Continue with defining dilution factors for the samples. Select identifier SM and enter a factor of 10 for samples SM1 to SM10.



NOTE: If dilution factors for samples are defined, they are considered by the calculation of the unknown sample concentration. E.g. entering a dilution factor of 10 means that the sample has been diluted by 1:10 dilution. The calculated concentration will therefore be multiplied by 10.

Step 3:

Add an Absorbance strip (Name = ABS) to the measurement workflow pane.

Analysis tab:

Step 4:

Add a new Calculation strip and call it ‘Blank reduction’. To define the formula, select the formula type **User-defined** and define:

Fx = x-BL1

The result is the subtraction of the blank value from every single well value.

Step 5:

Add a Standard Curve strip (Name = Standard Curve 1, Input data = Blank reduction) with the analysis type **Linear regression**.

Step 6:

To calculate the average concentration value for every sample, add the second Calculation strip with Input data = Standard Curve 1.

Use the formula type **Predefined** with the predefined formula **Average**.

This will calculate the average concentration value of all replicates for each sample.



NOTE: The predefined formula **Average** calculates the average value of all replicates for every identifier on the plate. The formula function **AVG** calculates the average value of a single identifier only, e.g. Fx = AVG(SM1).

5 Dose-Response Curve

5.1 Dose-Response Curve Strip

A dose-response curve is used to visualize the effect of a drug on the biological system dependent on its dose. To generate the curve, the system response on the y-axis is plotted against the drug concentration on the x-axis. The produced curve reveals valuable insights into the potency and efficacy of the drug.

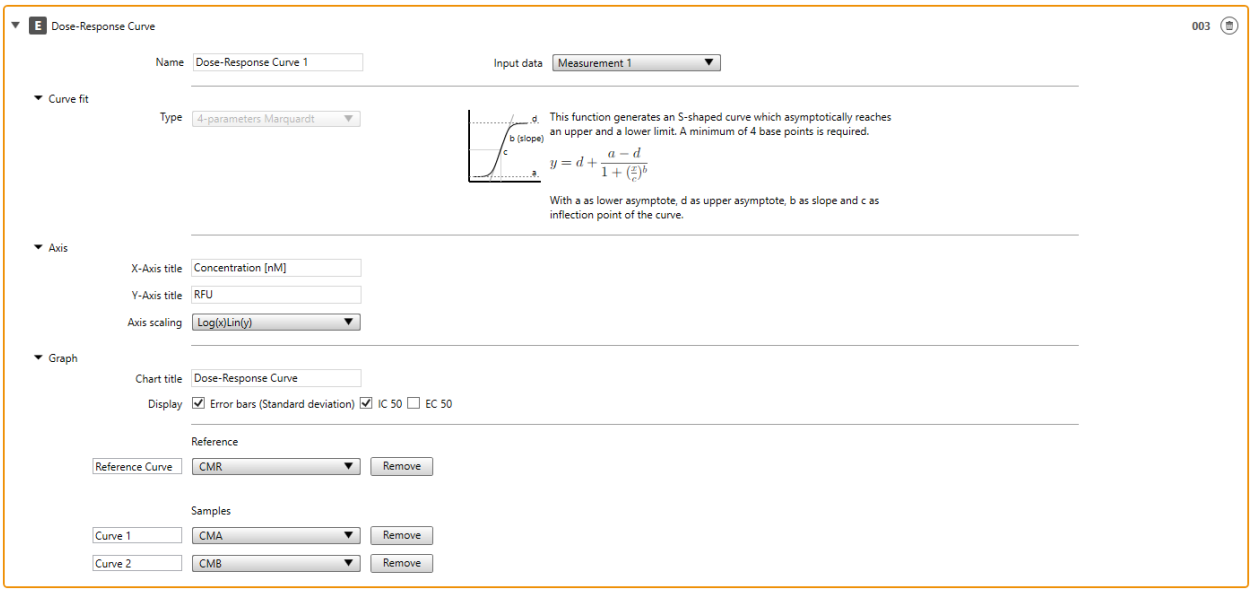


Figure 5: Dose-Response Curve strip

The **Dose-Response Curve** strip includes the following elements:

Name	Define a label name
Input data	Select the Input data to be used for the dose-response curve. Select measurement data or any available analysis results.
Curve fit	Includes information about the applied curve analysis type (Four parameters Marquardt). Not editable for the user.
Axis	
X-Axis title	Enter an axis label.
Y-Axis title	Enter an axis label.
Axis scaling	Select scaling of the axis (does not affect the calculation): • Lin(x)Lin(y): x and y axis are scaled linear • Lin(x)Log(y): x axis is scaled linear, y axis is scaled logarithmically • Log(x)Lin(y): x axis is scaled logarithmically; y axis is scaled linear • Log(x)Log(y): x and y axis are scaled logarithmically
Graph	
Chart title	Enter a label for the graph.
Display	If standards are defined as replicates, select **Error bars** to view a bar showing the range plus/minus standard deviation for each base point. Select **IC50** (half maximal inhibitory concentration)/**EC50** (half maximal effective concentration) to obtain the concentration value which results in 50% of the maximum response.
Samples	A dose-response curve will be created for every selected identifier.

NOTE: Include units in the x-axis and y-axis title to be displayed on the generated standard curve.

NOTE: The Dose-Response Curve strip supports definition of maximum 12 curves. Each curve is related to a compound on the plate. When multiple curves are defined, all curves are plotted on the same graphic.

5.2 Plate Layout

To generate a dose-response curve, the plate layout must contain wells identified as component (CMx, x = A-K) and/or component reference (CMR). The identifiers have to be provided with the corresponding component concentration value. To enter concentrations, open the table **Concentrations, dilution values** in the Plate Layout screen:

Select identifier	Select CMA, CMB, etc. to assign corresponding concentration values. All wells, which match the selected well type identifier, are listed.
Concentration/ Dilution	This table displays all wells in the Identifier column, which match the selected identifier (as from the Select identifier list). The corresponding concentrations values are inserted and listed in the Concentration/Dilution column.
Unit text field	The concentration unit can be determined.

NOTE: Concentration values have to be inserted manually. To save time, copy and paste values from an existing Excel table. In case of multiple dose-response curves, the inserted values for the first compound can be copied and pasted in the tables of the subsequent identifiers if applicable.

5.3 Analysis Results

5.3.1 Dose-Response Curve

The Evaluation plugin calculates and displays:

- Dose-response curve
- Curve parameters
- IC50, EC50 (if selected for display)

5.3.2 Error Handling

For error handling, see chapter 3.3.4.

5.4 Training Point

Exercise 4:

Cells are exposed to an increasing concentration of saponin. Their viability is assessed by measuring fluorescence intensity signal at excitation/emission wavelength = 560/590 nm. Generate the corresponding dose-response curve. Please consider the guidelines below.

Plate layout:

Six replicate wells for blank (cell medium only), untreated cells and cells treated with an increasing concentration of saponin.

Saponin concentration:

250, 62.5, 15.625, 3.906, 0.976, 0.244, 0.061, 0.015, 0.0038 and 0.00095 nM

Calculate for every defined group:

1) average signal of all replicates per compound concentration

2) standard deviation for signal of all replicates per compound concentration

3) blank-corrected average signal

4) normalized blank-corrected average signal in % i.e. treated cells/non-treated cells x 100

Dose-response curve: plot normalized blank-corrected average signal against saponin concentration

5) Z'-value

Solution

Measurement tab:

Step 1:

In the Plate strip, define the measurement area A1-F12. Click **Plate Layout**.

To define identifiers representing replicates of wells treated with different saponin concentrations, select the corresponding area (e.g. A1-F10). Select the identifier CMA with the number of replicates set to 6. Assign it to the selected wells. This will result in the plate layout containing CMA1, CMA2, ...CMA9 and CMA10.



NOTE: To select CMA, select the identifier CM first. This will open a subgroup of identifiers CMA-CMK. Select one identifier per compound used.

Continue with the group of wells filled with non-treated cells. Define them as negative control (NC). Finally, mark the blank wells with the identifier BL.

Step 2:

In the Plate Layout screen, go to **Concentration/Dilution values**.

Select the identifier **CMA** and define the following concentrations [nM]:

CMA1 = 250	CMA6 = 0.244
CMA2 = 62.5	CMA7 = 0.061
CMA3 = 15.625	CMA8 = 0.015
CMA4 = 3.906	CMA9 = 0.0038
CMA5 = 0.976	CMA10 = 0.00095

Step 3:

Add a Fluorescence Intensity strip (Name = FLU 560_590, Ex/Em = 560/590 nm) to the measurement workflow pane.

Analysis tab:

Step 4:

To calculate the average fluorescence intensity value for every identifier, add the first Calculation strip (Name = AVG, Input data = FLU 560/590).

Use the formula type **Predefined** with the predefined formula **Average**.

This will average replicates of all identifiers (CMA1, CMA2, ...) on the plate.

Step 5:

For standard deviation of the measured signals per defined identifier, use a new Calculation strip with the predefined formula **Standard Deviation**.

Step 6:

Add the second Calculation strip (Name = AVG-BL, Input data = AVG). Define blank-corrected average value by using the formula type **User-defined**:

Fx = x-BL1

Step 7:

Include an additional Calculation strip to define the last formula for the signal normalization:

Name = % Viability

Input data = AVG-BL

Formula type = User-defined, Fx = x/NC1*100

Step 8:

Add a Dose-Response curve (Name = Dose-Response curve 1, Input data = % Viability).

Step 9:

Drag & drop a new Calculation strip. Define Z'. Use the predefined formula **Z'**.

Incl. CM1 as positive and NC1 as negative control. Input data = % Viability.

Exercise 5:

Examine the dose-response of a biological system treated with three different compounds on the plate within the same measurement. Include also a known reference compound. Work with replicates. Plot the measured signal versus the corresponding compound concentrations.

Solution

Measurement tab:

Step 1:

In the Plate strip, define the measurement area according to your pipetting pattern. Click **Plate Layout**.

To identify replicates of wells treated with the increasing concentration of the first compound, select the corresponding wells and the identifier CMA.

For the second compound, select the corresponding wells and the identifier CMB.

For the third compound, select the corresponding wells and the identifier CMB.

For the reference compound, select the corresponding wells and the identifier CMR.



NOTE: To select CMA, CMB, CMC..., select the identifier CM first. This will open a subgroup of identifiers CMA-CMK. Select one identifier per compound used.

Step 2:

In the Plate Layout screen, go to **Concentration/Dilution values**.

Select the identifier **CMA** and define the concentrations used for the measurement. Repeat the same procedure for **CMB**, **CMC** and **CMR**.



NOTE: If the compounds are used at the same concentrations, copy and paste the values from CMA to the tables for remaining compounds.

Step 3:

Define the detection setup (e.g. absorbance, cell count, fluorescence intensity).

Analysis tab:

Step 4:

To calculate the average concentration value for every defined identifier, add a Calculation (Name = AVG, Input data as defined in Step 3).

Use the formula type **Predefined** with the predefined formula **Average**.

Step 5:

Add a Dose-Response curve (Name = Dose-Response curve 1, Input data = AVG)

6 ResultAnalyzer

The ResultAnalyzer software is designed for analyzing measurement results after method execution. Raw data, evaluation data and evaluation parameters can be viewed, and data can be re-analyzed.

ResultAnalyzer allows the user to add new analysis strips or modify the strips already included in the originally executed method. Every modification of the method will trigger a re-calculation of all data. These changes are saved in the workspace but will not modify the original method itself. For ResultAnalyzer's general description and structure, see the SparkControl manual.



NOTE: ResultAnalyzer supports post-analysis of measured data for all detection modes (absorbance, imaging, etc.). Few exceptions, though, are related to the applied measurement mode and selected method type: Tecan Apps (e.g. NanoQuant App, Cell Counting/Viability App) as well as workspace with scan data or multiple reads per well (incl. area scan) cannot be opened and subjected to further evaluation in ResultAnalyzer.



CAUTION: Avoid working with ResultAnalyzer and SparkControl in parallel to ensure maximum performance of the ResultAnalyzer software.

6.1 Measurement

When opening a workspace in ResultAnalyzer, all available, measured and analyzed data are shown under **Results/Measurement**. Depending on the executed method, these are raw data, image analysis data, calculated data and graphs.

6.2 Edit Method

To modify or add analysis strips provided by Evaluation plugin, go to **Edit method**. Select a strip in the workflow pane and modify it according to the assay needs.

To add a new analysis strip, select an appropriate strip in the Control panel and add it to the workflow pane by drag & drop or double clicking the strip. For strip parameters, see chapters 3 Calculation, 4 Standard Curve and 5 Dose-Response Curve.



NOTE: In measurements with imaging, analysis strips for (re)analysis of images are located in the **Images** tab. They have to be considered in a separate workflow before making changes in the **Result** screen. The generated image analysis data (e.g. cell count, fluorescence intensity) are available as Input data for the use in the evaluation strips.



NOTE: Every modification of the method will trigger a recalculation of evaluated data. These changes are saved in the workspace but will not modify the original method itself. They have to be exported from the ResultAnalyzer separately.

For recalculation of data, the ResultAnalyzer offers the following options:

Preview	Defined analysis parameters are applied to the subject of analysis but **not saved**. Choose the **Apply** button to save the data.
Apply	Defined analysis parameters are applied to the subject of analysis and **saved**.
Reset	Modified analysis parameters are cleared, and the last saved analysis parameters are restored.

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- 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.)