



Instructions for Use for
infinite M1000



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30036266 06



WARNING
**CAREFULLY READ AND FOLLOW THE INSTRUCTIONS PROVIDED IN
THIS DOCUMENT BEFORE OPERATING THE INSTRUMENT.**

Notice

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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.

Manufacturer



Tecan Austria GmbH
Untersbergstrasse 1A
A-5082 Grödig/Salzburg
AUSTRIA/EUROPE
T: +43 62 46 89 33
F: +43 62 46 72 770
E-mail: office.austria@tecan.com
www.tecan.com

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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 M1000** multifunctional microplate reader. It is intended as reference and instruction for the user.

This document instructs how to:

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

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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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Note

Gives helpful information.



Caution

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



WARNING

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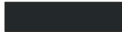
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INDICATES LASER. DO NOT STARE INTO THE BEAM!



WARNING
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THIS SYMBOL INDICATES THE POSSIBLE PRESENCE OF FLAMMABLE MATERIALS AND A RISK OF FIRE. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.



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NEGATIVE ENVIRONMENTAL IMPACTS ASSOCIATED WITH THE TREATMENT OF WASTE.

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- COLLECT WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT SEPARATELY.

Symbols



Manufactured by



Date of manufacture



USB label



Conformité Européenne



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Laser



Bio-hazardous

Table of Contents

1. Safety	9
1.1 Introduction	9
2. General Description	11
2.1 Instrument	11
2.1.1 <i>Instrument Features</i>	11
2.1.2 <i>Intended Use</i>	11
2.1.3 <i>Multifunctionality</i>	11
2.1.4 <i>Performance</i>	12
2.1.5 <i>User Friendliness</i>	12
2.1.6 <i>System Requirements</i>	14
2.2 Measurement Techniques	15
2.2.1 <i>Fluorescence</i>	15
2.2.2 <i>Absorbance</i>	17
2.2.3 <i>Luminescence</i>	18
2.3 Software	20
2.3.1 <i>i-control</i>	20
2.3.2 <i>Magellan</i>	20
3. Installation	21
3.1 Unpacking & Inspection	21
3.1.1 <i>Inspection of Delivered Packaging</i>	21
3.1.2 <i>Unpacking Instructions</i>	21
3.2 Plate Carrier Transport Lock	23
3.3 Power Requirements	25
3.4 Switching the Instrument ON	25
3.5 Preparing the Infinite M1000 for Shipping	27
3.6 Instrument Dimensions	28
3.6.1 <i>Infinite M1000 Instrument</i>	28
3.6.2 <i>Infinite M1000 Instrument with Built-in Stacker</i>	29
3.6.3 <i>Injector Module Dimensions</i>	30
4. Optical System	31
4.1 Fluorescence Intensity System	31
4.1.1 <i>Light Source System Fluorescence Intensity</i>	32
4.1.2 <i>Fluorescence Top/Bottom Optics</i>	34
4.1.3 <i>Fluorescence Intensity Detection</i>	35
4.1.4 <i>Luminescence Scan</i>	36
4.2 Fluorescence Polarization System	37
4.2.1 <i>Light Source System Fluorescence Polarization</i>	37
4.2.2 <i>Fluorescence Polarization Optics</i>	38
4.2.3 <i>Fluorescence Polarization Detection</i>	40
4.2.4 <i>Fluorescence Polarization Measurement parameters</i>	40
4.3 Absorbance System	41
4.3.1 <i>Absorbance Optics</i>	42
4.3.2 <i>Absorbance Detection</i>	43
4.4 Luminescence System	44

4.4.1	<i>Luminescence Optics</i>	45
4.4.2	<i>Luminescence Detection</i>	47
5.	Operating the Infinite M1000	49
5.1	Introduction	49
5.2	General Operating Features	50
5.2.1	<i>Instrument Start Up</i>	50
5.2.2	<i>Finish a Measurement Session</i>	51
5.2.3	<i>General Options</i>	51
5.3	Optimize Fluorescence Measurements	52
5.3.1	<i>FI Scanning (Spectral Intensity Calibration)</i>	52
5.3.2	<i>FP Measurements</i>	54
5.3.3	<i>Instrument Parameters</i>	56
5.3.4	<i>FI Ratio Mode</i>	61
5.3.5	<i>Optimal Read (FI Bottom measurements only)</i>	61
5.3.6	<i>Measurement Accessories</i>	63
5.4	Optimize Absorbance Measurements	63
5.4.1	<i>Measurement Parameters</i>	63
5.4.2	<i>Absorbance Ratio Mode</i>	64
5.4.3	<i>Measurement Accessories</i>	64
5.5	Optimize Luminescence Measurements	65
5.5.1	<i>Integration Time</i>	65
5.5.2	<i>Light Level Attenuation</i>	65
5.6	Injectors	66
5.6.1	<i>Measurement with Injectors</i>	67
5.6.2	<i>Storage Bottles and Bottle Holders</i>	67
5.6.3	<i>Injector Carrier</i>	68
5.6.4	<i>Priming and Washing of the Injector(s)</i>	69
5.6.5	<i>Injector Modes and Settings (i-control)</i> '	75
5.6.6	<i>Injector Cleaning and Maintenance</i>	78
5.6.7	<i>Injector Reagent Compatibility</i>	80
5.7	Built-in Stacker	81
5.8	Barcode Scanner	82
6.	Instrument Specifications	85
6.1	Introduction	85
6.2	Technical Specifications	86
6.3	Fluorescence Intensity and Time Resolved (TRF)	87
6.3.1	<i>Definition of the Detection Limit:</i>	87
6.3.2	<i>Fluorescein (Fluorescence Intensity) Top</i>	87
6.3.3	<i>Fluorescein (Fluorescence Intensity) Bottom</i>	88
6.3.4	<i>Europium (Time Resolved Fluorescence)</i>	88
6.3.5	<i>HTRF[®] (Time Resolved Fluorescence)</i>	88
6.4	Fluorescence Polarization	89
6.4.1	<i>Fluorescein 1nM (fluorescence polarization)</i>	89
6.5	Absorbance	89
6.6	Luminescence	90
6.6.1	<i>ATP Glow Luminescence</i>	90
6.7	Flash Type Luminescence	91

6.8	Dual Color Luminescence (e.g. BRET)	91
6.9	“On the Fly” Measurements.....	92
6.10	Injectors.....	92
6.10.1	Injector Performance.....	92
7.	Quality Control	93
7.1	Periodic Quality Control Tests	93
7.2	Definitions	94
7.2.1	Detection Limit (LOD)	94
7.2.2	Uniformity.....	94
7.2.3	Linearity	94
7.2.4	Accuracy	94
7.2.5	Cross-Talk.....	95
7.2.6	Repeatability (Reproducibility)	95
7.3	Acceptance Criteria	95
7.4	Test Instructions.....	96
7.4.1	Fluorescence Intensity	96
7.4.2	Time Resolved Fluorescence.....	104
7.4.3	Fluorescence Polarization (FP).....	106
7.4.4	Luminescence.....	108
7.4.5	Absorbance.....	112
8.	Cleaning & Maintenance	117
8.1	Introduction.....	117
8.2	Liquid Spills	117
8.3	Instrument Decontamination/Disinfection.....	118
8.3.1	Decontamination/Disinfection Solutions.....	118
8.3.2	Decontamination/Disinfection Procedure	119
8.3.3	Safety Certificate.....	120
8.4	Disposal.....	121
8.4.1	Disposal of Packing Material.....	121
8.4.2	Disposal of Operating Material.....	122
8.4.3	Disposal of the Instrument	122
9.	Error Messages and Troubleshooting	123
9.1	Error Messages Introduction	123
	Index.....	127
	Tecan Customer Support.....	129

1. Safety

1.1 Introduction

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 an **Infinite M1000** instrument.
5. Never force a microplate into the instrument.
6. Observe proper laboratory safety precautions, such as wearing protective clothing (powder-free gloves, safety glasses, surgical mask and protective clothing, etc. ...) and using approved laboratory safety procedures.



Caution

Tecan Austria GmbH have taken great care when creating the stored Plate Definition Files (.pdfx) that are received with the instrument. We take 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 adds 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. The safety gap cannot be calculated by Infinite M1000 if the plate used does not match the .pdfx selected.

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



Caution

Before starting measurements, make sure that the microplate position **A1** is inserted correctly.



Caution

To insure the optimal working of Tecan instruments we recommend a service interval of 1 year.

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 the INFINITE M1000 may come in contact with biohazardous/infectious material. Make sure that only qualified personnel is operating the instrument. In case of service or when dislocating or disposing the instrument, always disinfect the instrument according to the instructions given in this manual.

2. General Description

2.1 Instrument

2.1.1 Instrument Features

The Tecan **Infinite M1000** is a multifunctional monochromator based microplate reader that provides high performance for the vast majority of today's microplate applications and research. The **Infinite M1000** shows exceptional flexibility in wavelength selection for absorbance and fluorescence measurements and also enables the recording of absorbance and fluorescence spectra.

In addition to offering absorbance and fluorescence intensity measurements, the **Infinite M1000** allows the measurement of fluorescence polarization and luminescence. The **Infinite M1000** is also robotic compatible and offers a built-in stacker option as well as an external injector module (see picture below).

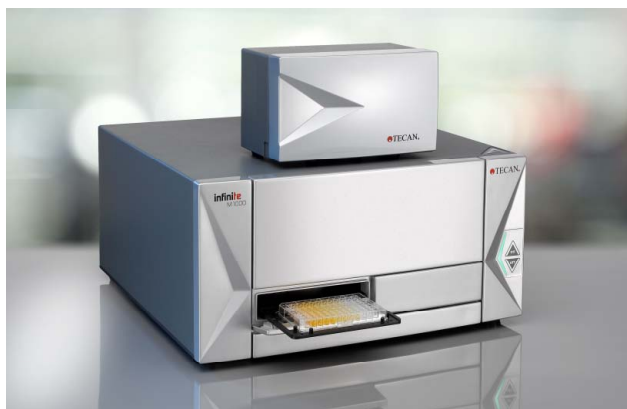


Figure 1: Infinite M1000 with injector box.

2.1.2 Intended Use

The **Infinite M1000** is intended as a general purpose laboratory instrument (Europe) and is a Class I General Controls medical device (U.S.) for professional use, supporting common microplates conforming to the ANSI/SBS standards.

2.1.3 Multifunctionality

The fully equipped instrument (all options installed) provides you with the following measurement techniques:

- Absorbance
- Fluorescence Intensity Top (FRET)
- Fluorescence Intensity Bottom
- Time Resolved Fluorescence (TRF, TR- FRET)
- Fluorescence Polarization
- Luminescence (Glow Type, Flash Type and Dual-color)
- Luminescence Scan

Every standard microplate ranging from 6 to 1536-well formats with a maximum plate height of 23 mm including lid can be measured with any of the above measurement techniques. Switching between measurement techniques or plate formats is fully automated: **NO** manual adjustments are necessary for the **Infinite M1000**. Injectors are available for microplates from 6 to 384 wells.

Tecan provides a cuvette adapter for four standard cuvettes (e.g. Hellma 110 QS). The cuvette has to be inserted in a horizontal direction and must be closed tightly to avoid any liquid leakage.

2.1.4 Performance

The **Infinite M1000** has been designed to be sensitive while fast. Specifications of sensitivity or precision are related to the corresponding measurement time per microplate.

Measurement results can be optimized for different assay types (cell-based or homogeneous), for different microplate types, and for different volume dispensing per well. For Fluorescence Top Reading this is accomplished by a lens system, which can be positioned within the instrument to a specific measurement height. This adjustment can be made automatically.

2.1.5 User Friendliness

The **Infinite M1000** offers unparalleled flexibility in wavelength selection for fluorescence intensity and absorbance measurements. Any wavelength within the specified wavelength range can be easily adjusted by the user via software.

In fluorescence mode also the bandwidth can be selected by software. In addition to single wavelength measurements, absorbance and fluorescence spectra can be recorded. The measurement of spectra is possible over the entire wavelength range.

Onboard Control Buttons

In addition to the main power switch on the back panel of the instrument, the **Infinite M1000** also has onboard control buttons to simplify some common tasks (see picture below).

An '**On/Off**' button is available on the front to easily switch the instrument on and off. The '**Retract/Eject**' button allows microplates to be inserted or removed from the instrument without software activation. The '**Quick-Start-Script**' button is used to start favorite measurement scripts directly from the instrument (for further details, see the Instructions for Use for i-control software).



Figure 2: Onboard control buttons of **Infinite M1000**. The '**Quick-Start-Script**'-Button and the **Retract-/Eject-Button** are located in the front right corner of the top cover. The '**on/off**'-button is located on the front of the instrument.



Caution

If the instructions given in this Instructions for Use are not performed correctly, the instrument will either be damaged or the procedures will not be performed correctly and the safety of the instrument cannot be guaranteed.

2.1.6 System Requirements

	Minimum	Recommended
PC	<u>Windows XP/Vista (32-bit)/Windows 7 (32- or 64-bit):</u> Windows compatible PC with a Pentium compatible processor running at 1 GHz	2 GHz (Dual Core)
Operating System	Windows XP (32-bit) SP3 Windows Vista (32-bit) Windows 7 (32-bit) Windows 7 (64-bit)	Windows XP (32-bit) SP3
Memory	<u>Windows XP:</u> 512 MB RAM <u>Windows Vista (32-bit):</u> 1 GB RAM <u>Windows 7 (32-bit):</u> 1 GB RAM <u>Windows 7 (64-bit):</u> 2 GB RAM	1 GB RAM 2 GB RAM 2 GB RAM 3 GB RAM
Space Requirements	700 MB	1 GB
Monitor	Super VGA Graphics	
Resolution	1024 x 768	1280 x 1024
Color Depth	256	
Mouse	Microsoft mouse or compatible pointing device	
Communication	1 x USB 2.0	2 x USB 2.0, 1 x RS232 (Serial)
Devices	1 x CD-ROM drive <u>Windows Vista:</u> DirectX 9 graphics and 32 MB of graphics memory (for Home Basic); 128 MB of graphics memory plus WDDM support for all other versions <u>Windows 7:</u> DirectX 9 graphics device with WDDM 1.0 or higher driver	
.NET	Microsoft .NET Framework 2.0 If this version is not present, the install/upgrade program will install it side-by-side with any existing installations of the .NET Framework.	
Windows Installer	3.1 If this version is not present, the install/upgrade program will install it.	
Microsoft Excel	2002 2003 2007 2010 (32-bit) – Starter edition NOT supported!	

2.2 Measurement Techniques

The following sections provide an introduction to the **Infinite M1000** measurement techniques. To keep this chapter compact, a few simplifications have been made. For details see the references.

2.2.1 Fluorescence

Infinite M1000 offers the basic fluorescence measurement technique and some even more sophisticated variants:

- A. Fluorescence Intensity (FI, or simply Fluorescence)
- B. Fluorescence Time Resolved (TRF)
- C. Fluorescence Polarization (FP)

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 fluorescence polarization (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 (TR-FRET). Fluorescence Time Resolved (TRF) should not be confused with Fluorescence Lifetime Measurements.

Fluorescence (An Abstract)

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 fast 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.

Flash Fluorescence and FI Kinetic

For high sensitivity Flash Fluorescence assays, the measurement is done just after dispensing the activating reagent or after a short delay time.

The measurement position is not identical to the injector position. The movement between measurement position and inject position takes ≤ 500 ms.

Fluorescence Resonance Energy Transfer (FRET)

Some microplate applications utilize a sophisticated dual labeling strategy. The Fluorescence Resonance Energy Transfer effect (FRET) 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. And secondly, the emission spectrum of the donor has to 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 – see section 19 Homogeneous, Time-Resolved Fluorescence Method for Drug Discovery* by Alfred J. Kolb et al.).

B) Fluorescence Time Resolved (TRF)

TRF applies to a class of fluorescent labels (chelates) of lanthanides like Europium, (Ref. *Europium and Samarium in Time-Resolved Fluoroimmunoassays* by T.Ståhlberg et.al. - *American Laboratory*, December 1993 page 15), some of them having fluorescence lifetimes in excess of 100 microseconds. The **Infinite M1000** 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.

Homogenous Time Resolved Fluorescence (HTRF)

HTRF technology combines both, time-gated fluorescence (commonly referred to as time-resolved fluorescence = TRF) and fluorescence resonance energy transfer (FRET). HTRF is based on the energy transfer between two fluorescent labels, a long-lifetime Eu^{3+} -cryptate donor and the XL665 acceptor (chemically modified allophycocyanin). The main benefit of time gated measurement is the efficient reduction of background fluorescence by temporal discrimination. The addition of energy transfer further minimizes several undesired assay interferences and side effects (e.g. volume/meniscus, quenching, light scattering, autofluorescence, molecular size, etc.). Furthermore, the homogenous format of these assays, so called 'mix and measure' protocols, satisfies the demand from the industry for one-step, non-separating applications for high throughput screening (HTS).

The measurement is based on sequential detection of donor intensity (620 nm) and acceptor intensity (665 nm) using the multilabeling setup. A ratio of the two intensities (acceptor:donor) is calculated and the relative energy transfer rate for each sample is determined as Delta F (%). The fluorescence ratio is a correction method developed by CIS bio international, which application is limited to the use of HTRF[®] reagents and technology, and for which CIS bio international has granted a license to Tecan. The method is covered by the US patent 5,527,684 and its foreign equivalents.

C) Fluorescence Polarization (FP)

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

Fluorescence Polarization is defined by the following equation:

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

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

FP is suitable for binding studies, because tumbling of molecules may be dramatically reduced after binding to a much larger site, and vice versa.

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

The FP measurement result will be calculated from two successive Fluorescence Intensity measurements. They differ in the mutual orientation of polarizing filters, one being placed behind the excitation filter, another ahead of the emission filter. 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.

For further information see:

High Throughput Screening by Marcel Dekker Inc. 1997 New York, Basel, Hong Kong – see section Fluorescence Polarization by J.R. Sportsman et al.

Polarization De La Lumière De Fluorescence Vie Moyenne Des Molécules Dans L'état Excité by M. Francis Perrin (Journal de Physique No:12, 1926).

2.2.2 Absorbance

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

$$A = \text{LOG}_{10} (I_0 / I_{\text{SAMPLE}}).$$

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 O.D. (Optical Density).

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

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

0.1 O.D. means $10^{0.1}$ or 1.26-fold attenuation (3.85% 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 Law).

2.2.3 Luminescence



Caution

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

Glow Type Chemi- or Bioluminescence

The **Infinite M1000** 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. Like enzyme-based assays, control of environmental conditions is rather critical (temperature, pH-value).

For practical aspects of luminescence assays see:

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

Flash Type Luminescence (with Injectors)

In flash type luminescence assays the measurement is only done during the dispensing of the activating reagent or after a short delay time.

Flash type luminescence is one of the measurement modes that can be performed with injectors.



Note

The plate detection sensor is only active if one of the injectors is in use (strips "injection" or "dispense").



Note

During luminescence measurements it is important to close the lid covering the syringes and bottles of the reagent system to minimize background signal.

Dual Color Luminescence

Selected assays emit light of two different wavelengths at the same time. For these assays wavelength discrimination during luminescence detection may be required.

Tecan luminescence filters are optimized for the Chroma-Glo™ Luciferase assay system, for BRET and for BRET²™. Filters are built into the luminescence filter wheel according to the demands of the applied assay:

- 'Lumi Magenta': wavelength range of 370 to 450 nm and 610 to 700 nm
- 'Lumi Green': wavelength range of 510 to 540 nm
- 'Lumi Blue 1': wavelength range of 370 to 480 nm
- 'Lumi Green 1': wavelength range of 520 to 570 nm

The Chroma-Glo luciferase assay generates red and green (dual-color) luminescence from two luciferases within a single well and upon a single reagent addition. This homogenous dual-reporter gene assay permits each reporter to be measured independently by detecting one well at two different wavelengths (red and green).

Luminescence Scan

The **Infinite M1000** is capable of recording emission spectra of luminescent signals. Luminescence substrates providing stable light output are required for luminescence scans.

As an example, emission spectra of different luciferase types (new recombinantes of Renilla or Firefly luciferase) can be recorded in order to define emission maxima. Also environmental influences on the spectral behaviour of luciferases can be studied (pH-value, solvent, buffer).

The luminescence scanning procedure is operated by the fluorescence emission optics, therefore additional information on the luminescence scan can be found in chapter 4.1 Fluorescence Intensity System and chapter 5.3 Optimize Fluorescent Measurements.



Note

Luminescence scan is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

2.3 Software

The **Infinite M1000** is delivered with the *i-control* software including online-help and a printed Instructions for Use. The software is formatted as a self-extracting archive on CD-ROM.

For advanced data reduction, *Magellan* software can be used to control the **Infinite M1000**.

For robotic automation **Infinite M1000** is compatible with *EVOware* (For more information, contact your local Tecan representative).

2.3.1 *i-control*

The *i-control* software is a user interface for stand-alone operation of the **Infinite M1000**. (For more detailed information, please refer to the Instructions for Use for *i-control*). The *i-control* software presents the raw data for further use in Excel.

2.3.2 *Magellan*

One main advantage of *Magellan* is that data processing capabilities are included. In *Magellan*, data is organized and managed as follows:

Methods can be defined around a test. Within *Magellan* a method includes a test, measurement parameters, and several options for data handling. Methods are assay and instrument specific.

Workspaces can be built around methods. After performing a method, the processed data will be addressed with unique sample identifiers for reporting within a *Magellan* workspace. The workspace integrates sample, assay, and instrument specific data.

The *Magellan* architecture provides a safe and easy to use interface, especially in a multi-user laboratory environment. **Magellan Tracker** offers all the functionality to become compliant with the FDA Regulation 21 CFR part 11.

Magellan provides measurement data acquisition and customized data reduction for your specific assays. For details see the Instructions for Use for *Magellan*.

3. Installation

3.1 Unpacking & Inspection

3.1.1 *Inspection of Delivered Packaging*

The delivered packaging includes the following:

- OOB Quality Report
- Final test protocol
- Software (disk or CD-ROM)
- Cables (USB 2.0 and main)
- Transport lock (mounted)
- This Instructions for Use for Infinite M1000 and the IFU for i-control

Each injector module packaging includes the following:

- Bottle holder
- Beaker for priming
- 125 ml bottle (light protective)
- 15 ml bottle (light protective)
- Injector dummy (mounted)
- Waste tub for plate carrier

3.1.2 *Unpacking Instructions*

Before installing abide by the following instructions:

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 and injector 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 fully equipped Infinite M1000 is a precision instrument and weights approximately 29.5 kg. At least two people must carefully lift the instrument from the box.

**Caution**

The maximum load for the Infinite M1000 cover is 20 kg, however the load must be distributed evenly across the entire surface of the cover.

**Caution**

The maximum load for the Infinite M1000 plate transport is 300 g. Overloading of the plate carrier cause instrument damages and service.

Plate carrier testing and wavelength calibration with Multicheck-Plus Testplate should be done annually to assure good performance of the Infinite M1000.

**Caution**

Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment. Do not cover instrument while operating.

3.2 Plate Carrier Transport Lock



Caution

Remove the transport lock before operating the instrument.

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



Caution

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

1. Switch ON the computer and install the corresponding software on the computer (i-control, Magellan or EVOware).
2. 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.
3. Connect the computer to the instrument only with the delivered USB interface cable.
4. Insert the power cable into the main power socket (with protective earth connection) in the back panel of the instrument.
All connected devices must be approved and listed as per IEC 60950-1 Information Technology Equipment – Safety or equivalent local standards.
5. Open the plate door manually and loosen the two outer screws from the Transport Lock (2.5 mm Allen key is supplied).
6. Switch ON the instrument using the main power switch on the back panel of the instrument.
7. Switch ON the computer and start the corresponding software on the computer (i-control, Magellan or EVOware).
8. Connect the Infinite M1000 instrument via the software.
9. The software displays a message stating that the instrument is parked and requests the loosening of the two outer screws from the Transport Lock - confirm with **OK**.



Figure 3

- 10. The plate carrier moves out.
- 11. The software displays a message requesting the loosening of the two remaining screws of the Transport Lock.
- 12. Loosen the two remaining screws and remove the transport lock and confirm the software message by clicking **OK**.

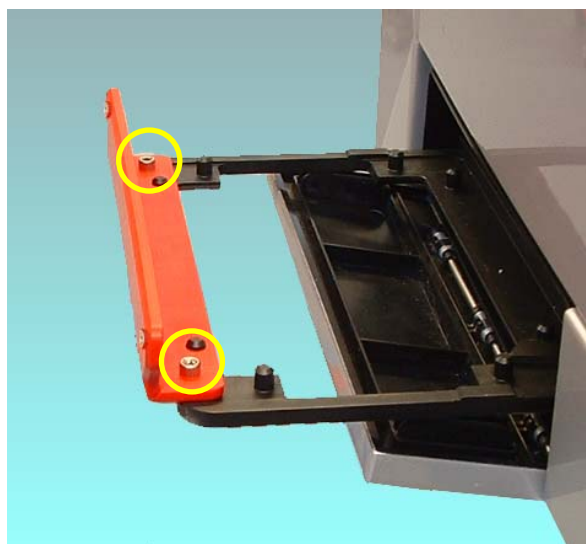


Figure 4

- 13. The instrument will initialize and is then ready for use.



Caution
Save packing materials and transport locks for further transportation purposes. The Infinite M1000 must be shipped only with the original packing and installed transport locks.

3.3 Power Requirements

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

The voltage range is from 100 – 120 V and 220 – 240 V, 50/60 Hz.

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

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



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.

3.4 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.

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



WARNING

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



Caution

When installing or uninstalling the instrument, ensure that the instrument and the computer are both switched off and disconnected from the main power supply before the USB interface cable or any other cables are connected or removed.

Rear View

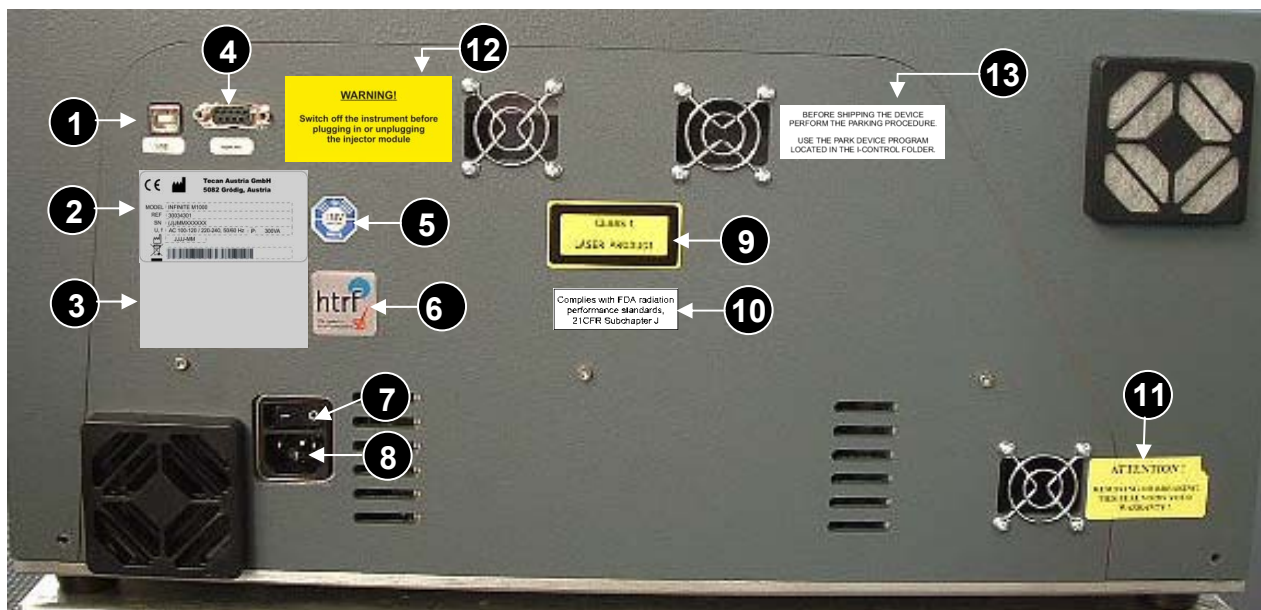


Figure 5

- 1 USB Connection
- 2 Name Plate
- 3 Label – Options/Configuration
- 4 RS 232 Serial Connection
- 5 Label – Technical Inspection Agency
- 6 HTRF Label
- 7 Main Power Switch
- 8 Main Power Socket
- 9 Label – Class 1 Laser Product
- 10 Complies with FDA radiation performance standards, 21 CFR Subchapter J
- 11 Warranty Label
- 12 Warning Label: Warning! Switch off the instrument before plugging in or unplugging the module
- 13 Label: Before shipping the device perform the parking procedure. Use the park device program located in the i-control folder.

**Caution**

Only Tecan authorized service technicians are allowed to open the instrument. Removing or breaking the warranty seal voids the warranty.

**WARNING**

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

3.5 Preparing the Infinite M1000 for Shipping

Before shipping the **Infinite M1000**, the measurement head has to be parked to avoid any damage to the optics and plate transport. This must be performed only by a Tecan service technician; please contact your local Tecan representative.

**BEFORE SHIPPING:**

THE MEASUREMENT HEAD MUST BE PARKED AND THE TRANSPORT LOCK MUST BE MOUNTED BEFORE SHIPPING AND THIS MUST BE PERFORMED ONLY BY A TECAN SERVICE TECHNICIAN.

IF THE INSTRUMENT IS SHIPPED WITHOUT THESE SAFETY MEASURES, THE INSTRUMENT GUARANTEE IS RENDERED NULL AND VOID. USE ORIGINAL PACKAGING FOR SHIPPING.

3.6 Instrument Dimensions

3.6.1 Infinite M1000 Instrument

Front View

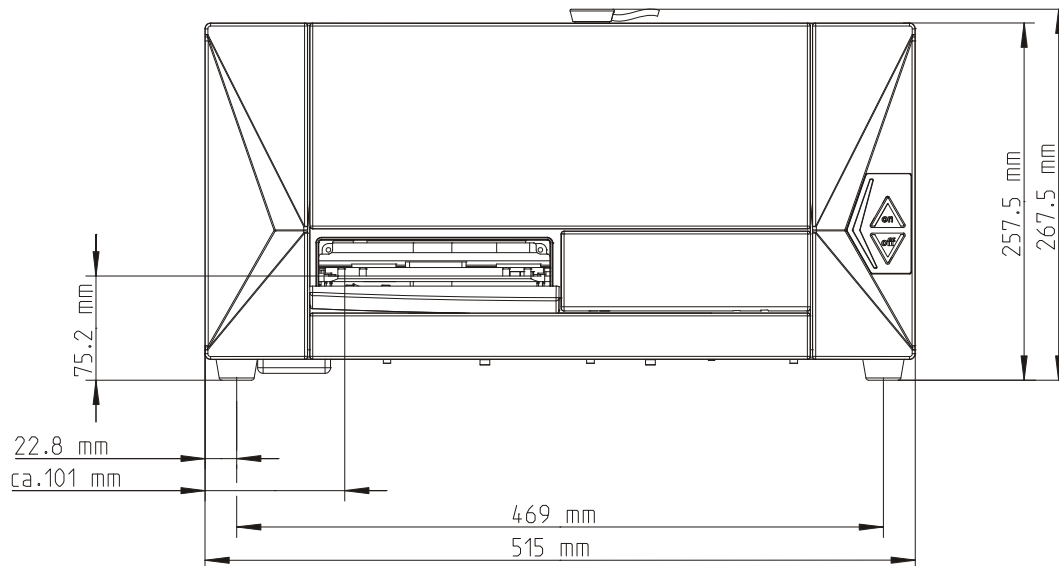


Figure 6

Side View

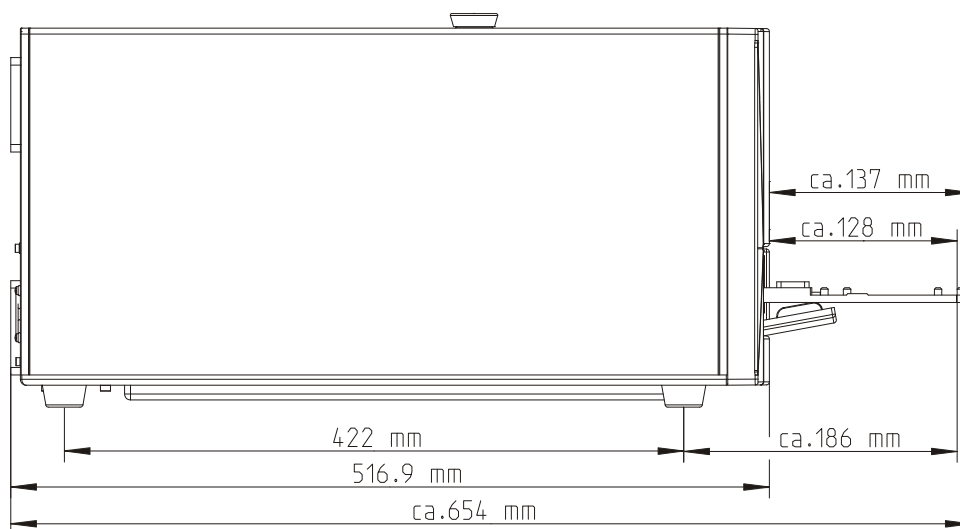


Figure 7

3.6.2 Infinite M1000 Instrument with Built-in Stacker

Front View with Built-in Stacker

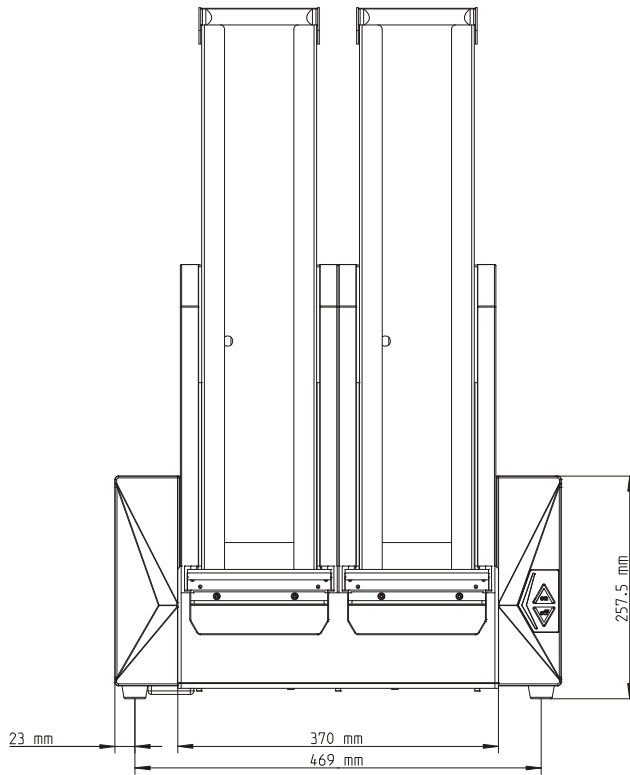


Figure 8

Side View with Built-in Stacker

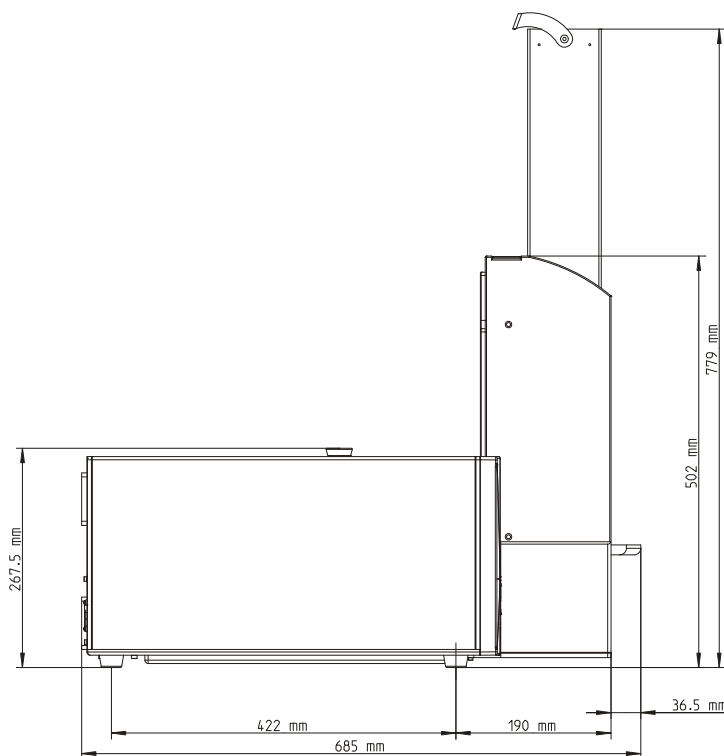


Figure 9

3.6.3 Injector Module Dimensions

Front View

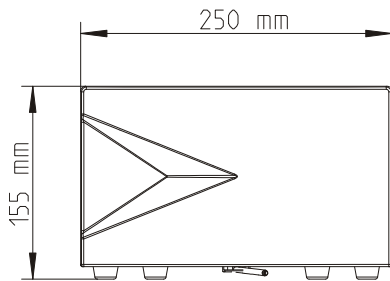


Figure 10

Side View

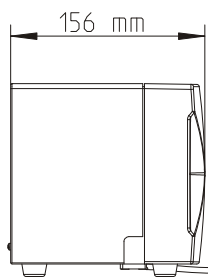


Figure 11

4. Optical System

4.1 Fluorescence Intensity System

The **Infinite M1000** fluorescence optical system is sketched below. The path of fluorescence top light goes from the light source, to and from the top measurement head and to the PMT. The path of fluorescence bottom light goes from the light source, to and from the bottom measurement head and to the PMT. The system consists of:

1) the light source system, 2) the fluorescence top optics, 3) the fluorescence bottom optics and 4) the fluorescence detection unit.

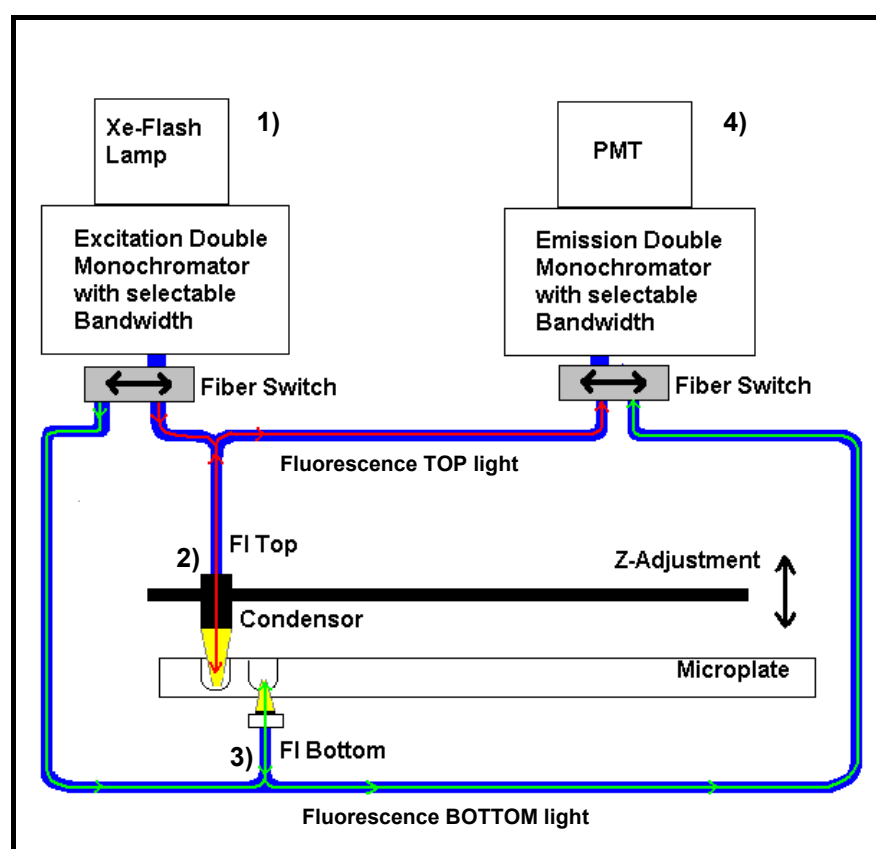


Figure 12: Optical System Fluorescence Top and Bottom

4.1.1 Light Source System Fluorescence Intensity

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

The **Infinite M1000** light source system is built from the following components:

1. Flash lamp
2. Condensing optics
3. Order sorting filter wheel
4. Excitation double monochromator
5. Fiber optic bundle
6. Flash lamp monitor

Flash Lamp

The **Infinite M1000** 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 **Infinite M1000** 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:

- a) High intensity from the deep UV to the near IR
- b) Very long lifetime
- c) Many applications - only one kind of lamp
- d) No warm up time required

Condenser

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

Order Sorting 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 monochromator is used to select any desired wavelengths from the flash lamp spectrum in the range from 230 nm to 850 nm for fluorescence intensity and from 230 nm to 1000 nm for absorbance applications.

Fluorescence emission spectra in many cases do not depend on the exact excitation wavelength. For a maximum total fluorescence signal, therefore, rather broad excitation bandwidth may be used. **For measurements > 300 nm the bandwidth can be selected continuously from 5 nm to 20 nm in 1 nm steps. For measurements ≤ 300 nm the bandwidth can be selected continuously from 2.5 to 10 nm in 0.5 nm steps.**

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

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 **Infinite M1000** 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 (bandpass) passes through the exit slit. This means that when the monochromator entrance slit is illuminated with white light, only light with a specific wavelength (monochromatic light) passes through the exit slit. The wavelength of this light is set by the rotation angle of the optical grating. The bandwidth is set by the width of the exit slit. The bandwidth is defined as Full Width at Half Maximum intensity (FWHM).

Monochromators block undesired wavelengths, typically amounting to 10^3 . This means when the monochromator is set for light with a wavelength of 500 nm and the 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 **Infinite M1000**, a double monochromator is installed on both the excitation and detection side. This allows easy selection of arbitrary excitation and emission wavelengths.

Fiber optic bundle

From the exit slit of the excitation monochromator, the light will be coupled into a fiber optic bundle guiding the light either to the top measuring optics or the bottom measuring optics (Figure 12). 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 lamp 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.

4.1.2 Fluorescence Top/Bottom Optics

Flash light enters the optical system being focused by the condenser onto the entrance slit of the excitation monochromator. The wavelength and bandwidth of the excitation light is selected within the monochromator. After passing the monochromator, the excitation light is coupled into a fiber bundle guiding the light to the top or bottom measuring head. The light is then focused into the sample by the top/bottom lens system (Figure 13, left hand side).

The fluorescence emission light is collected by the top/bottom lens system again, coupled into the fluorescence fibers bundle (Figure 13, right hand side) and guided to the detection system.

Z-Positioning (Top Fluorescence only)

The **Z-position** of the fluorescence top optics fixed onto the optics carrier can be adjusted. As light is refracted at the sample liquid surface z-adjustment helps to maximize signal to noise.

The fluorescence measuring Optics Top and Bottom is built from the following components:

1. Fluorescence Intensity Lens system Top/Bottom
2. Fluorescence Fiber Bundle

Fluorescence Intensity Lens System

The exit side of the bundle acts as a color specific light source. The lens system at the end of the excitation top fiber and excitation bottom fiber is designed to focus the excitation light into the sample and also 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.

Fluorescence Fiber Bundle

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.

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 **Infinite M1000** can automatically select between two available orifice diameters depending on the type of microplate required. **For microplates up to 384 wells a spot size of about 2 mm is used. For microplates with 1536 wells a spot size of 1 mm is used.**

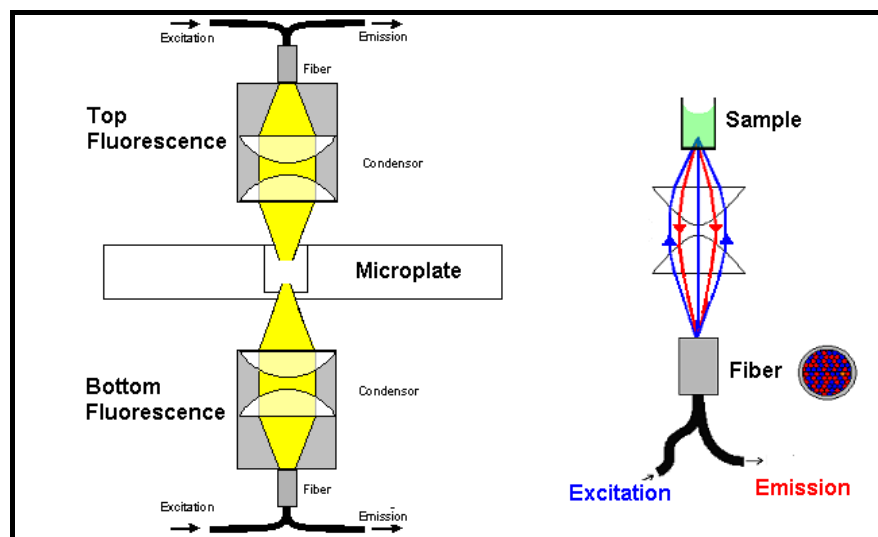


Figure 13: Fluorescence Optics used for Top/Bottom Fluorescence intensity measurement (the fiber details on the right hand side are shown for the bottom measurement which is comparable to the top optics).

4.1.3 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: photo-multiplier tube, Figure 12). Between the monochromator and the PMT a filter wheel is located (read below).

The Fluorescence Detection system is built from the following components:

1. Emission Monochromator
2. Filter wheel PMT
3. PMT Detector

Emission Monochromator

Similar to the excitation monochromator, the emission monochromator is used to select any wavelength of the fluorescence signal. It acts like an adjustable filter in wavelength and bandwidth to discriminate scatter of excitation light and nonspecific fluorescence. For a maximum total fluorescence signal, therefore, rather broad excitation bandwidth may be used. **The bandwidth can be selected from 5 nm to 20 nm in 1 nm steps.**

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 dedicated fluorescence PMT of the **Infinite M1000** 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 chapter 5.3.3 Instrument Parameters.

4.1.4 Luminescence Scan

The **Infinite M1000** is capable of recording emission spectra of luminescent signals by using the fluorescence top or bottom emission optics. The light emitted by the luminescent sample is collected by the top/bottom lens system, coupled into the emission fiber bundle and guided to the emission monochromator. The emission monochromator is used to select any wavelength from 280 nm to 850 nm of the luminescent signal, after passing the emission monochromator the light is focused onto the detector (PMT: photo-multiplier tube). Results are given in relative luminescence units (RLU). The bandwidth can be selected from 5 nm to 20 nm in 1 nm increments. The integration time can be selected from 1 ms to 1 s.



Note

Luminescence scan is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

4.2 Fluorescence Polarization System

The **Infinite M1000** Fluorescence Polarization System consists of the following parts (see figure below): LEDs 1), polarization optics 2), emission double monochromator unit (3), and detection unit (4).

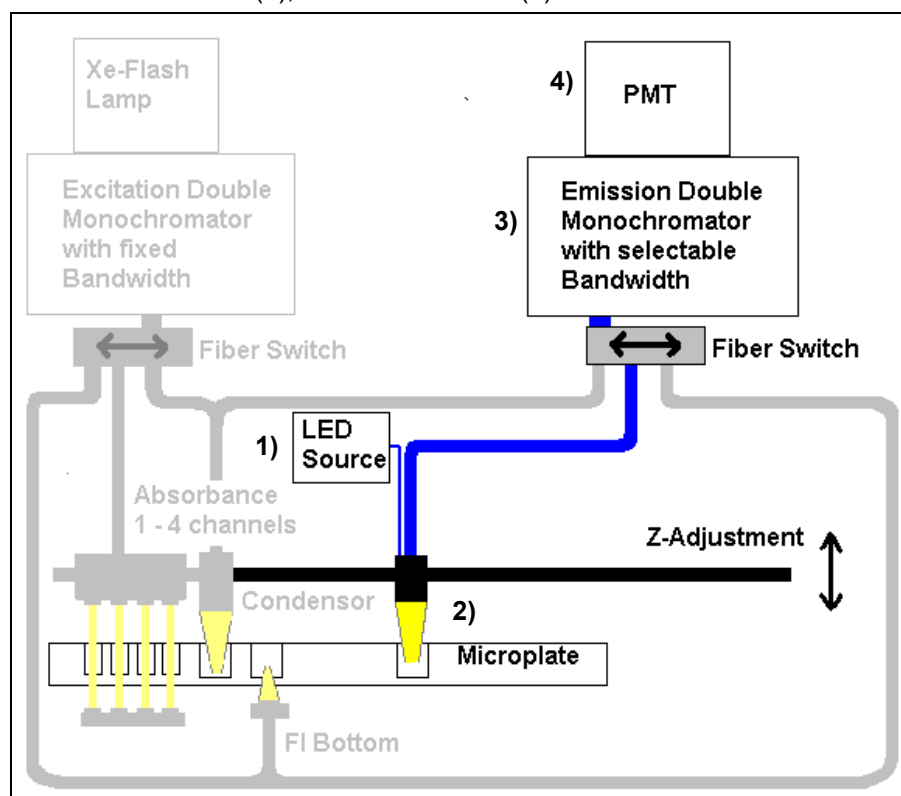


Figure 14: Optical System Fluorescence Polarization.

4.2.1 Light Source System Fluorescence Polarization

The Polarization light source system is built from the following components:

1. LED Light Source
2. Lens system
3. Polarization Optics
4. Fiber optic bundle

LED Light Source

For uncompromising performance, the **Infinite M1000** utilizes high-performance LEDs for fluorescence polarization measurements instead of a Xenon flash lamp used for fluorescence intensity measurements. Four different LEDs with the following central wavelength are installed on **Infinite M1000**: LED 1: 470 nm; LED 2: 530 nm; LED 3: 590 nm; LED 4: 635 nm.

The main benefits of LEDs are:

- Improved excitation energy compared to monochromator system
- No warm up time required

Lens System

This system is made from 3 lenses. Lens 1 collects and aligns the LED light so that it is parallel, lens 2 focuses the polarized light into the wells and collects the more or less depolarized emission light from the sample, and lens 3 focuses the emission light onto the fiber optic bundle (Figure 14) guiding the light to the emission monochromator system. The lenses are made from fused silica. This material provides high UV transmission and is virtually void of auto-fluorescence.

Fiber Optic Bundle

The fiber bundle guides the emission light to the detector.

4.2.2 Fluorescence Polarization Optics

The excitation light for the FP measurement is generated by the 4 different LED passing appropriate interference filter and dichroic mirrors (Figure 14).

Figure 15 shows the spectrum of the resulting light exciting the sample.

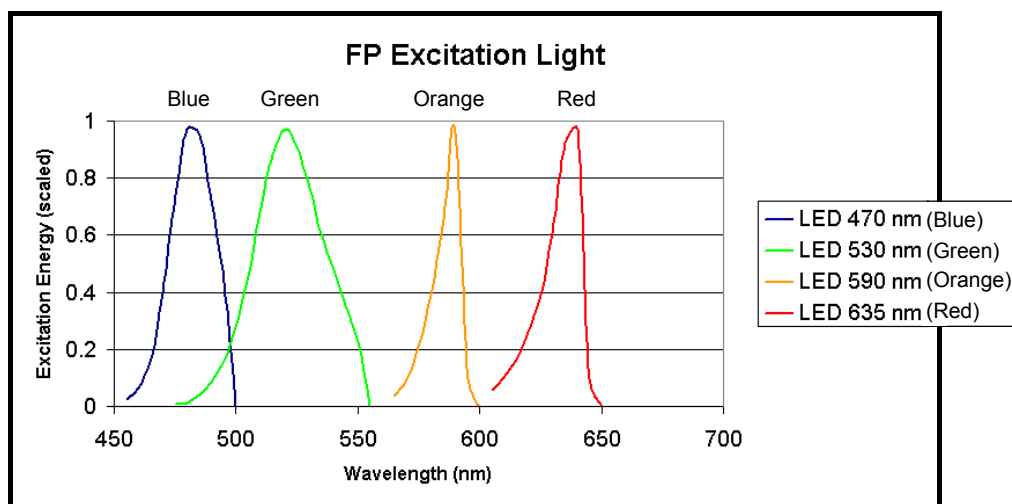


Figure 15: Fluorescence Polarization Excitation light spectra.

Z-Positioning

The Z-position of the polarization optics that is fixed onto the optics carrier can be adjusted (Figure 14). As light is refracted at the sample liquid surface z-adjustment helps to maximize signal to noise.

The Fluorescence Polarization Optics (Figure 16) consists of the following components:

- Polarizer
- Rotator
- EX Filter 1 – 4
- Dichroic mirror 1 – 4
- Analyzer

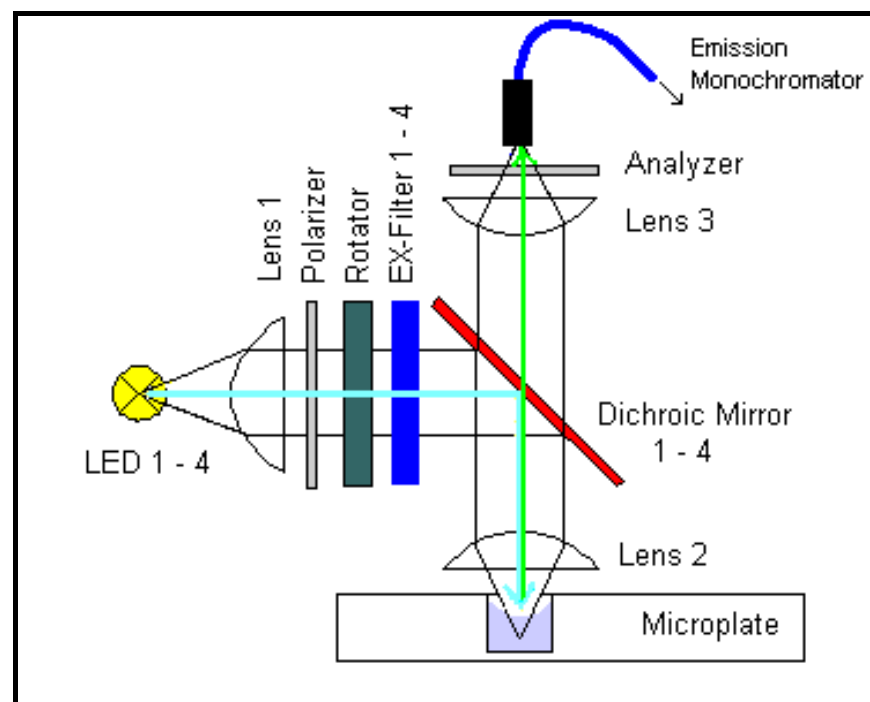


Figure 16: Fluorescence Polarization Optics.

Polarizer

The Polarizer is a device for producing plane-polarized light.

Rotator

A LC (Liquid Crystal) rotator changes the plane of the polarized excitation light.

EX-Filter

Fluorescence emission spectra in many cases do not depend on the exact excitation wavelength. For a maximum total fluorescence signal, therefore, rather broad excitation band pass filters (10 – 40 nm) may be used. For each LED an appropriate EX-filter is installed.

Dichroic Mirror

A dichroic mirror is designed to reflect a range of wavelengths almost perfectly. This range is used for excitation. On the other hand, that dichroic does transmit most of the fluorescence light. This usually gives a better signal to noise ratio when compared with the 50% mirror. For each LED in combination with an EX-filter an appropriate dichroic mirror is installed.

Analyzer

The Polarizer is a device for analyzing plane-polarized light. Only light with a specific type of plane can pass the Analyzer.

4.2.3 Fluorescence Polarization Detection

A fiber bundle guides the polarized light, which passed the Analyzer, to the emission monochromator. The light is detected by the PMT (Figure 14).

4.2.4 Fluorescence Polarization Measurement parameters

The light source is switched on during the whole measurement. Therefore it is not recommended to use a "settle time" for fast bleaching samples because it increases the total time the sample is exposed to light. However, when using stable samples a 'settle time' might increase the FP performance.

4.3 Absorbance System

For absorbance measurements, a similar optical path is used as for fluorescence excitation. For details of the light source(1) and the excitation monochromator (2), please refer to chapter 4.1.1 Light Source System Fluorescence Intensity.

A fiber bundle guides the light from the excitation monochromator(2) to the absorbance optics(3), which focus the light into the wells. The transmitted light is measured by silicon photodiodes(4) located beneath the plate carrier (see figure below).

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

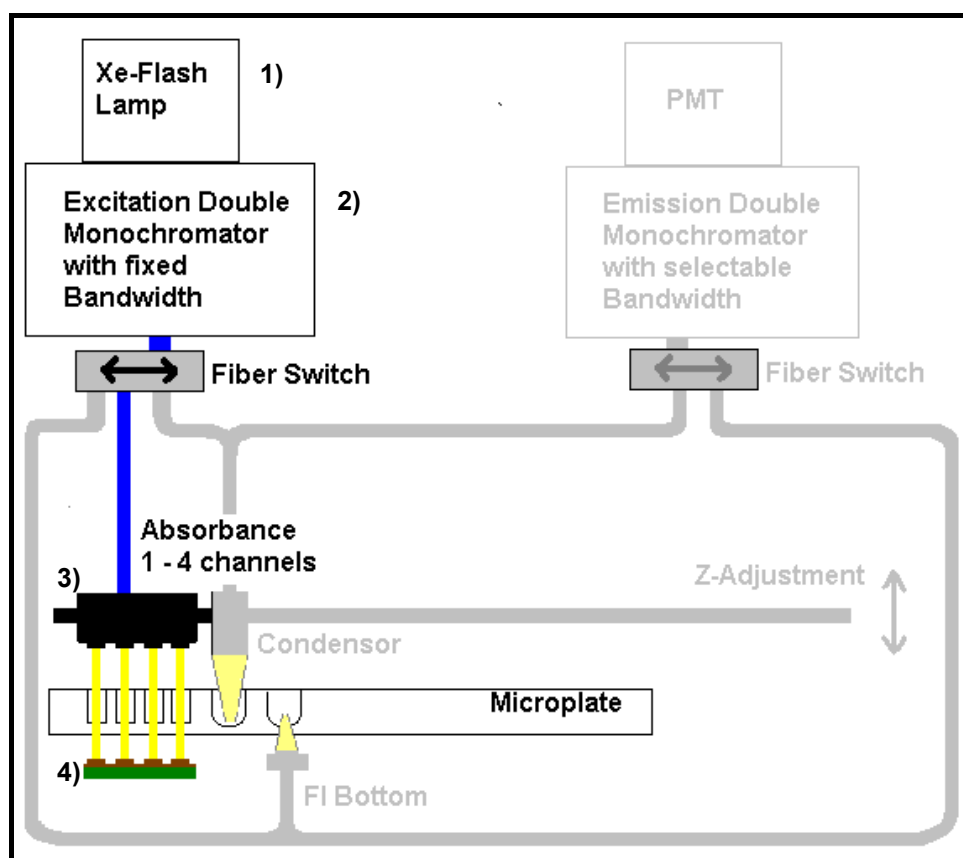


Figure 17: Optical System Absorbance.

4.3.1 Absorbance Optics

Up to 4 fiber bundles guide the light from the excitation monochromator system to the absorbance optics (Figure 17). The absorbance optics consists of a pair of lenses focusing the light beam into the well of the microplate (Figure 18).

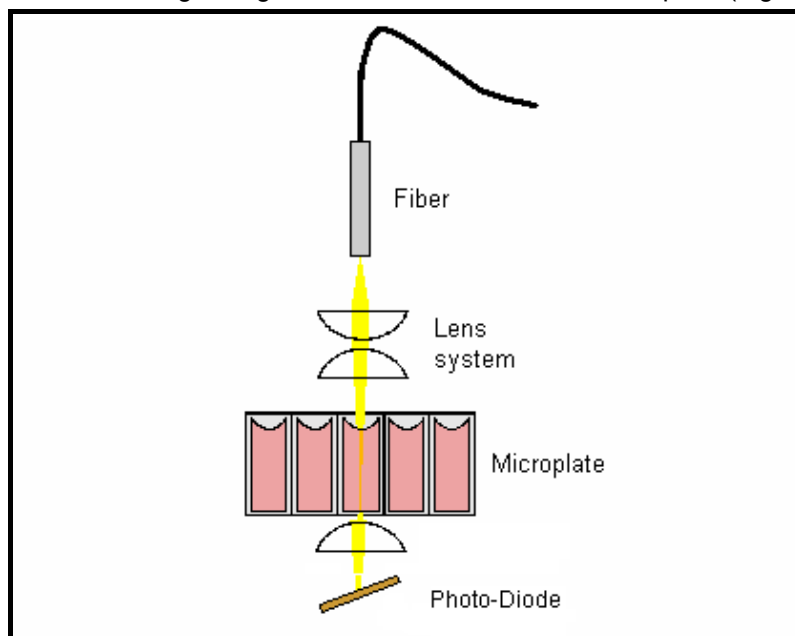


Figure 18: Absorbance Optics, one channel.

The absorbance channels are spaced to read a 96-well plate. The optical alignment for a 384- or 1536-well plate is shown in Figure 19. Software automatically sorts the data to report in the correct order. Each well is measured with only one channel in absorbance mode. If a plate type other than 96/384/1536 is used, only a single optic channel will be used for the absorbance reading.

The **light beam diameter** of the absorbance optics is **about 1 mm**.

Measurements using the injector are performed with one Absorbance channel only.

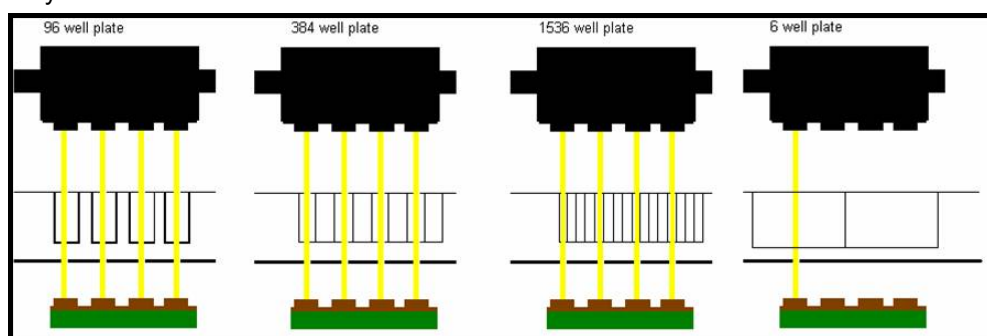


Figure 19: From left to right: Schematic view of the channel usage of an absorbance measurement of a 96-, 384-, 1536-, 6-well plate.

4.3.2 Absorbance Detection

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



Note

The NanoQuant™ 'Applications'-tab in i-control is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

4.4 Luminescence System

For uncompromising performance, the **Infinite M1000** has a dedicated luminescence detection module. The luminescence optics have been designed to meet requirements different from dedicated fluorescence optics. The much lower light levels involved when compared to flash lamp induced fluorescence require the benefits of a photon counting detection technique.

The **Infinite M1000** Luminescence System consists of the following parts (see figure below): luminescence fiber bundle, the filter wheel, and detection unit (PMT). The luminescence fiber bundle guides the light from the sample to the detector passing the filter wheel. Three different fibers are available for **Infinite M1000**, the fibers are optimized for different plate types: 96-well, 384-well, or 1536-well.

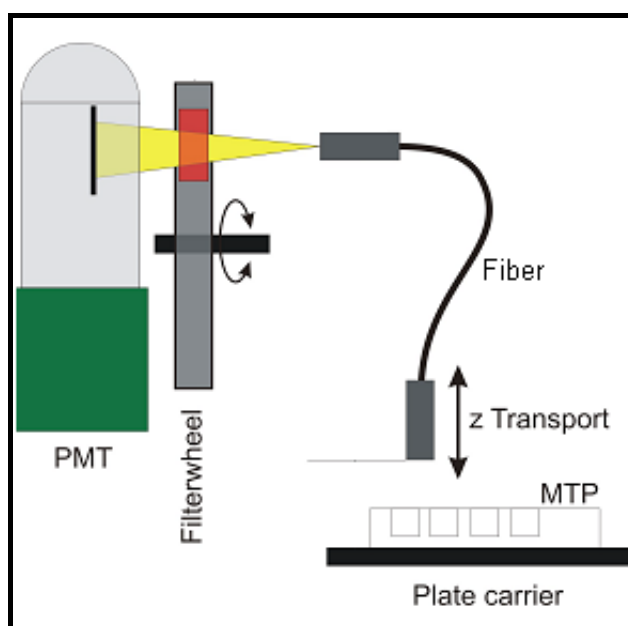


Figure 20: Optical System Luminescence.



Note

The power-on LED is switched off during luminescence measurements to gain the highest possible sensitivity.

4.4.1 Luminescence Optics

The Z-position of the luminescence fiber bundle fixed onto the optics carrier can be adjusted. As light is refracted at the sample liquid surface, z-adjustment helps to maximize signal to noise and minimize cross-talk. The software does the adjustment automatically once the user has selected the corresponding plate type in the software dialog.

Fibers

A fiber guides the light from the sample to the detection unit. Three different fibers to measure 96-well plates, 384-well plates, or 1536-well plates are available.

The orifices in the ceiling of the measurement chamber are designed to receive as much light as possible from wells of 96, 384 or 1536, well plates, respectively. Thus, luminescence signal is maximized. A particular orifice does not receive substantial amounts of light from neighboring wells. Thereby, cross talk is minimized.

Filter Wheel

A filter wheel with 6 filter positions in front of the PMT window is switched for 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 (OD2) across the selected fiber exit. This will be done automatically by the instrument.



Note

Attenuation filter OD2 is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

Filter Wheel Position	Filter
Position 1	Green (Chroma-Glo, BRET ²)
Position 2	Magenta (Chroma-Glo, BRET ²)
Position 3	OD2 neutral density filter
Position 4	No attenuation
Position 5	Blue 1 (BRET)
Position 6	Green 1 (BRET)

Figure 21 to Figure 24 show the transmission spectra of the different luminescence filters accordingly.

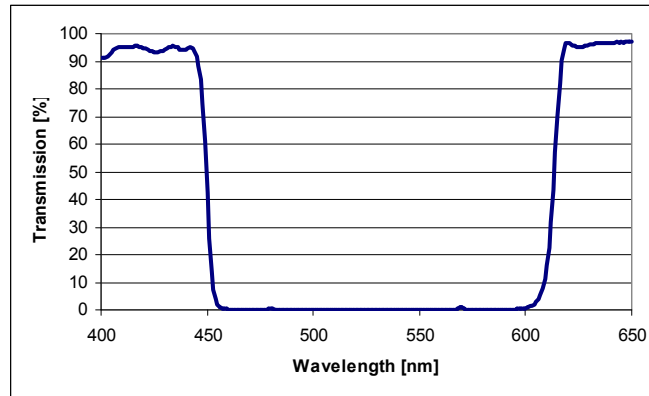


Figure 21: Transmission spectrum of filter 'Lumi Magenta'.

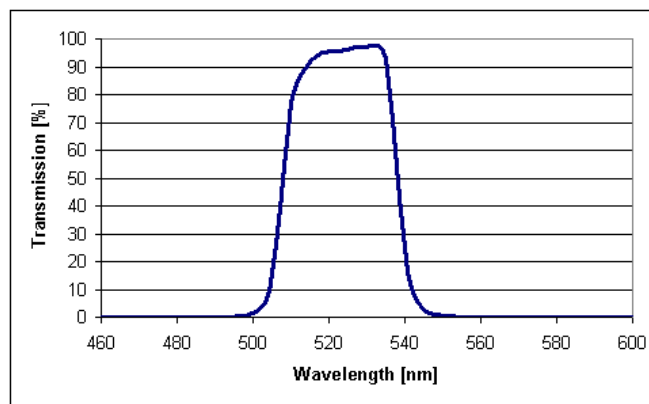


Figure 22: Transmission spectrum of filter 'Lumi Green'.

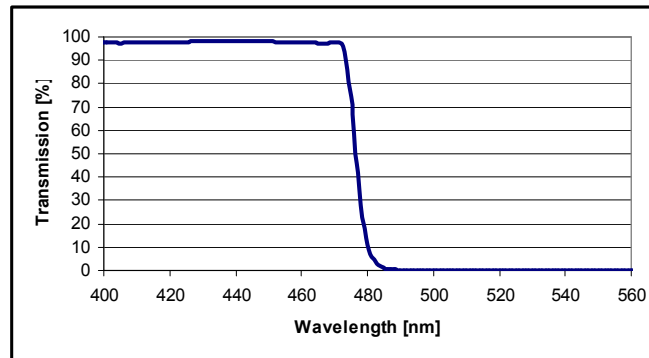


Figure 23: Transmission spectrum of filter 'Blue 1'.

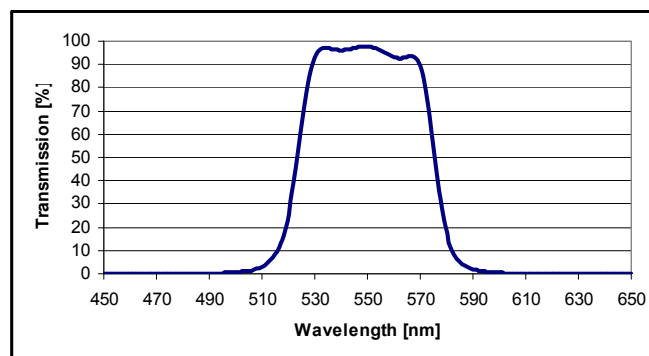


Figure 24: Transmission spectrum of filter 'Green 1'.

Photon Counting Module (PCM)

The PCM, containing a channel photomultiplier, is designed for applications in chemo- and bioluminescence. The channel photomultiplier provides a high dynamic range enabling luminescence measurement with strong variations in light levels. The exceptional low noise and high sensitivity allows the detection of very low light levels.

4.4.2 Luminescence Detection



Caution

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

The **Infinite M1000** 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 measurements of very low light levels.

For best performance, it is recommended to use white plates for luminescence measurements.



Note

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

5. Operating the Infinite M1000

5.1 Introduction



WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESSES RUN ON THE INFINITE M1000.

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.

The **Infinite M1000** is operated under personal computer based software control. *i-control*, *Magellan*, or *EVOware* software may be used as the user interface. For details see the corresponding software manual. This chapter is for a general understanding of instrument parameters and operation. Suggestions are made how to optimize instrument parameters for your applications.

Every effort has been made 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 has to be on the upper left side.



Important

When operating the Infinite M1000 always work according to GLP (good laboratory practice) guidelines.



Caution

The Infinite M1000 has a fan on the backside of the instrument that draws in air. The air filter has to be checked every 4 weeks and be replaced when dirty. The air filter must be replaced after 6 months.

5.2 General Operating Features

The **Infinite M1000** has some general behavior and options, which are independent from a particularly selected measurement technique.

5.2.1 Instrument Start Up

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



Caution

When the USB interface cable is being plugged or unplugged, the instrument and the PC should be powered off.

It is highly recommended to use the USB interface cable provided by Tecan only to ensure a good performance of the instrument.

Instrument Power On

When switching the instrument ON, 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. All movable parts (e.g. slits, gratings, order sorting filter wheels, plate transport, z-transport) are initialized and moved to the home position. The instrument is ready to be operated.



Note

When connecting to the instrument the functionality of the photo multiplier tube (PMT) is checked. This can take some time.



Caution

It is necessary to check the functionality of the PMT annually with the MultiCheck-Plus test plate.

Loading Microplates

Infinite M1000 is equipped with a **'Retract/Eject'** button which allows microplates to be inserted or removed from the instrument without software activation.

5.2.2 Finish a Measurement Session

Disconnect from Instrument

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



Note

Remove the microplate before disconnecting.

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.

5.2.3 General Options

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

Temperature Control

Some assays ask for an exact operating temperature. The **Infinite M1000** can set up a specific temperature within some range, provide uniformity across the plate, and keep temperature constant above ambient. The main cooling fans stop ventilation.

Temperature range: 4°C above ambient up to 42°C

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.

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.

Microplate Shaking

The **Infinite M1000** is capable of plate shaking before start of a measurement or in between kinetic cycles. Three shaking modes are available: linear, orbital and double orbital. The shaking amplitude can be selected from 1 to 6 mm in steps of 0.5 mm for linear and orbital shaking modes, and from 1 to 3.5 mm for double orbital shaking mode. The frequency is a function of the amplitude. The shaking duration is selectable from 1-1000 s.

**Note**

Double orbital shaking is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

Multi Labeling

i-control provides a basic Multi Labeling capability. Up to ten sets of instrument parameters can be edited. The corresponding plate measurements will be executed in order. For example, when using more than one fluorescent label, different wavelength combinations could be selected.

5.3 Optimize 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.

5.3.1 FI Scanning (Spectral Intensity Calibration)

Due to wavelength dependence of the intensity of the exciting light and instrument components (gratings; lenses; PMT) being passed by the excitation and emission light distortions of measured spectra might be possible.

Excitation spectra are distorted primarily by the wavelength dependence of the intensity of the exciting light **Infinite M1000** allows you to correct the spectra.

To calculate corrected emission spectra, one needs to know the wavelength-dependent efficiency of the detection system. Therefore a calibration curve is saved on **Infinite M1000** for correction.

For more details see also 'Principles of Fluorescence Spectroscopy', Third Edition; Joseph R. Lakowicz.

What is the reason for intensity differences between scan measurement values and fixed wavelength measurement values?

Excitation scan versus fixed wavelength

Infinite M1000 uses a reference fiber to compensate for fluctuations of the flash lamp. The sensitivity of the reference fiber needs to be adjusted (automatically done by the software) before each measurement to make sure that the fiber is working in an optimal sensitivity range and does not show overflow values. This reference measurement is performed differently for scan measurements and fixed wavelength measurements. For fixed wavelength measurements the calibration of the reference fiber is performed at the selected measurement wavelength.

For scan measurements the same reference method would be possible. But in the worst case (3D scan over full wavelength range) over 600 reference points (one per wavelength) have to be measured and saved.

Depending on the number of measurement points, this would take a few seconds up to nearly one minute. To improve the measurement speed we decided to perform the reference measurement at one wavelength, which is expected to give the highest light intensity. This procedure has proven successful in avoiding overflow errors and in providing sufficient sensitivity. The fixed wavelength measurement and scan measurement performed with the same measurement parameters (gain, number of flashes, z-position), have one side effect the results do not show the same RFU values.

Emission scan versus fixed wavelength

The reference measurement is performed at the selected excitation wavelength in both modes. Fixed wavelength values might deviate from scan wavelength values $\pm 10\%$ due to energy fluctuations of the flash lamp.

Luminescence Scan

Results of luminescence scans may be optimized by selecting appropriate instrument parameters. In comparison to fluorescence intensity scans longer integration times are necessary to detect luminescent signals. Long signal integration combined with very high gain value may result in objectionable noise of the detector. Each **Infinite M1000** instrument offers a default gain value keeping the noise level low at maximum signal integration. Of course gain value and integration time may be optimized in assays by the user according to the demands of the application. Gain optimizations are also necessary if temperature-dependent experiments are performed.

A company preset gain value for the luminescence measurement is provided. Especially if measurements at temperatures well below 30°C are performed, it may be useful to increase the sensitivity by increasing the gain value compared to the company preset gain. On the other hand if measurements well above 30°C are performed it may be necessary to decrease the gain value, in order to avoid a dark signal overflow.

Standard or dual colour luminescence measurements are performed by the luminescence module whereas luminescence scans are performed by the fluorescence intensity module (refer to chapter 4.1.4 Luminescence Scan). Various detector types with totally different technical specifications are installed on the different modules. The luminescence module is equipped with a single photon-counting detector and the results of standard or dual colour luminescence measurements are given in counts per second (refer to chapter 4.4.2 Luminescence Detection). Luminescence scanning results are given in relative luminescence units (RLU). Direct data comparison of measured intensities between standard/dual colour luminescence measurements and luminescence scan measurements are therefore not possible.



Note

Luminescence scan is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

5.3.2 FP Measurements

Fluorescence Polarization

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

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

I^{par} and I^{cross} 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.

G-Factor

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 FP measurement.

In order to perform a G-factor calibration, please define:

- **Polarization reference:** select a polarization value for the reference used, e.g. 20 mP for a 1nM fluorescein solution in 0.01 M NaOH. Select all wells filled with fluorescein.
- **Reference blank:** select all wells filled with blank. Select "same as measurement blank" if the reference blank is the same as the sample 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.

Settle time

Due to the stop and go motion of the carrier the dispensed liquids meniscus may vibrate during signal integration. Vibrations can cause fluctuations in the measured values, therefore to minimize this effect and to obtain optimal FP performance select a time between move and flash of 100ms.

Calculation of FP Parameters

G-factor:

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

P_{ref} ...Polarization value of reference

\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.

$$\Delta RFU^{par} = \begin{cases} RFU_{ref}^{par} - \overline{RFU}_{buf}^{par} \\ RFU_{buf}^{par} - \overline{RFU}_{buf}^{par} \\ RFU_{smp}^{par} - \overline{RFU}_{blk}^{par} \\ RFU_{blk}^{par} - \overline{RFU}_{blk}^{par} \end{cases}$$

$$\Delta RFU^{cross} = \begin{cases} RFU_{ref}^{cross} - \overline{RFU}_{buf}^{cross} \\ RFU_{buf}^{cross} - \overline{RFU}_{buf}^{cross} \\ RFU_{smp}^{cross} - \overline{RFU}_{blk}^{cross} \\ RFU_{blk}^{cross} - \overline{RFU}_{blk}^{cross} \end{cases}$$

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:

$$I_{total} = I^{par} + 2 * I^{cross}$$

5.3.3 Instrument Parameters

Gain Settings

The **Infinite M1000** fluorescence detection system uses analog to digital (A/D convert) conversion of PMT signal. The gain setting controls the amplification of the PMT when converting fluorescence light into electrical current. The AD converter 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 manual).

Gain Adjustment

The gain for fluorescence intensity and polarization measurements is selectable from 1 – 255. The performance of the PMT depends on the supply voltage (see figure below). The **Infinite M1000** PMT is specified from 300 to 1250 V. The relationship between the gain setting of **Infinite M1000** and the voltage supply is described in the equation below. The intended use of the **Infinite M1000** PMT is specified for gain settings from 60 – 255. Gain settings below 60 are possible and might be useful for special applications 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 M1000** when using gain settings below 60.

$$U = \frac{\text{Gain}}{255} * 1250V$$

- U** Voltage
- Gain** Infinite M1000 Gain
- 255** maximum gain on Infinite M1000
- 1250V** maximum voltage supply of PMT

Example:

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

$$U = \frac{100}{255} * 1250 = 490V$$

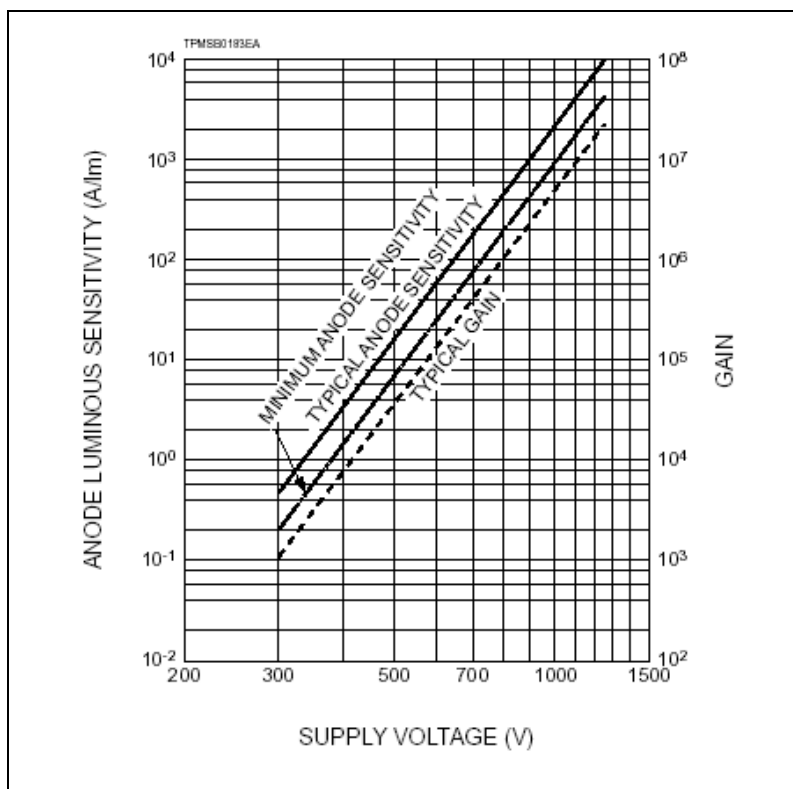


Figure 25: Sensitivity of PMT in relation to supply voltage. Sensitivity below 300 V is not specified.

Z-Optimization

Z-optimization is only available for FI Top and FP measurements with the **Infinite M1000**. 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 stripe.

(2) **'Calculated from well'**:

When using the option 'calculated from well', the **Infinite M1000** will automatically identify the z-position of maximum signal in the selected well for further measurements.

(3) **'Same as'** for multilabeling measurements:

When using the option 'same as', the **Infinite M1000** 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'.

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

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 the label(s) for which the z-position optimization shall be performed. The optimal z-position can be simultaneously determined for up to 5 labels. The label selection/number of labels depends on the measurement script previously defined in i-control.

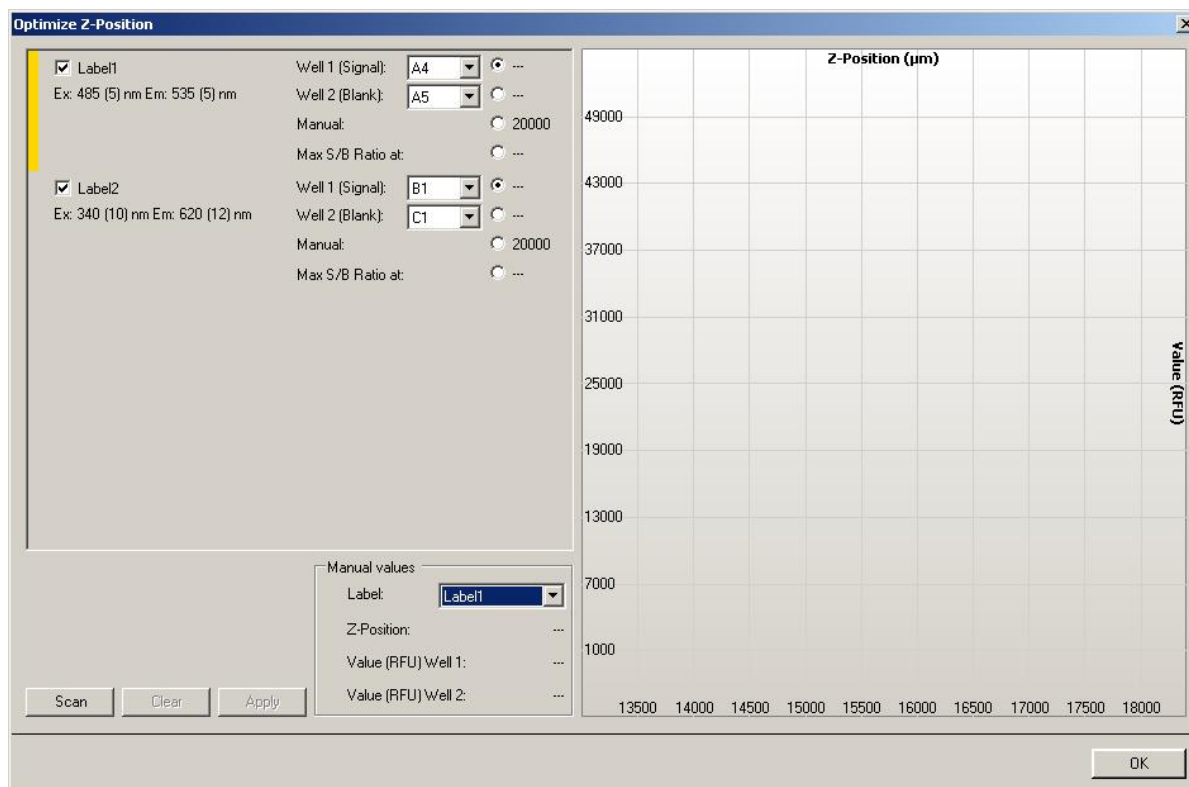


Figure 26

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 fluorophor 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.

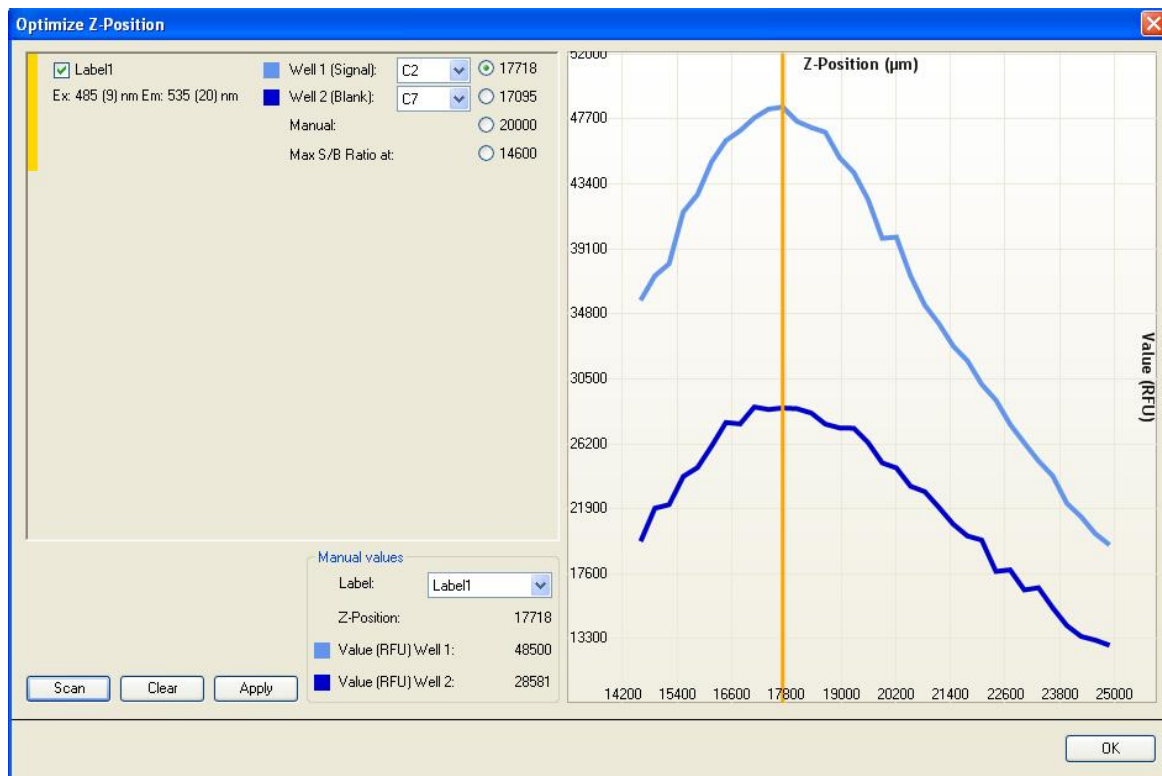


Figure 27



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. The vertical yellow bar of the graph can be moved to the desired z-position. Upon clicking 'Apply', the selected z-position will be automatically applied to i-control script and used for the subsequent measurement.

Flash Settings

On the fly measurements with 1 flash 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. 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.



Note

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

Flash Frequency Mode

Infinite M1000 allows switching between two flash frequencies for the Fluorescence Intensity and Fluorescence Intensity Scan mode: 100 and 400 Hz (100/400 flashes per second). As a standard, it is recommended to use the 400 Hz mode and 50 flashes. A higher number of flashes and therefore a higher number of single measurement values result in more accurate final measurement values.

For time resolved fluorescence (TRF) measurements we recommend using the 100 Hz mode to improve results.

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:

- 1) As a starting point you may take the **Fluorescence Lifetime** of the label for both **Integration Time** and **Lag Time**.
- 2) Coarse tuning: With Integration Time being fixed reduce the Lag Time to maximize **Signal to Background (S/B)**.
- 3) Fine tuning: With Lag Time being fixed extends the Integration Time and check, if S/B further improves.
- 4) Optional Fine-tuning: With either timing parameter being fixed you may vary the other one and check, if S/B further improves.

Comparing S/B at different timing parameters is valid if gain is fixed. For dual TRF labels, establish the procedure for the label with the shorter fluorescence lifetime (label 1). Compromise the Integration Time of label 1 with the Lag Time of label 2.

Time between Move and Flash

If selecting more than one flash per well a time delay between move and flash 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.

5.3.4 FI Ratio Mode

Ratio Mode

Up to 5 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.

5.3.5 Optimal Read (FI Bottom measurements only)

The "Optimal Read" function is available for Fluorescence Bottom measurements only. The "Optimal Read" function is a measurement on multiple, spatially separated spots inside the well. The spots are arrayed to cover the whole well area in order to achieve maximal well illumination. The total number of individual measurement spots per well is reflected by the size of the beam diameter of the Fluorescence Intensity Bottom fiber and is optimized for plate formats from 12 to 96 wells (see table below).

Plate	Pattern	Number of Spots
1536-well	'Optimal Read' option not available	
384-well	'Optimal Read' option not available	
96-well	Circle (filled)	5
48-well	Circle (filled)	21
24-well	Circle (filled)	37
12-well	Circle (filled)	61
6-well	'Optimal Read' option not available	

"Optimal Read" spot patterns in different plate formats

The flash number per measurement spot is selectable via software (1-200 flashes) and the number of measurement spots per well is displayed as soon as the 'Optimal Read' function is activated for a certain plate format in the fluorescence bottom measurement stripe. The 'Optimal Read' function is available in combination with the 400 Hz flash frequency mode only.

The user-defined total number of flashes is automatically distributed over all measured spots per well. A minor imprecision occurs if an entered flash number is not divisible without a remainder by the default number of spots for the used plate format. In this case the next possible flash distribution that is integrally divisible by the number of spots per well is calculated, e.g. a measurement with a total of 26-30 flashes in a 96-well plate (5 single spots) is performed with 6 flashes per spot, whereas a total flash number of 31 results in 7 flashes per spot.

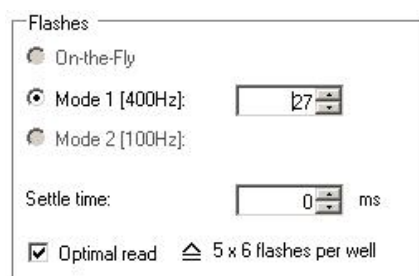


Figure 28

Result Display in MS Excel

The MS Excel results sheet generated by i-control software displays a single average measurement value for each well that has been measured using the Optimal Read function. The employed Optimal Read settings, i.e. the overall number of flashes as well as the number of flashes per well, are also displayed.

Label: Label1				
Mode				Fluorescence Bottom Reading
Optimal read				
Excitation Wavelength				485 nm
Emission Wavelength				535 nm
Excitation Bandwidth				10 nm
Emission Bandwidth				20 nm
Gain				82 Optimal (100%)
Number of Flashes				105 (21 x 5 flashes per well)
Flash Frequency				400 Hz
Integration Time				20 µs
Lag Time				0 µs
Settle Time				0 ms
Part of Plate				B2-C4
Start Time				21.02.2011 09:01:51
				Temperature: 21.9 °C
<>	2	3	4	
B	4146	2202	255	
C	2913	2265	221	

Figure 29 Results output for a measurement with optimal read (example for a 48-well plate).

Miscellaneous Features of Optimal Read

Optimal Read is only available for Fluorescence Intensity Bottom measurements. The Optimal Read feature is not available when performing well-wise measurements. The standard MRW function for Fluorescence Intensity Bottom reads is disabled when “Optimal Read” is activated and vice versa. The Optimal Read feature is not available in combination with the gain setting extended dynamic range.

5.3.6 Measurement Accessories

Recommended Types of Microplates

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.

With the z-Positioning capability the **Infinite M1000** can optimize signal for a particular volume of dispensed sample. However, 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.

5.4 Optimize Absorbance Measurements

5.4.1 Measurement Parameters

Flash Settings

'On-the-fly' measurements with 1 flash 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.

For Absorbance measurements only the 400 Hz flash frequency mode is available. By increasing the number of flashes more accurate results can be achieved.



Note

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

Time between Move and Flash

If selecting more than one flash per well, a time delay between move and flash may be set (100-300 ms). 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.

5.4.2 Absorbance Ratio Mode

Ratio Mode

Using the „Standard“-tab in i-control up to 5 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.



Note

The NanoQuant™ 'Applications'-tab in i-control is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

5.4.3 Measurement Accessories

Recommended Types of Microplates

Generally, for absorbance measurements transparent or UV-transparent microplates are used. For high OD values black microplates with transparent bottom seem superior.



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.



Note

The NanoQuant™ 'Applications'-tab in i-control is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

5.5 Optimize Luminescence Measurements

5.5.1 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. It rather 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 to discriminate electronic noise.

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 relevant signal to (shot) noise ratio can be improved by a factor when measurement time is multiplied with the square of the desired factor.

5.5.2 Light Level Attenuation

When using photon counting detection, optical attenuation of higher luminescence light levels (> 10,000,000 counts per second) is necessary. In that case, too many photons entering the PCT at a time cannot be distinguished as distinct exit pulses. Count rates would even fall behind values at lower light levels. Therefore, values >10,000,000 counts/s (without attenuation) are marked as "INVALID" on the result sheet.

'The OD2 neutral density filter serves to attenuate high light levels by a factor of 100. The resulting values are automatically scaled to counts per second and displayed accordingly in the software result output.'



Note

Attenuation filter OD2 is available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

5.6 Injectors

The **Infinite M1000** 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 (see figure below). Needles are designed to inject liquid into any well of microplates from 6 to 384 well.

Three different syringe volumes are available. The standard volume is 1000 μl , additionally syringes with volumes of 500 μl and 2500 μl are offered.

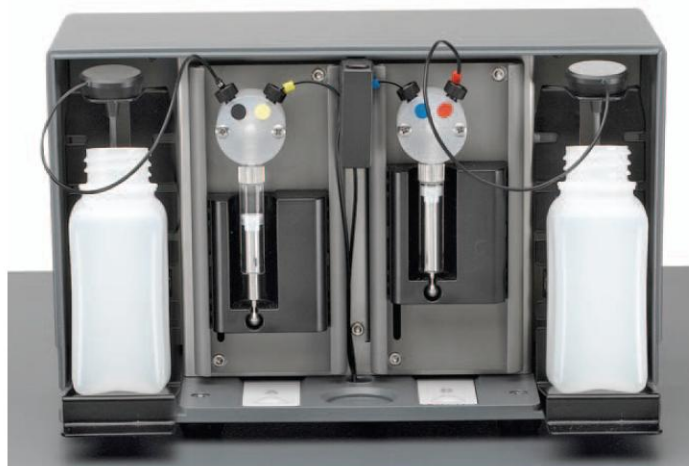


Figure 30: View of the injector module.

WARNING!

Switch off the instrument before plugging in or unplugging the injector module

This label appears on the rear panel of the injector module.

The **Infinite M1000** can be equipped with one pump or two pumps (Figure 29).

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

One Injector Option (one pump): A **Infinite M1000** equipped with one pump allows injections in all wells of 6- to 384-well plates that comply to SBS standard. All reactions requiring injection of only one liquid per well can be performed with this option.

Two Injector Option (two pumps): Several reactions, 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. By using pumps A and B, two independent liquids can be dispensed into the same well of microplates from 6- to 384-well.



WARNING

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

5.6.1 Measurement with Injectors

The injectors of the **Infinite M1000** can be used with the following measurement modes: Fluorescence Intensity top and bottom, Time Resolved Fluorescence, Absorbance, Flash and Glow Type Luminescence and Dual Color Luminescence. However, 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.

Schematic Diagram of the Two Injector Option

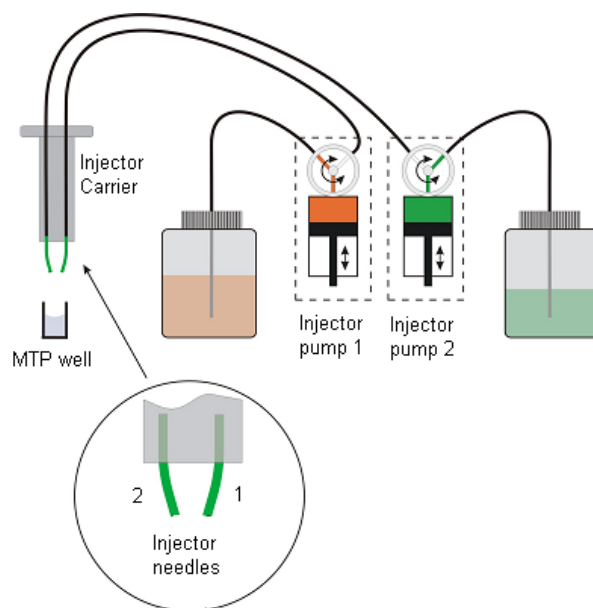


Figure 31: Liquid path in *Infinite M1000* with two pumps.

5.6.2 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 Injector option: one 125 ml bottle and one 15 ml bottle
- Two Injectors option: one 125 ml bottle and two 15 ml bottles

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 stably 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 32: Bottle holders

5.6.3 Injector Carrier



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

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 gently into the port until you hear a clicking noise.

The instrument contains an injector sensor that checks the correct position of the injector carrier in the instrument. If the injector 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 (Figure 33).

Figure 33: Injector carrier with injector in 'service position'.



Caution

The injector carrier must be in the service position for washing and priming. Prime and wash must not be performed when the injector is in the instrument.



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.

The dead volume of the injection system (injector needles, syringes, valves and tubing) is approximately 100 µl after using '**backflush**' 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 optimum injection speed is dependent on the assay parameters, such as viscosity of fluids, the plate format and the measuring behavior of the liquids. The removable injector carrier allows this process to be done outside of the instrument where a visual inspection can be easily performed.

Before starting a measurement make sure that:

1. The tubes are clean. If not please refer to chapter 5.6.4 'Priming and Washing of the injector(s)' for details.
2. The injector tubes are correctly inserted into the storage bottles and fixed.
3. The injector system is primed. It is not possible to start a measurement without priming the system.

When priming the system:

1. Check the tubes for leaks.
2. Check the tubes for kinks.
3. 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.

5.6.4 **Priming and Washing of the Injector(s)**



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. 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 (41.34 Inch) and 'short' = 80 cm (31.5 Inch).

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

**Note**

The options 'Minimal priming volumes' and 'Refill volumes' are available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

**Caution**

Do not touch the injector needles, as they can become easily bent or misaligned, which can cause injection problems or damage the instrument.

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 started by using the i-control software or by using the hardware buttons on the injector box:

To perform the priming procedure:

1. Fill the storage bottles with the necessary reagents and insert the feeding tube(s). Make sure, that the tube(s) reaches the bottom of the bottle.
2. Remove the injector from the carrier slot, check the injector needles for damages, and insert it into the service position of the injector box.
3. Position an empty container under the injector needles.
4. Adjust parameters at the prime tab of the 'injector maintenance' dialog box in the settings menu.
5. Press 'Start prime' to activate the priming procedure in the 'injection maintenance' dialog box.
6. Visually inspect the syringes for air bubbles. Any bubbles should be removed after priming to ensure good injection performance.
7. Visually inspect the tube(s) for leaks and kinks.
8. Check the liquid jet during priming.

Prime

1. Select one of the injectors (Injector A or Injector B) or Injector A and B!
2. Select the 'Prime Volume' (5-60000 μ l).
3. Select the 'Prime Speed' (100-300 μ l/sec).
4. Select the 'Refill Speed' (100-300 μ l/sec) or select 'Refill Speed equal to Prime Speed'.
5. Press 'Start Prime' to activate the priming procedure.
6. 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. Start the prime procedure by pressing and holding the Prime/Wash button for more than 3 seconds.
7. Select 'Close' to exit the dialog box.
8. 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 μ l of liquid are dispensed into a small container on the plate carrier. This initial dispense step makes sure that the injection/dispense conditions are equal for each well.



Note

After priming procedure the syringe remains empty.



Caution

Close the lid of the pump module (injector box) completely before starting a measurement.

Reagent Backflush

Prior to the cleaning of the injector system, reagent back-flushing allows the remaining reagent in the liquid system (injector needles, syringes, valves and tubing) to be pumped back into the storage bottles. This procedure is a cost effective solution for minimizing reagent consumption.

To perform the reagent back-flushing procedure:

1. Remove the injector from the carrier slot and insert it into the service position of the injector box.
2. Place the feeding tubing into the appropriate storage bottle.
3. Adjust parameters on the 'Backflush' tab of the 'Injector Maintenance' dialog box in the 'Settings' menu.
4. Select 'Start **Backflush**' to start the procedure.

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).
3. Select the 'Backflush Speed' (100-300 $\mu\text{l}/\text{sec}$).
4. Select the 'Refill Speed' (100-300 $\mu\text{l}/\text{sec}$) or select 'Refill Speed equal to Backflush Speed' check box.
5. Press 'Start Backflush' to start the reagent backflush procedure.
6. Select '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.

Washing

Before the instrument is switched off, a wash procedure should be run to clean the injector system.

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

To perform a typical wash procedure:

1. Remove injector carrier and bring it into the 'service position'.
2. Perform a backflush procedure to feed unused reagent back into the storage bottle.
3. Fill the storage bottles with the appropriate wash reagents (distilled or deionized water, 70 % ethanol, ...) and insert feeding tubes of the injector system.
4. Put an empty container under the injector.
5. Adjust the parameters on the wash tab of the 'Injector Maintenance' dialog box in the 'Settings' menu.
6. Start the washing procedure by clicking the 'Start wash' button.

Wash

1. Select one of the injectors (Injector A or Injector B) or Injector A and B!
2. Select the 'Piston Strokes' (1-60).
3. Select the 'Wash Speed' (100-300 $\mu\text{l}/\text{sec}$).
4. Select the 'Refill Speed' (100-300 $\mu\text{l}/\text{sec}$) or select 'Refill Speed equal to Wash Speed' check box.
5. Press 'Start Wash' to start the wash procedure.
6. 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 washing these settings will be applied. Start the wash procedure by pressing and holding the Prime/Wash button for less than 3 seconds.
7. Select 'Close' to exit the dialog box.



Caution

**The injector carrier must be in the service position
for the action 'wash'.**

Do not perform wash when the injector is in the instrument.



Important

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.



Important

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 exchanging the injector together with the corresponding tubing.



Important

The button(s) on the injector box include two functions:
 Press the button for more than 3 seconds to start 'prime'.
 Press the button for less 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 container on the plate carrier. 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.

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

The waste tub must therefore be emptied from time to time. The maximum filling volume is 2 ml. The internal counter checks the dispensed liquid volumes and the software alert the user when it is time to empty the waste tub.

To empty the waste tub 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.



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.

5.6.5 Injector Modes and Settings (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:

Figure 34

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. The **Refill volume** can be defined for each injector separately and allows economical handling of solutions.

Select '**Refill for every dispense**', if refill should be performed for every dispense step.

The option '**Read time like dispense time**' aligns the measurement time to the dispensing time in order to achieve same reaction times for each measured well.

Using the Dispense Strip:

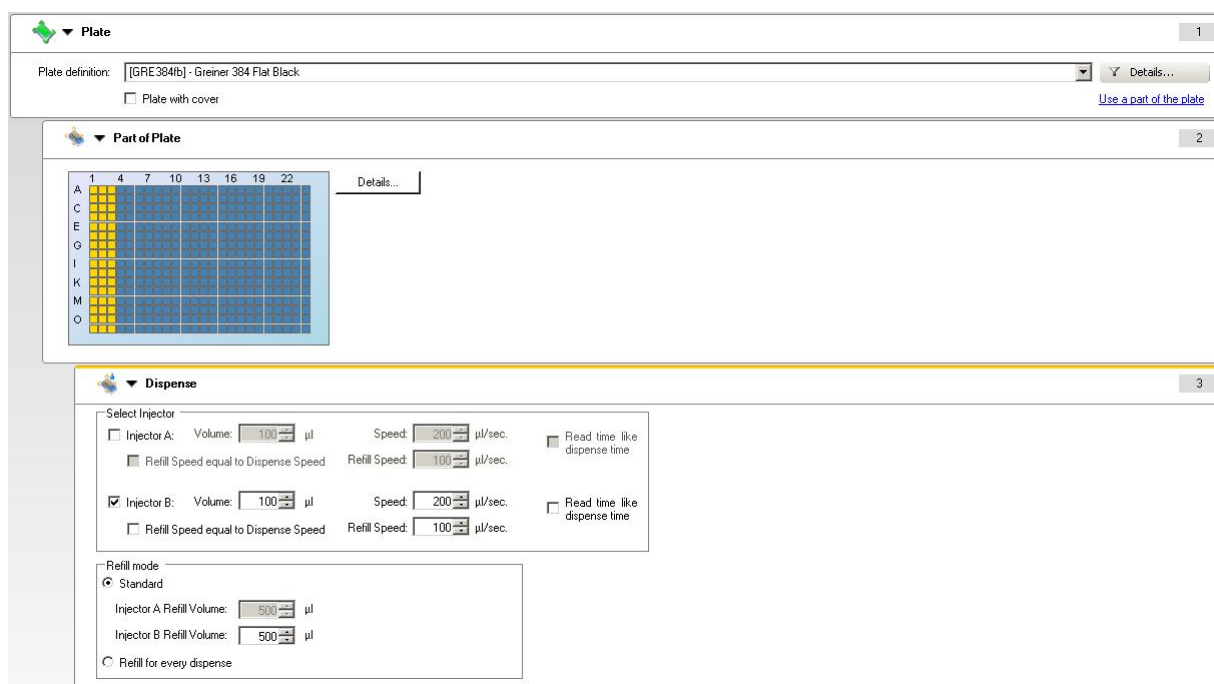


Figure 35

Plate: Select an appropriate plate type

Part of the plate: 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 afterwards with injector B.

The dispense strip does not require an additional measurement strip.

Dispense volume: The dispense volume depends on the microplate type. The plate definition files include the working volume of the microplate. This working volume defines the maximum volume to be dispensed into the selected microplate. Therefore, ensure that the selected plate definition file contains the correct value for the working volume. The maximum dispense volume is also limited by the installed syringe size (500 µl; 1 ml; 2.5 ml).

Injection Mode

The injection settings can be adjusted via the software:

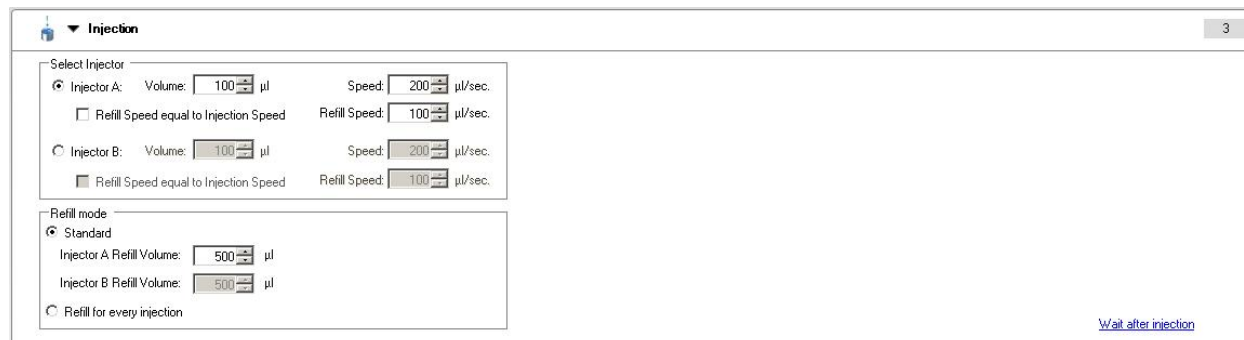


Figure 36

Injection

Select Injector: Injector A or Injector B can be selected. It is not possible to select both injectors using 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 $\mu\text{l}/\text{sec}$ for each injector. Select a '**Refill speed**' from 100 – 300 $\mu\text{l}/\text{sec}$. 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. The Refill volume can be defined for each injector separately and allows economical handling of solutions.

Select '**Refill for every injection**' if refill should be performed for every injection step.

Using the Injection Strip:

The screenshot displays the software interface for configuring an injection strip. The top bar shows the plate type as 'GRE384f - Greiner 384 Flat Black'. The 'Part of Plate' section shows a grid of wells with columns 1, 4, 7, 10, 13, 16, 19, and 22 highlighted in yellow. The 'Injection' section is expanded, showing the following settings:

- Select Injector:**
 - Injector A: Volume: 100 μl , Speed: 200 $\mu\text{l}/\text{sec}$.
 - Refill Speed equal to Injection Speed: Refill Speed: 100 $\mu\text{l}/\text{sec}$.
 - Injector B: Volume: 100 μl , Speed: 200 $\mu\text{l}/\text{sec}$.
 - Refill Speed equal to Injection Speed: Refill Speed: 100 $\mu\text{l}/\text{sec}$.
- Refill mode:**
 - Standard:
 - Injector A Refill Volume: 500 μl
 - Injector B Refill Volume: 500 μl
 - Refill for every injection

Additional options include 'Wait after injection' (a link), 'Wavelength' (Measurement: 492 nm), 'Flashes' (Number of flashes: 50), 'Settle time' (0 ms), 'Reference' (checkbox), 'Multiple Reads per Well' (checkbox), and 'Label' (Name: Label1).

Figure 37

Plate: Select an appropriate plate type.

Part of the plate: Select the wells to be dispensed

Well: The well strip is mandatory. Injection is only possible with a 'well' strip.

Injection: Set up the injection parameters.

Only one injector can be selected per strip. If both injectors are required an additional injection strip has to be inserted.

Injection volume: The dispense volume depends on the microplate type. The plate definition files include the working volume of the microplate. This working volume defines the maximum volume to be dispensed into the selected microplate. Therefore, ensure that the selected plate definition file contains the correct value for the working volume. The maximum dispense volume is also limited by the installed syringe size (500 μl ; 1 ml; 2.5 ml).

Measurement strip (Absorbance in the presented example): 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.



Note

Ensure that the correct value for the Working Volume is inserted in the plate definition file.



Note

The options 'Minimal priming volumes' and 'Refill volumes' are available for Infinite M1000 (REF 30061442) with main firmware V 2.0 or higher and i-control V 1.8 or higher. For further information please contact your local Tecan customer support office.

5.6.6 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.

Daily Maintenance:

If not otherwise stated by the manufacturer of the kit to be used, at least the following tasks shall be performed 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.

Weekly/Periodical Maintenance:

The fluid path must be cleaned weekly to remove precipitates such as salts, eliminate bacterial growth, and so on:

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 carefully with a cotton swab soaked in 70 % ethanol or isopropanol.

5.6.7 Injector Reagent Compatibility

The injector system of the Infinite M1000 instruments consists of the following materials:

- a. Teflon (PTFE): Tubing, valve plug, seal
- b. Kelf: Valve body
- c. 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
Acetonitrile	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, 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.

5.7 Built-in Stacker

**Caution**

When using the stacker, always make sure that the correct plate definition file (plate height) has been selected in the software before you start the measurement. Maximum plate height is 23 mm.

The **Infinite M1000** may be optionally equipped with a built-in stacker for loading and un-loading standard microplates automatically (Figure 38). Only plates without lids can be executed with the stacker.

The optional stacker is made from the following components:

- Input stack
- Output stack
- 2 stack columns
- Stack holder



Figure 38: Built-in stacker.

**WARNING**

NEVER TOUCH THE INSIDE OF THE INPUT STACK OR THE OUTPUT STACK WHEN THE STACKER IS BUSY. NEVER INSERT OR REMOVE PLATES MANUALLY WHEN THE STACKER IS WORKING.

The stacker comes with two thin metal plates (one for each stack). These plates are designed to weight down the microplates in the stacks. Put one plate on top of the plates in the input stack and the second one into the empty output-stack to improve the stacking performance.

**WARNING**

THE PLATE SENSOR IN THE STACKS DOES NOT RECOGNIZE THE METAL PLATES. THEREFORE DO NOT FORGET TO REMOVE THE METAL PLATE FROM THE EMPTY INPUT STACK BEFORE REFILLING THE STACK WITH MICROPLATES. MAKE SURE THAT THE METAL PLATE IS ALWAYS ON TOP OF THE PLATE STACK.

5.8 Barcode Scanner

Infinite M1000 may be optionally equipped with a barcode scanner mounted on the left or right side of the plate transport.

Infinite M1000 is a LASER CLASS 1 product. **Infinite M1000** complies with FDA radiation performance standards, 21 CFR Subchapter J, IEC/EN60825-1:2007.

**WARNING**

**LASER RADIATION – DO NOT STARE INTO THE BEAM!
CLASS I LASER PRODUCT INSIDE.**

The barcode is to be applied on the right/left side of the microplate depending on which side the barcode scanner is mounted. The distance to the front edge of the microplate must be at least 7 mm, the total length of the barcode may be up to 48 mm.

The specified barcode types are Class A, B, or C ANSI/CEN/ISO:

- CODE 39
- UPC A
- UPC E
- EAN 8
- CODE 128

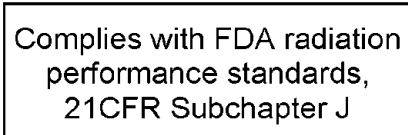
**Note**

The barcodes must have the following quality: Class A, B, or C/ANSI/CEN/ISO standard. Yellowed, dirty, folded, wet, or damaged barcode labels must not be used. The adhesive labels must be flat and without peeled edges. We recommend assuring the quality of the barcodes, by means of a local SOP.

Because the **Infinite M1000** contains a barcode scanner, the instrument is a class I laser device.



The following label is attached to the rear of the instrument:



The production date of the barcode scanner may be found on the rear of the barcode reader inside the instrument.



Warning

The use of controls or adjustments, or the performance of procedures other than those specified herein may result in hazardous radiation exposure.

6. Instrument Specifications

6.1 Introduction

**Note**

All specifications are subject to change without prior notification with appropriate validation, verification, and approvals.

The following types of measurement are provided with the fully equipped **Infinite M1000** microplate reader:

- Fluorescence Intensity Top/Bottom (FRET)
- Fluorescence Time Resolved (TRF, TR-FRET)
- Fluorescence Polarization
- Absorbance
- Glow Type Luminescence
- Dual Color Luminescence
- Flash Type Luminescence
- Injector options for Fluorescence, Absorbance, Luminescence

All standard microplates (SBS) from 6 to 1536-wells may be measured in any of the above measurement types. The plates must comply with ANSI/SBS 1-4 2004 standards.

Injection is possible for all standard microplates from 6 to 384-wells in any of the above measurement types.

The instrument allows for kinetic measurements.

Reading is no longer restricted to one part of the microplate, several areas can easily be selected for measurement at the same time.

6.2 Technical Specifications

The table below lists the technical specifications of the instrument.

Parameters	Characteristics
Measurement	Software controlled
Interface	USB
Filter handling	Monochromator based system
Microplates	From 6 well to 1536 well SBS plates
Plate definition	Definable via software
Temperature control	From 4° C above ambient up to 42°C
Plate shaking	Linear, orbital and double orbital shaking
Parameters	Characteristics
Light source	High energy Xenon flash lamp
Optics	Fused Silica Lenses
Fluorescence Detector	Low dark current photomultiplier tube
Luminescence Detector	Low dark count photomultiplier tube
Absorbance Detector	Silicon photodiode
Power supply	Auto-sensing: 100 – 120 V and 220 – 240 V, 50/60 Hz
Power consumption	300 VA
Physical	
Outer dimensions	Width: 515 mm (20.3 inches) Height: 257.5 mm (10.1 inches) Depth: 516.9 mm (20.4 inches)
Weight	29.5 kg
Environmental	
Environment	+15°C to +35°C 59°F - 95°F
Transportation	-20°C - +60°C -4°F - +140°F
Relative humidity	Up to 90% (non-condensing)
Overvoltage category	II
Pollution degree	2
Usage	Commercial
Noise level	< 60 dBA
Method of disposal	Electronic waste (infectious waste)

6.3 Fluorescence Intensity and Time Resolved (TRF)

Parameters	Characteristics
Wavelength Range	230 - 850 nm, selectable in 1 nm steps
Bandwidth ≤ 300 nm (excitation)	Selectable from 2.5 to 10 nm in 0.5 nm steps
Bandwidth > 300 nm (excitation) > 280 nm (emission)	Selectable from 5 to 20 nm in 1 nm steps

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	20 - 2000 μ s
Lag Time	0 - 2000 μ s

6.3.1 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. If selecting 1 flash per well, the plate carrier does not stop at the measurement position (fast mode; 'on the fly'). Using more flashes per well may improve the detection limit, but the total measurement time will be longer.

6.3.2 Fluorescein (Fluorescence Intensity) Top

Plate type (number of wells)	384	1536
Dispensed Volume [μ l]	100	10
Flashes per Well	12	12
Flash frequency	100 Hz	100 Hz
Fluorescein Detection Limit [pM]	≤ 3	≤ 10

6.3.3 *Fluorescein (Fluorescence Intensity) Bottom*

Plate type (number of wells)	384	1536
Dispensed Volume [μ l]	100	10
Flashes per Well	12	12
Flash frequency	100 Hz	100 Hz
Fluorescein Detection Limit [pM]	≤ 35	≤ 40

6.3.4 *Europium (Time Resolved Fluorescence)*

Plate type (number of wells)	384	1536
Dispensed Volume [μ l]	100	10
Flashes per Well	12	12
Flash frequency	100 Hz	100 Hz
Europium Detection Limit [pM]	≤ 0.75	≤ 0.90

6.3.5 *HTRF[®] (Time Resolved Fluorescence)*

Plate type (number of wells), white	384
Dispensed Volume [μ l]	50
Integration Time [μ s]	500
Lag Time [μ s]	60
Flashes per Well	50
Flash frequency	100 Hz
Signal/Background-Ratio	≥ 4
Delta F, Low Calibrator [%]	≥ 15
Delta F, High Calibrator [%]	≥ 600

6.4 Fluorescence Polarization

Parameters	Characteristics
Standard LED Excitation	470 nm, 530 nm, 590 nm, 635 nm
Emission Wavelength Range	280-850 nm, selectable in 1 nm steps
Emission Bandwidth	Selectable from 5 to 20 nm
Gain setting	see 6.3 Fluorescence Intensity and Time Resolved (TRF)

6.4.1 Fluorescein 1nM (fluorescence polarization)

Plate type (number of wells)	384	1536
Dispensed Volume [μ l]	100	10
Flashes per Well (1 flash = 10 ms integration time)	10	10
Fluorescein 1 nM Precision [mP]	≤ 2	≤ 10

6.5 Absorbance

Parameters	Characteristics
Wavelength range	230 - 1000 nm, selectable in 1 nm steps
Bandwidth Absorbance Scan	2.5 nm <i>for meas. ≤ 300 nm</i> 5 nm <i>for meas. > 300 nm</i>
Bandwidth Fixed Wavelength	5 nm
Measurement range	0 - 4 OD
Resolution	0.0001 OD
Accuracy (0-2 OD)	$\leq \pm (1 \% + 6 \text{ mOD})^*$
Precision (0-2 OD)	$\leq \pm (0.5 \% + 5 \text{ mOD})^*$
Linearity (0-2 OD)	$R^2 \geq 0.999^*$

**Specifications valid only for 384-well plates.*

6.6 Luminescence



Caution

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

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	400-700 nm
Dynamic Range	6 decades
Cross Talk (96 well)	≤ 0.035 % ATP
Integration Time/well	100 - 20000 ms
Attenuation	No attenuation, Automatic (OD2)

6.6.1 ATP Glow Luminescence

Plate type (number of wells)	384	1536
Total Dispensed Volume[μl]	100	10
Integration Time/well [ms]	1000	1000
ATP Detection Limit [fmol/well]	≤ 1	≤ 10

6.7 Flash Type Luminescence



Caution

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

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	400 – 700 nm
Measurement Range	> 6 orders of magnitude
Integration Time/well	100 – 20000 ms

6.8 Dual Color Luminescence (e.g. BRET)



Caution

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

Parameters	Characteristics
Integration Time:	100 – 20000 ms. Different integration times are possible for each wavelength.
Plate Type:	96-, 384-, and 1536-well microplates
Dynamic Range:	6 decades

6.9 “On the Fly” Measurements

“On the Fly” measurements are the fastest measurements possible using the **Infinite M1000**. These measurements are performed with one flash only, a lag time of 0, and an integration time shorter than 100 μ s.

Measurement Technique	Measurement Time		
	96-well	384-well	1536-well
Absorbance	11s	15s	23s
Fluorescence Intensity TOP	17s	24s	36s
Fluorescence Intensity BOTTOM	19s	25s	38s

6.10 Injectors

Parameters	Characteristics
Plate Types	6- to 384-well plates
Injector Syringe Volumes	500 μ l, 1000 μ l, 2500 μ l

6.10.1 Injector Performance

Injector Syringe Volume	500 μ l	1000 μ l	2500 μ l
Accuracy at 10 μ l	$\leq 10\%$	$\leq 10\%$	$\leq 20\%$
Accuracy at 100 μ l	$\leq 2\%$	$\leq 2\%$	$\leq 5\%$
Accuracy at 450 μ l	$\leq 0.7\%$	$\leq 0.7\%$	$\leq 2\%$
Precision at 10 μ l	$\leq 10\%$	$\leq 10\%$	$\leq 20\%$
Precision at 100 μ l	$\leq 2\%$	$\leq 2\%$	$\leq 5\%$
Precision at 450 μ l	$\leq 0.7\%$	$\leq 0.7\%$	$\leq 2\%$

Specifications are valid for 96-well plates only.

7. Quality Control

7.1 Periodic Quality Control Tests

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

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

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

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



Caution

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



Caution

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

7.2 Definitions

7.2.1 Detection Limit (LOD)

The detection limit is defined as concentration of the fluorophore, at which the corresponding signal can be distinguished from the background with a certain statistical probability. The LOD is calculated according to the following formula:

$$\text{LOD} = \frac{C}{(F - B)} * 3 * \text{Stdev}_B$$

C: Concentration of the fluorophore (concentration is at least 100 times higher than the LOD).

Stdev_B: standard deviation of the blank.

F: Measured RFU value of the fluorophore.

B: Measured RFU value of the blank.

7.2.2 Uniformity

Uniformity is a measure for the well-to-well variations when measuring a multi-well-plate. The uniformity is either calculated as percentage deviation from the mean value or (for low OD values) as a maximal deviation in mOD.

7.2.3 Linearity

Linearity is defined as relationship between concentration of fluorophore/dye and corresponding signal probability. For evaluation of linearity a dilution series of the appropriate dye is measured and the r-square value is assessed according to the following formula:

$$r^2 = \frac{SSR}{SST}$$

$$r^2 = \frac{SSR}{SST}$$

SSR: Sum of squares of the regression.

SST: Total sum of squares.

$$SSR = \sum_{i=1}^n \omega_i (\hat{y}_i - \bar{y})^2$$

$$SST = \sum_{i=1}^n \omega_i (y_i - \bar{y})^2$$

7.2.4 Accuracy

Accuracy is the ability of a measuring instrument to give responses close to a true value. The accuracy is either calculated as percentage deviation from the true value or for low OD values as deviation from the true value in mOD.

7.2.5 Cross-Talk

Cross-talk is defined as a measure of the influence of a well on its adjacent wells due to optical leakage.

7.2.6 Repeatability (Reproducibility)

Repeatability is the ability of the instrument to provide closely similar responses. It is calculated according to the following formula:

$$CV\% = \frac{stdev * 100}{average}$$

CV: Coefficient of variation.

Stdev: standard deviation of one well over time.

Average: average of the measurement values of one well over time.

7.3 Acceptance Criteria



Note

All specifications are subject to change without prior notification, with appropriate validation, verification, and approvals.

The following table gives an overview of the passed/failed criteria for the specification test of **Infinite M1000**.

Specification	Acceptance Criteria	
	384	1536
Fluorescence Top Sensitivity	≤ 3 pM Fluorescein	≤ 10 pM Fluorescein
Fluorescence Top Uniformity	≤ 3.5 % CV	≤ 5 % CV
Fluorescence Bottom Sensitivity	≤ 35 pM Fluorescein	≤ 40 pM Fluorescein
Fluorescence Bottom Uniformity	≤ 3 % CV	≤ 5 % CV
Time Resolved Fluorescence Sensitivity	≤ 0.75 pM Europium	≤ 0.90 pM Europium
Fluorescence Polarization Precision	< 2 mP	< 10 mP
Luminescence Sensitivity Glow Type	≤ 1 fmol/well ATP	≤ 10 fmol/well ATP
Luminescence Cross talk	≤ 0.5% ATP	≤ 5% ATP
Absorbance Accuracy (0 – 2 OD)	≤ ± (1 % + 6 mOD)	
Absorbance Precision (0 – 2 OD)	≤ ± (0.5 % + 5 mOD)	
Absorbance Reproducibility (0 – 2 OD)	≤ ± (1 % + 20 mOD)	
Absorbance Linearity (0 – 2 OD)	R ² > 0.999	
Absorbance Baseline Flatness (300-700 nm)	± 10 mOD	

7.4 Test Instructions

7.4.1 Fluorescence Intensity

7.4.1.1 Detection Limit Top Fluorescein 384-well Plate

Pipette 100 μ l of the 1 nM Fluorescein solution and 100 μ l of the blank solution (0.01 M NaOH) into the wells of a Greiner 384-well plate (black, flat bottom) according to the plate layout.

Material:

Greiner 384-well plate, flat bottom, black
 100 μ l Pipette (8-channel) + Tips
 1 nM Fluorescein (diluted in 0.01 M NaOH)
 0.01 M NaOH

Plate Layout:

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

(F.....100 μ l 1 nM Fluorescein; B.....100 μ l 0.01 M NaOH = Blank)

Measurement Parameters:

Measure the plate with the following measurement parameters:

Measurement mode: Fluorescence Top
 Excitation wavelength: 483 nm
 Emission wavelength: 525 nm
 Excitation bandwidth: 20 nm
 Emission bandwidth: 20 nm
 Read Mode: Mod 2 (100 Hz)
 Gain: Optimal
 Number of flashes: 12
 Lag time: 0 µs
 Integration time: 40 µs
 Plate definition file: GRE384fb.pdfx
 Z-Position: Calculate from A1

Evaluation:

Calculate the detection limit in pM:

$$\text{DetectionLimit} = \frac{1000}{(\text{mean}_F - \text{mean}_B)} * 3 * \text{stdev}_B$$

mean_F average of wells filled with 1 nM Fluorescein
 mean_B average of wells filled with Blank
 stdev_F standard deviation of wells with Blank

7.4.1.2 Uniformity Top Fluorescein 384-well Plate:

Pipette 100 µl of the 1 nM Fluorescein solution into the wells of a Greiner 384-well plate (black, flat bottom) as described in chapter 7.4.1.1 Detection Limit Top Fluorescein 384-well Plate.

Material:

See chapter 7.4.1.1 Detection Limit Top Fluorescein 384-well Plate

Measurement Parameters:

See chapter 7.4.1.1 Detection Limit Top Fluorescein 384-well Plate

Evaluation:

Calculate the uniformity:

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

mean_F average of wells filled with 1 nM Fluorescein
 stdev_F standard deviation of wells 1 nM Fluorescein

7.4.1.3 Detection Limit Top Fluorescein 1536-well Plate

Pipette 10 µl of a 1 nM Fluorescein solution (diluted in 0.01 M NaOH) and 10 µl of the blank solution (0.01 M NaOH) into the wells of a Greiner 1536-well plate (black, flat bottom) according to the plate layout.

Material:

Greiner 1536-well plate, flat bottom, black
 10 µl Pipette + Tips
 1 nM Fluorescein (diluted in 0.01 M NaOH)
 0.01 M NaOH

Plate layout:

<>	1	...	5	...	9	...	13	...	17	...	21	...	25	...	29	...	33	...	37	...	41	...	45	...
A	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
E	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
I	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
M	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
Q	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
U	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
Y	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
AC	F		B		F		B		F		B		F		B		F		B		F		B	
...																								

(F.....10 µl 1 nM Fluorescein; B.....10 µl 0.01 M NaOH = Blank)

Measurement Parameters:

Measurement mode: Fluorescence Top
 Excitation wavelength: 483 nm
 Emission wavelength: 525 nm
 Excitation bandwidth: 20 nm
 Emission bandwidth: 20 nm
 Gain: Optimal
 Read Mode: Mod 2 (100 Hz)
 Number of flashes: 12
 Lag time: 0 µs
 Integration time: 40 µs
 Plate definition file: GRE1536fb.pdf, whole plate
 Z-Position: Calculate from A1

Evaluation:

Calculate the detection limit in pM:

$$\text{DetectionLimit} = \frac{1000}{(\text{mean}_F - \text{mean}_B)} * 3 * \text{stdev}_B$$

mean_F average of wells filled with 1 nM Fluorescein
mean_B average of wells filled with Blank
stdev_F standard deviation of wells with Blank

7.4.1.4 Uniformity Top Fluorescein 1536-well Plate:

Pipette 10 µl of the 1 nM Fluorescein solution into the wells of a Greiner 1536-well plate (black, flat bottom) as described in chapter 7.4.1.1 Detection Limit Top Fluorescein 384-well Plate.

Material:

See chapter 7.4.1.3 Detection Limit Top Fluorescein 1536-well Plate (without pipetting the Blank)

Measurement Parameters:

See chapter 7.4.1.3 Detection Limit Top Fluorescein 1536-well Plate.

Evaluation:

Calculate the uniformity:

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

mean_F average of wells filled with 1 nM Fluorescein
stdev_F standard deviation of wells 1 nM Fluorescein

7.4.1.5 Detection Limit Bottom Fluorescein 384-well Plate

Pipette 100 μ l of a 1 nM Fluorescein solution (diluted in 0.01 M NaOH) into the wells of a Greiner 384-well plate (black, μ Clear, flat bottom) according to the plate layout.

Material:

Greiner 384-well plate, flat bottom, μ Clear (black with transparent bottom)
 100 μ l Pipette (8-channel) + Tips
 1 nM Fluorescein (diluted in 0.01 M NaOH)
 0.01 M NaOH

Plate Layout:

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

(F.....100 μ l 1 nM Fluorescein; B.....100 μ l 0.01 M NaOH = Blank)

Measurement Parameters:

Measurement mode: Fluorescence Bottom
 Excitation wavelength: 483 nm
 Emission wavelength: 525 nm
 Excitation bandwidth: 20 nm
 Emission bandwidth: 20 nm
 Gain: Optimal
 Read Mode: Mod 2 (100 Hz)
 Number of flashes: 12
 Lag time: 0 μ s
 Integration time: 40 μ s
 Plate definition file: GRE384fb.pdfx, whole plate

Evaluation:

Calculate the detection limit in pM:

$$\text{DetectionLimit} = \frac{1000}{(\text{mean}_F - \text{mean}_B)} * 3 * \text{stdev}_B$$

mean_F average of wells filled with 1 nM Fluorescein
 mean_B average of wells filled with Blank
 stdev_F standard deviation of wells with Blank

7.4.1.6 Uniformity Bottom Fluorescein 384-well Plate:

Pipette 100 µl of the 1 nM Fluorescein solution into the wells of a Greiner 384-well plate (black; µClear, flat bottom) as described in chapter 7.4.1.5 Detection Limit Bottom Fluorescein 384-well Plate.

Material:

See chapter 7.4.1.5 Detection Limit Bottom Fluorescein 384-well Plate (except Blank = 0.01 M NaOH).

Measurement Parameters:

See chapter 7.4.1.5 Detection Limit Bottom Fluorescein 384-well Plate

Evaluation.

Calculate the uniformity:

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

mean_F average of wells filled with 1 nM Fluorescein
 stdev_F standard deviation of wells 1 nM Fluorescein

7.4.1.7 Detection Limit Bottom Fluorescein 1536-well Plate

Pipette 10 µl of a 1 nM Fluorescein solution (diluted in 0.01 M NaOH) into the wells of a Greiner 1536-well plate (transparent, flat bottom) according to the Plate Layout.

Material:

Greiner 1536-well plate, flat bottom, transparent
 10 µl Pipette + Tips
 1 nM Fluorescein (diluted in 0.01 M NaOH)
 0.01 M NaOH

Plate layout:

<>	1	...	5	...	9	...	13	...	17	...	21	...	25	...	29	...	33	...	37	...	41	...	45	...
A	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
E	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
I	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
M	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
Q	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
U	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
Y	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
AC	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
AE																								
...																								

(F....10 µl 1 nM Fluorescein; B.....10 µl 0.01 M NaOH = Blank)

Measurement Parameters:

Measurement mode: Fluorescence Bottom
 Excitation wavelength: 485 nm
 Emission wavelength: 525 nm
 Excitation bandwidth: 20 nm
 Emission bandwidth: 20 nm
 Gain: Optimal
 Read Mode: Mod 2 (100 Hz)
 Number of flashes: 12
 Lag time: 0 µs
 Integration time: 40 µs
 Plate definition file: GRE1536ft.pdfx, whole plate

Evaluation:

Calculate the detection limit in pM:

$$\text{DetectionLimit} = \frac{1000}{(\text{mean}_F - \text{mean}_B)} * 3 * \text{stdev}_B$$

mean_F average of wells filled with 1 nM Fluorescein
 mean_B average of wells filled with Blank
 stdev_F standard deviation of wells with Blank

7.4.1.8 Uniformity Bottom Fluorescein 1536-well Plate:

Pipette 10 µl of the 1 nM Fluorescein solution into the wells of a Greiner 1536-well plate (transparent, flat bottom) as described in chapter 7.4.1.7 Detection Limit Bottom Fluorescein 1536-well Plate.

Material:

See chapter 7.4.1.7 Detection Limit Bottom Fluorescein 1536-well Plate (0.01 M NaOH).

Measurement Parameters:

See chapter 7.4.1.7 Detection Limit Bottom Fluorescein 1536-well Plate.

Evaluation:

Calculate the uniformity:

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

mean_F

average of wells filled with 1 nM Fluorescein

stdev_F

standard deviation of wells 1 nM Fluorescein

7.4.2 Time Resolved Fluorescence

7.4.2.1 Detection Limit Europium 384-well Plate:

Pipette 100 µl of the reagents into the wells of a Greiner 384-well plate (white, flat bottom) according to the Plate Layout.

Material:

Greiner 384-well plate, flat bottom, white
200 µl Pipette (8-channel) + Tips
1 nM Europium Standard (Wallac)
Enhancement Solution

Plate Layout:

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

(Eu....100 µl 1 nM Europium; B....100 µl Enhancement Solution = Blank)

Measurement Parameters:

Measurement mode: Fluorescence Top
Excitation wavelength: 345 nm
Emission wavelength: 617 nm
Excitation bandwidth: 20 nm
Emission bandwidth: 20 nm
Gain: Optimal
Read Mode: Mod 2 (100 Hz)
Number of flashes: 12
Lag time: 100 µs
Integration time: 400 µs
Plate definition file: GRE384fw.pdfx, part of the plate: A1 – O13
Z-Position: Calculate from A1

Evaluation:

Calculate the detection limit in pM:

$$\text{DetectionLimit} = \frac{1000}{(\text{mean}_{\text{Eu}} - \text{mean}_{\text{B}})} * 3 * \text{stdev}_{\text{B}}$$

mean_{Eu} average of wells filled with 1 nM Europium
mean_B average of wells filled with Blank
stdev_B standard deviation of wells filled with Blank

7.4.2.2 Detection Limit Europium 1536-well Plate:

Pipette 10 µl of the reagents into the wells of a Greiner 1536-well plate (white, flat bottom) according to the Plate Layout.

Material:

Greiner 1536-well plate, flat bottom, white
 10 µl Pipette + Tips
 1 nM Europium Standard (Wallac)
 Enhancement Solution

Plate Layout:

<>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	...	25	...	33	...	41	...
A	Eu	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B							
B	Eu	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B							
C	Eu	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B							
D																								
E																								
...																								
I																								
...																								
M																								
...																								
Q																								
...																								
U																								
...																								
Y																								
...																								
AC																								
...																								

(Eu....10 µl 1 nM Europium; B.....10 µl Enhancement Solution = Blank)

Measurement Parameters:

Measurement mode: Fluorescence Top
 Excitation wavelength: 345 nm
 Emission wavelength: 617 nm
 Excitation bandwidth: 20 nm
 Emission bandwidth: 20 nm
 Gain: Optimal
 Read Mode: Mod 2 (100 Hz)
 Number of flashes: 12
 Lag time: 100 µs
 Integration time: 400 µs
 Plate definition file: GRE1536fw.pdfx, part of the plate: A1 – C17
 Z-Position: Calculate from A1

Evaluation:

Calculate the detection limit in pM:

$$\text{DetectionLimit} = \frac{1000}{(\text{mean}_{\text{Eu}} - \text{mean}_{\text{B}})} * 3 * \text{stdev}_{\text{B}}$$

mean_{Eu} average of wells filled with 1 nM Europium
 mean_B average of wells filled with Blank
 stdev_B standard deviation of wells filled with Blank

7.4.3 Fluorescence Polarization (FP)

7.4.3.1 FP Precision Fluorescein 384-well Plate:

Pipette 100 µl of the reagents into the wells of a Greiner 384-well plate (black, flat bottom) according to Plate Layout.

Material:

Greiner 384-well plate, flat bottom, black
 100 µl Pipette (8-channel) + Tips
 1 nM Fluorescein
 0.01 nM NaOH (=Blank)
 High Polarization Standard (Invitrogen)

Plate Layout:

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

(F.....100 µl 1 nM Fluorescein; B.....100 µl 0.01 M NaOH = Blank, P...100 µl Invitrogen High Pol Standard)

Measurement Parameters:

Measurement mode: Fluorescence Polarization
 Excitation wavelength: 470 nm
 Emission wavelength: 525 nm
 Emission bandwidth: 20 nm
 Gain: 'Optimal'
 Lag time: 0 µs
 Number of reads: 10 (= 100 ms integration time)
 Settle time: 50 ms
 Plate definition file: GRE384fb.pdfx
 Z-Position: Calculated from Well A1
 G-Factor reference: Calibrated from well A1, ref. value = 20 mP;
 G-Factor reference blank: I3; sample blank same as reference blank

Evaluation:

Calculate the lower detection limit [mP] = 'precision':

FP Precision (mP) = $Stdev_F$

$Stdev_F$ Standard deviation of wells filled with Fluorescein (except reference well)

7.4.3.2 FP Precision Fluorescein 1536-well Plate:

Pipette 10 µl of the reagents into the wells of a Greiner 1536-well plate (black, flat bottom) according to the Plate Layout.

Material:

- Greiner 384-well plate, flat bottom, black
- 10 µl Pipette (8-channel) + Tips
- 1 nM Fluorescein
- 0.01 nM NaOH (=Blank)
- High Polarization Standard (Invitrogen)

Plate Layout:

<>	1	...	5	...	9	...	13	...	17	...	21	...	25	...	29	...	33	...	37	...	41	...	45	...
A	F		P		F		B		F		B		F		B		F		B		F		B	
...																								
E	F		P		F		B		F		B		F		B		F		B		F		B	
...																								
I	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
M	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
Q	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
U	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
Y	F		B		F		B		F		B		F		B		F		B		F		B	
...																								
AC	F		B		F		B		F		B		F		B		F		B		F		B	
...																								

(F....10 µl 1 nM Fluorescein; B.....10 µl 0.01 M NaOH = Blank, P...10 µl Invitrogen HighPolStandard)

Measurement Parameters:

- Measurement mode: Fluorescence Polarization
- Excitation wavelength: 470 nm
- Emission wavelength: 525 nm
- Emission Bandwidth: 20 nm
- Gain: 'Optimal'
- Lag time: 0 µs
- Number of reads: 10 (= 100 ms integration time)
- Settle time: 50 ms
- Plate definition file: GRE1536fb.pdf
- Z-Position: Calculated from Well A1
- G-Factor Reference: Calibrated from well A1; ref. value = 20 mP;
- Reference blank: I5; sample blank is same as reference blank

Evaluation:

Calculate the lower detection limit [mP] = 'precision':

FP Precision (mP) = Stdev_F

Stdev_F Standard deviation of wells filled with Fluorescein (except reference well)

7.4.4 Luminescence

7.4.4.1 Cross Talk ATP 96-well Plate:

Pipette the reagents into the wells of a Greiner 96 well plate (white, flat bottom) according to the Plate Layout.

Material:

ATP Kit SL 144-041, BioThema AB
Greiner 96 well plate, flat bottom, white
200 µl Pipette + tips

Plate layout:

Pipette 200 µl of the Blank into the wells A1 – D1 and A3 – D10.

Pipette 40 µl of ATP standard 10^{-7} M into the wells A2 – D2, add 160 µl of ATP reagent and mix in well (**use fresh tip for each well**); **ATP reagent must NOT be contaminated with ATP standard!**

<>	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....200 µl 2×10^{-8} M ATP; B....200 µl Blank, Bx...200µl Blank used for cross talk check

Measurement Parameters:

Before pipetting the plate, prepare instrument for measurement:

Parameters:

Measurement mode: Luminescence

Integration time: 1000 ms

Plate definition file: GRE96fw.pdfx

Part of the plate: A1 – D10

Start measurement immediately after pipetting!

Evaluation:

Calculate Cross Talk for each well separately. The following wells are charged together: A2 with A1, A2 with A3, B2 with B1, B2 with B3, C2 with C1, C2 with C3, D2 with D1, and D2 with D3. The average of these 8 values must be within the specified limits, see chapter 7.3 Acceptance Criteria (under Luminescence Cross talk).

$$CT\% = \frac{(B_x - B) * 100}{(ATP_{wellx} - B)}$$

B_x Blank wells A1 – D1 and A3 – D3, respectively

ATP_{wellx} ATP wells A2 – D2, respectively

B Average of wells A4- D10 (Blank)

7.4.4.2 Detection Limit ATP 384-well Plate

Pipette the reagents into the wells of a Corning 384-well plate (white, flat bottom) according to the Plate Layout

Material:

ATP Kit SL 144-041, BioThema AB
 Corning 384-well plate, flat bottom, white
 100 µl Pipette + tips

Plate layout:

Pipette 100 µl of the Blank into the wells A1 – D1 and A3 – D10
 Pipette 20 µl of ATP standard 10⁻⁷ M into the wells A2 – D2, add 80 µl of ATP reagent and mix in well (**use fresh tip for each well**); **ATP reagent must NOT be contaminated with ATP standard!**

< >	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	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																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

ATP....100µl 2*10-8 M ATP; B....100 µl Blank, Bx...100 µl Blank used for cross talk check, not included in calculations below.

Measurement Parameters

Before pipetting the plate, prepare instrument for measurement:

Parameters:

- Measurement mode: Luminescence
- Integration time: 1000 ms
- Plate definition file: COS384fw.pdfx
- Part of the plate: A1 – D10

Start measurement immediately after pipetting!

Evaluation:

Calculate the detection limit in fmol/well:

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

- 2*10⁻⁸ Concentration of ATP standard [M]
- Stdev_B Standard deviation of Blank (B: A3 – D9)
- mean_{ATP} Average of wells filled with ATP standard
- mean_B Average of Blank wells (B: A3 – D9)
- 0.0001 Conversion into mol/well
- 1/1e⁻¹⁵ Conversion into fmol/well

7.4.4.3 Cross Talk ATP 384-well Plate:

Material:

Same plate/plate layout and reagent as described in chapter 7.4.4.2 Detection Limit ATP 384-well Plate

Evaluation:

Calculate Cross Talk from measurement results of chapter 7.4.4.2 Detection Limit ATP 384-well Plate for each well separately. The following wells are charged together: A2 with A1, A2 with A3, B2 with B1, B2 with B3, C2 with C1, C2 with C3, D2 with D1, and D2 with D3. The average of these 8 values must be within the specified limits, see chapter 7.3 Acceptance Criteria (under Luminescence Cross talk).

$$CT\% = \frac{(B_x - B) * 100}{(ATP_{wellx} - B)}$$

B_x	Blank wells A1 – D1 and A3 – D3, respectively
ATP_{wellx}	ATP wells A2 – D2, respectively
B	Average of wells A4- D10 (Blank)

7.4.4.4 Detection Limit ATP 1536-well Plate

Pipette the reagents into the wells of a Greiner 1536-well plate (white, flat bottom) according to the Plate Layout

Material:

ATP Kit SL 144-041, BioThema AB
 Greiner 384-well plate, flat bottom, white
 10 µl Pipette + tips

Plate layout:

Pipette 10 µl of the Blank into the wells A1 – D1 and A3 – D10

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

<>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	...	25	...	33	...	41	...	
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																									
...																									
I																									
...																									
M																									
...																									
AA																									
...																									
AE																									
...																									
AI																									
...																									
AM																									
...																									

ATP....10 µl 2×10^{-8} M ATP; B....10 µl Blank, Bx...10 µl Blank used for cross talk check, not included in calculations below.

Measurement Parameters

Before pipetting the plate, prepare instrument for measurement:

Parameters:

Measurement mode: Luminescence
 Integration time: 1000 ms
 Plate definition file: GRE1536fw.pdfx
 Part of the plate: A1 – D10

Start measurement immediately after pipetting!

Evaluation:

Calculate the detection limit in fmol/well:

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

$2 \cdot 10^{-8}$	Concentration of ATP standard [M]
Stdev_B	Standard deviation of Blank (B: A3 – D9)
mean_{ATP}	Average of wells filled with ATP standard
mean_B	Average of Blank wells (B: A3 – D9)
0.00001	Conversion into mol/well
$1/1e^{-15}$	Conversion into fmol/well

7.4.4.5 Cross Talk ATP 1536-well Plate:**Material:**

Same plate/plate layout and reagent as described in chapter 7.4.4.4 Detection Limit ATP 1536-well Plate

Evaluation:

Calculate Cross Talk from measurement results of chapter 7.4.4.4 Detection Limit ATP 1536-well Plate for each well separately. The following wells are charged together: A2 with A1, A2 with A3, B2 with B1, B2 with B3, C2 with C1, C2 with C3, D2 with D1, and D2 with D3. The average of these 8 values must be within the specified limits, see chapter 7.3 Acceptance Criteria (under Luminescence Cross talk).

$$CT\% = \frac{(B_x - B) * 100}{(ATP_{\text{well}x} - B)}$$

B_x	Blank wells A1 – D1 and A3 – D3, respectively
$ATP_{\text{well}x}$	ATP wells A2 – D2, respectively
B	Average of wells A4- D10 (Blank)

7.4.5 Absorbance**7.4.5.1 Absorbance Baseline Flatness:****Material:**

No plate required; measurement is performed with empty plate carrier

Measurement Parameters:

Measurement mode:	Absorbance
Wavelength scan type:	Absorbance
Wavelength start:	300 nm
Wavelength end:	700 nm
Wavelength step size:	1 nm
Number of flashes:	50
Plate definition file:	GRE96ft.pdfx
Part of the plate:	A1 - A1

Evaluation:

Calculate the deviation from 0 (unit mOD)

7.4.5.2 Absorbance Uniformity 384-well Plate:

Pipette 100µl the reagents into the wells of a Greiner 384-well plate(flat bottom, transparent) as described under Plate Layout

Material:

Greiner, 384-well plate, flat bottom, transparent

Orange G (60 mg/l)

100 µl-8-channel-pipette + tips

Plate layout:

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

OG...100µl 60 mg/l Orange G

Measurement Parameters OG:

Measurement mode: Absorbance
 Measurement wavelength: 492 nm
 Number of flashes: 50
 Plate definition file: GRE384ft.pdfx

Evaluation:

Calculate the CV%:

$$Uniformity(CV\%) = \frac{stdev_{OG} * 100}{mean_{OG}}$$

mean_{OG} average of wells filled with OG
 stdev_{OG} standard deviation of well filled with OG

7.4.5.3 Absorbance Linearity 384-well Plate:

Pipette 100 µl of the reagents into the wells of a Greiner 384-well plate (flat bottom, transparent) as described under Plate Layout.

Material:

- Greiner 384-well plate, flat bottom, transparent
- Orange G (120 µg/l; 60 µg/l, 40 µg/l, 20 µg/l, 10 µg/l) diluted in water
- Distilled water (Blank)
- 100 µl pipette + tips

Plate layout:

	1	2	3	4	5	6	7	8	9	10	
A	120 mg/l Orange G	60 mg/l Orange G	40 mg/l Orange G	20 mg/l Orange G	10 mg/l Orange G	Blank							
B													
C													
D													
E													
F													
G													
H													
::													

100 µl/well

Measurement Parameters OG:

- Measurement mode: Absorbance
- Measurement wavelength: 492 nm
- Number of flashes: 50
- Plate definition file: GRE384ft.pdfx
- Part of the plate: A1 – H6

Evaluation:

Calculate the average of each concentration (and blank) and subtract blank from samples. Plot the blank subtracted mean measurement values of Orange G against the concentration values. Calculate the coefficient of correlation (r^2).

7.4.5.4 Absorbance Precision 384-well Plate:

Use plate of chapter 7.4.5.3 Absorbance Linearity 384-well Plate:

Material:

For plate/plate layout and materials, see chapter 7.4.5.3 Absorbance Linearity 384-well Plate:

Measurement Parameters OG:

Measurement mode: Absorbance
 Measurement wavelength: 492 nm
 Number of flashes: 50
 Plate definition file: GRE384ft.pdfx
 Part of the plate: A1 and A5
 Number of kinetic cycles: 20
 Kinetic interval (Minimal)

Evaluation:

Calculate the precision in mOD (well A5) and the CV% (well A1):

$$\text{Precision}(mOD) = \text{Average} - \text{Measured}$$

Average Average of well over 20 cycles
 Measured Measured value of one well @ one time point

$$\text{Precision}(CV\%) = \frac{\text{stdev} * 100}{\text{mean}}$$

mean average of well over 20 cycles
 stdev standard deviation of well over 20 cycles

7.4.5.5 Absorbance Reproducibility 384-well plate:

Use plate of chapter 7.4.5.3 Absorbance Linearity 384-well Plate:

Material:

For plate/plate layout and materials see chapter 7.4.5.3 Absorbance Linearity 384-well Plate:

Measurement Parameters OG:

Measurement mode: Absorbance
 Measurement wavelength: 492 nm
 Number of flashes: 50
 Plate definition file: GRE384ft.pdfx
 Part of the plate: A1 to A5
 Number of kinetic cycles: 20
 Kinetic interval (Minimal)

Evaluation:

Calculate the reproducibility (CV%):

$$\text{Reproducibility}(CV\%) = \frac{\text{stdev} * 100}{\text{average}}$$

average average of well over 20 cycles
 stdev standard deviation of well over 20 cycles

8. Cleaning & Maintenance

8.1 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 in order to prolong the instrument's life and to reduce the need for servicing.



Caution

Keep the plate transport clean, especially take care of the clip mechanism to fix microplates. Insufficient plate fixing lead to instrument damages. Strong soiling needs servicing.

This section contains the following procedures:

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

8.2 Liquid Spills

1. Wipe up the spill immediately with absorbent material.
2. Dispose of contaminated material appropriately.
3. Clean the instrument surfaces with a mild detergent.
4. For biohazard spills, clean with a 5-10 % solution of bleach in de-ionized water.
5. Wipe cleaned areas dry.

8.3 Instrument Decontamination/Disinfection



WARNING

ALL PARTS OF THE INSTRUMENT THAT COME INTO CONTACT WITH POTENTIALLY INFECTIOUS MATERIAL, BIOLOGICAL SAMPLES, PATIENT SAMPLES, POSITIVE CONTROL SAMPLES OR ANY HAZARDOUS MATERIAL MUST BE TREATED AS POTENTIALLY INFECTIOUS AREAS.

IT IS ADVISABLE TO ADHERE TO APPLICABLE SAFETY PRECAUTIONS, (INCLUDING THE WEARING OF POWDER-FREE GLOVES, SAFETY GLASSES, SURGICAL MASK AND PROTECTIVE CLOTHING) TO AVOID POTENTIAL INFECTIOUS DISEASE CONTAMINATION WHEN PERFORMING THE DECONTAMINATION/DISINFECTION PROCEDURE.



Warning

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



Caution

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

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

8.3.1 Decontamination/Disinfection Solutions

The instrument (Front, Cover, Plate transport) should be decontaminated/disinfected using one of the following solutions:

- Areades B (Schülke & Mayr GmbH, A-1070 Wien)
- Dodacarna rapid neu (Schülke & Mayr GmbH, A-1070 Wien)



Caution

The Decontamination/Disinfection procedure should be performed by authorized trained personnel in a well ventilated room wearing powder-free gloves, safety glasses, surgical mask and protective clothing.

Please note that the decontamination/disinfectant solution can influence the performance of your instrument if applied inside the instrument.

8.3.2 Decontamination/Disinfection Procedure

**WARNING**

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

**WARNING**

THE DECONTAMINATION/DISINFECTION PROCEDURE SHOULD BE PERFORMED ACCORDING TO NATIONAL, REGIONAL, AND LOCAL REGULATIONS.

**Caution**

The surface decontamination/disinfectant solution can negatively influence the performance of your instrument, if it is applied or accidentally gets inside the instrument.

**Caution**

Make sure that the microplate has been removed from the instrument before starting Decontamination/Disinfection.

Perform the Decontamination/Disinfection procedure as follows:

1. Wear powder-free gloves, safety glasses, surgical mask and protective clothing.
2. Prepare a suitable container for all disposables used during the Decontamination/Disinfection procedure.
3. Move the plate carrier into the load position; If a microplate is on the plate carrier, remove it.
4. Disconnect the instrument from the main power supply and let it cool down to ambient temperature to avoid any risk of fire and explosion.
5. Disconnect the instrument from the computer and from any accessories.
6. Carefully apply the decontamination/disinfectant solution according to the manufacturer's instructions for use on the plate transport of the instrument.
7. After the required contact time (according to the manufacturer's instructions for use) wipe the plate carrier using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the decontamination/disinfectant solution.
8. Move the plate carrier into the instrument by gently pressing its front end (of the plate transport) until the front plate transport door is completely closed.
9. Carefully apply the decontamination/disinfectant solution according to the manufacturer's instructions for use on all outer surfaces of the instrument.
10. After the required contact time (according to the manufacturer's instructions for use) wipe the instrument using a soft paper towel moistened with a mild detergent or distilled water to remove all traces of the decontamination/disinfectant solution.
11. Wipe dry the outer surface of the instrument with a soft paper towel.

12. Repeat the Decontamination/Disinfection procedure on any accessories which are being moved or returned.
13. Wash your hands with a mild detergent and then disinfect them.
14. Pack the instrument and any accessories.
15. Dispose of the container with the disposables according to the relevant national, regional and local laws and regulations.
16. Complete a safety certificate and attach it to the outside of the box so that it is clearly visible.

**Caution**

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

8.3.3 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 decontaminated/disinfected at the operating authority's site before shipping.

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, surgical mask 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.

8.4 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 waste.

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

8.4.1 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.

8.4.2 Disposal of Operating Material



WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESS RUN ON THE INFINITE M1000.

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.

8.4.3 Disposal of the Instrument

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



Caution

Always disinfect the instrument before disposing.

Pollution degree	2 (IEC/EN 61010-1)
Method of disposal	Contaminated waste



WARNING

Depending on the applications, parts of the Infinite M1000 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.

9. Error Messages and Troubleshooting

9.1 Error Messages Introduction

The internal microprocessor controls and checks all electronic functions as well as measurements, operations and results. If the microprocessor detects a fault or an incorrect operating procedure, an error message is displayed on the computer. The following table gives a brief description of the error messages and the troubleshooting actions.



Note

If other error messages appear that are not mentioned in the table below, contact your local Tecan customer support office.

Error #	Error Text	Description
ERR: 1	Invalid Command	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 2	Parameter out of range	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 3	Invalid number of parameters	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 4	Invalid parameter	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 5	Invalid Parameter at pos	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 6	[prefix] is missing	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 7	RS485 Timeout	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
ERR: 8	Invalid module	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 9	Binary Transfer command: [cmd] at module [n]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
ERR: 10	Error at module	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
ERR: 11	Error lid check	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.
ERR: 12	Error fiber check	Hardware Failure Luminescence Module Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
ERR: 15	X drive init error	Hardware Failure Plate Transport Module Please report this error to your local Tecan customer support office.
ERR: 16	Y drive init error	Hardware Failure Plate Transport Module Please report this error to your local Tecan customer support office.
ERR: 17	z drive init error	Hardware Failure z-drive Module Please report this error to your local Tecan customer support office.
ERR: 18	Injector A not available	Hardware Failure Injector A Please report this error to your local Tecan customer support office.
ERR: 19	Injector B not available	Hardware Failure Injector A Please report this error to your local Tecan customer support office.
ERR: 30	Reference error	Unspecific Hardware failure Please report this error to your local Tecan customer support office.
ERR: 31	[ERR] at module [mod] (cmd:[cmd])	Unspecific Hardware failure Please report this error to your local Tecan customer support office.
ERR: 32	MTP In-/Out-Position	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 33	Error value not set	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 34	Injector not enabled	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
ERR: 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.
ERR: 38	Instrument Initialization Error	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.
ERR: 44	Steploss Error	Actuator failure. Please report this error to your local Tecan customer support office.
ERR: 53	No memory	Module has no user memory. Please report this error to your local Tecan customer support office.
ERR: 54	Memory access	Can not access memory. Please report this error to your local Tecan customer support office.
ERR: 56	Not implemented	Option not implemented.
ERR: 57	USB Time out	USB time out. Please report this error to your local Tecan customer support office.
ERR: 58	Invalid prefix	Invalid prefix. Please report this error to your local Tecan customer support office.
ERR: 59	Parameter out of range STRLEN	Invalid parameter. Please report this error to your local Tecan customer support office.
ERR: 60	OS steploss	Steploss of the order sorting filter wheel. Switch instrument off and on, if error persists contact your local Tecan customer support office.
ERR: 61	Fiber switch steploss	Fiber switch steploss. Switch instrument off and on, if error persists contact your local Tecan customer support office.

Error #	Error Text	Description
ERR: 62	Slit steploss	Slit steploss. Switch instrument off and on, if error persists contact your local Tecan customer support office.
ERR: 63	Polarization steploss	Polarization steploss. Switch instrument off and on, if error persists contact your local Tecan customer support office.
ERR: 64	Invalid wavelength table	Wrong wavelength settings for Absorbance scans. Please report this error to your local Tecan customer support office.
ERR: 65	Lamp low	Insufficient light on reference fiber. Please report this error to your local Tecan customer support office.
ERR: 66	Em data overflow	Emission data overflow. Please report this error to your local Tecan customer support office.
ERR: 67	Ex data overflow	Excitation data overflow. Please report this error to your local Tecan customer support office.
ERR: 68	Error load position	MTP not in load position. Please report this error to your local Tecan customer support office.
ERR: 70	Error stacker not ready	Stacker not ready. Please report this error to your local Tecan customer support office.
ERR: 71	No injector needle	No injector in the instrument.
ERR: 72	Stacker error	Stacker error. Please report this error to your local Tecan customer support office.
ERR: 73	Flash CNT error	Flash counter error. Please report this error to your local Tecan customer support office.
ERR: 74	Checksum Error Excitation	Checksum error on excitation data channel. Please report this error to your local Tecan customer support office.
ERR: 75	Checksum Error Emission	Checksum error on emission data channel. Please report this error to your local Tecan customer support office.
ERR: 76	CAN device error	CAN device error. Please report this error to your local Tecan customer support office.
ERR: 77	Module not enabled	Module not enabled. Please report this error to your local Tecan customer support office.
ERR: 78	Check GRATE error	Grating not stable. Please report this error to your local Tecan customer support office.
ERR: 79	Check GRATE error EX DIFF	Grating difference at excitation. Please report this error to your local Tecan customer support office.
ERR: 80	Check GRATE error EX MOVE	Grating not moving at excitation. Please report this error to your local Tecan customer support office.
ERR: 81	Check GRATE error EM DIFF	Grating difference at emission. Please report this error to your local Tecan customer support office.
ERR: 82	Check GRATE error EM MOVE	Grating not moving at emission. Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
ERR: 83	Check no plate	No plate on transport. Please report this error to your local Tecan customer support office.
ERR: 84	Check no plate detect	No plate detection. Please report this error to your local Tecan customer support office.
ERR: 86	ST MEAS HW: Error 10-99	Emission or excitation busy. Please report this error to your local Tecan customer support office.

Index

A	
Absorbance	17, 89
Absorbance Detection	43
Absorbance Optics	42
Absorbance System	41
Acceptance Criteria	95
Accuracy	94
Analyzer.....	<i>See</i> Polarizer
Area of Application	20
ATP.....	90
B	
Bandwidth	33
Blocking	33
C	
Channel Photomultiplier	47
Condenser	32
Cross-Talk	95
D	
Decontamination/Disinfection	118
Detection Limit.....	94
Dichroic mirror	40
Disconnect.....	51
Disinfection	
Safety Certificate.....	120
Disposal	
Instrument	121
Operating Material	122
Packing Material	121
E	
Emission Monochromator.....	35
Excitation Spot Size.....	34
F	
Filter Wheel PMT.....	35
Finish a Measurement Session	51
Flash.....	60
Flash Frequency Mode.....	60
Flash Lamp	32
Flash Monitor.....	33
Fluorescein	87, 88, 89
Fluorescence	15, 96
Fluorescence Optics	34, 38
Fluorescence Fiber Bundle.....	34
Fluorescence Intensity Detection	35
Fluorescence Intensity Lens System	34
Fluorescence Intensity System	31
Fluorescence Polarization.....	17, 89
Fluorescence Polarization Detection.....	40
Fluorescence Polarization System.....	37
Fluorescence Resonance Energy Transfer (FRET).....	16
Fluorescence Time Resolved (TRF)	16
FRET . <i>See</i> Fluorescence Resonance Energy Transfer	
Full width at half maximum.....	33
FWHM	<i>See</i> Full width at half maximum
G	
Gain	56
Gain adjustment	57
General.....	11
Glow Type Luminescence.....	90, 91
I	
i-control.....	20
Instrument Power On	50
Instrument Specifications	85
Instrument Start Up	50
Integration Time	65
Introduction.....	9
K	
Kinetic Measurements.....	51
L	
LC.....	<i>See</i> Liquid Crystal
LED Light Source	38
Light Source System	32
Light Source System Fluorescence Polarization	37
Linearity.....	94
Liquid Crystal.....	39
LOD	<i>See</i> Detection Limit
Luminescence	18
Luminescence Detection Unit	44
Luminescence Optics	44
Luminescence System	44
M	
Magellan.....	20
magellan Tracker.....	20
Maintenance.....	117
Measurement Techniques.....	15
Monochromator	33

Multi Labeling.....	52	R	Reagent Compatibility	80
O		S	Safety	9
On the Fly	92	Safety Certificate	120	
Optical System.....	31	Shaking.....	51	
Optical System Absorbance	41	Shut Down	51	
Optical System Fluorescence Top and Bottom.....	31	silicon photodiode.....	33	
Optical System Luminescence	44	Software	20	
OVER.....	<i>See Overflow</i>	Spectral Intensity Calibration.....	52	
overflow	56	T		
P		Temperature Control	51	
Packing Material		Test Instructions	96	
Disposal	121	Time between Move and Flash	61	
Returning.....	121	Timing Parameters	60	
Performance	12	TRF.....	<i>See Fluorescence Time Resolved</i>	
Plate Carrier Transport Lock	23	U		
PMT Detector.....	35	Uniformity	94	
Polarizer.....	39	V		
Power Requirements	25	voltage range.....	25	
Q				
Quality Control	93			

Tecan Customer Support

	Address	Contact
Tecan Asia Pte Ltd.	18 Boon Lay Way, #09-140 TradeHub 21 Singapore 609966, Singapore	T +65 6444 1886 F +65 6444 1836 tecan@tecan.com.sg
Tecan Australia Pty Ltd.	10-22 Hornibrook Esplanade Clontarf Qld 4019 Australia	T 1300 808 403 (free in Australia) T +61 7 3105 5010 F +61 7 3283 7922 support@tecan.com.au
Tecan Austria GmbH	Untersbergstrasse 1a A-5082 Grödig Austria	T +43 6246 8933 256 F +43 6246 72 770 helpdesk-at@tecan.com
Tecan Benelux bvba	Businesspark E19 Battelsesteenweg 455 B 2800 Mechelen, Belgium	T +32 15 709 054 (English) T +32 15 709 055 (French) F +32 15 421 612 helpdesk.benelux@tecan.com
Tecan Benelux bvba	Industrieweg 30 NL-4283 GZ Giessen Netherlands	T +31 20 708 4773 F +31 183 44 80 67 helpdesk.benelux@tecan.com
Tecan Group Ltd., Beijing Representative Office	Room 2502, Building A Jianwai SOHO No. 39 Dongsanhuan Zhong Rd. 100022 Beijing, China	T +86 10 5869 5936 F +86 10 5869 5935 helpdesk-cn@tecan.com
Tecan Deutschland GmbH	Werner-von-Siemens-Straße 23 D-74564 Crailsheim Germany	T +49 1805 8322 633 or T +49 1805 TECAN DE F +49 7951 9417 92 helpdesk-de@tecan.com
Tecan France S.A.S.	26 avenue Tony Garnier F-69007 Lyon France	T +33 820 88 77 36 F +33 4 72 76 04 99 helpdesk-fr@tecan.com
Tecan Ibérica (Spain)	Sabino de Arana 32 E-08028 Barcelona Spain	T +34 93 409 1237 F +34 93 411 2407 helpdesk-sp@tecan.com
Tecan Ibérica (Spain)	N VI - km 23,300, c/ Pollensa, 4 - Oficina 8 E-28230 Las Rozas de Madrid, Spain	T +34 91 151 71 07 F +34 91 151 7120 helpdesk-sp@tecan.com
Tecan Ibérica (Portugal)	Quinta da Fonte Edificio Pedro I P-2780-730 Paço D'Arcos, Portugal	T +35 21 000 8216
Tecan Italia S.r.l.	Via Brescia, 39 I-20063 - Cernusco Sul Naviglio (MI) Italy	T +39 (02) 800 112 291 F +39 (02) 92 72 90 47 helpdesk-it@tecan.com
Tecan Japan Co. Ltd	Kawasaki Tech Center 580-16, Horikawa-cho, Saiwai-ku Kawasaki, Kanagawa 212-0013 Japan	T +81 44 556 7311(Kawasaki) F +81 44 556 7312 (Kawasaki) T +81(0) 6305 8511 (Osaka) helpdesk-jp@tecan.com
Tecan Nordic AB	Taljegårdsgatan 11B SE-431 53 Mölndal Sweden	T +46 317 54 40 00 F +46 317 54 40 10 helpdesk@tecan.se
Tecan (Shanghai) Trading Co., Ltd.	Unit 621, Tower 1, German Center 88, Keyuan Road, Zhangjiang Hi-tech Park Pudong New Area 201203 Shanghai, P.R. China	T +86 21 2898 6333 F +86 21 2898 6844 tecan-cn@tecan.com
Tecan Schweiz AG	Seestrasse 103 CH-8708 Männedorf Switzerland	T +41 44 922 82 82 F +41 44 922 84 84 helpdesk-ch@tecan.com
Tecan Systems Inc. Technical support for components	2450 Zanker Road San Jose, CA 95131 USA	T +1 408 953 3100 F +1 408 953 3101 Toll Free in the US: T +1 866 798 3226 helpdesk-sy@tecan.com
Tecan UK	Theale Court 11-13 High Street, Theale UK-Reading RG7 5AH, United Kingdom	T +44 118 930 0300 F +44 118 930 5671 helpdesk-uk@tecan.com
Tecan US Technical support for Tecan instruments	4022 Stirrup Creek Road, Suite 310 Durham, NC 27703 USA	T +1 919 361 5200, F +1 919 361 5201 Toll Free in the US: T +1 800 TECAN US or T +1 800 832 2687 helpdesk-us@tecan.com

Declaration of Conformity

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

Product Type: Microplate Reader
Model Designation: Infinite® 1000

Variants: Infinite M1000

Options: Stacker, Injector, Barcode

Address: Tecan Austria G.m.b.H.
Untersbergstraße 1A
A-5082 Grödig Austria/ Europe

is in conformity with the provisions of the following EC Directive(s) when installed in accordance with the installation instructions contained in the product documentation:

2006/95/EC – Low Voltage Directive

2004/108/EC – EMC Directive

2006/42/EC – Machinery Directive

and that the standards referenced below were taken in consideration:

EN 61010-1:2001	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
EN 61010-2-081/A1:2003	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 2-081: Particular requirements for automatic and semiautomatic laboratory equipment for analysis and other purposes
EN 60825-1:2007	Safety of laser products - Part 1: Equipment classification and requirements
EN 61326-1:2006	Electrical Equipment for Measurement, Control, and Laboratory Use - EMC Requirements - Part 1: General requirements
EN ISO 14121-1:2007	Safety of machinery - Risk assessment - Part 1: Principles

Year of CE-marking: 2010