TECAN.

Instructions for Use for

INFINITE M1000 PRO

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WARNING

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

Notice

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

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

We would appreciate any comments on this publication.

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Copyright Information

Declaration for EU Certificate

See the last page of these Instructions for Use.

About the Instructions for Use

Original Instructions. This document describes the **INFINITE M1000 PRO** 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

Remarks on Screenshots

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

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Warnings, Cautions and Notes

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

Note
Gives helpful information.

Caution

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

WARNING

INDICATES THE POSSIBILITY OF SEVERE PERSONAL INJURY, LOSS OF LIFE OR EQUIPMENT DAMAGE IF THE INSTRUCTIONS ARE NOT FOLLOWED.

WARNING

INDICATES LASER. DO NOT STARE INTO THE BEAM!

WARNING

INDICATES THE POSSIBLE PRESENCE OF BIOLOGICALLY HAZARDOUS MATERIAL. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

WARNING

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

ATTENTION

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.**

Symbols

Manufactured by

Date of manufacture

USB label

Conformité Européenne

Consult Instructions for Use

Directive 2002/96/EC on waste electrical and electronic equipment (WEEE) symbol

Laser

Biohazardous

Table of Contents

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 PRO** 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 has taken great care when creating the stored Plate Definition Files (.pdfx) that are supplied with the instrument. We have taken every precaution to ensure that the plate heights and well depths are correct according to the defined plate type.

These parameters are used to determine the minimum distance between the top of the plate and the ceiling of the measurement chamber.

Additionally, Tecan Austria has added a very small safety gap to prevent any damage from occurring to the measurement chamber due to small changes in plate height. This has no affect on the performance of the instrument.

Users MUST ensure that the plate definition file selected corresponds to the actual plate being used. The safety gaps cannot be calculated by the INFINITE M1000 PRO 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 measurements are performed.

Caution

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

Caution

To ensure the optimal performance of the INFINITE M1000 PRO instrument, 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 PRO may come in contact with biohazardous/infectious material. Make sure that only qualified personnel operate the instrument. In case of service or when relocating or disposing of the instrument, always disinfect the instrument according to the directions given in these Instructions for Use.

2. General Description

2.1 Instrument

2.1.1 Instrument Features

The Tecan **INFINITE M1000 PRO** 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 PRO** 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 PRO** can also perform fluorescence polarization and luminescence measurements (including luminescence scans) as well as Amplified Luminescent Proximity Homogeneous Assays (AlphaScreen and AlphaLISA).

The **INFINITE M1000 PRO** 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 PRO with injector box.

2.1.2 Intended Use

The **INFINITE M1000 PRO** 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
- Absorbance Scan
- Fluorescence Intensity Top
- Fluorescence Intensity Bottom
- Fluorescence Scan (Top/Bottom)
- Time Resolved Fluorescence (TRF, TR-FRET)
- Fluorescence Polarization (FP)
- Luminescence (Glow Type, Flash Type and Dual-Color)
- Luminescence Scan (Top/Bottom)
- AlphaScreen/AlphaLISA

Any standard microplate (ranging from 6 to 1536-well formats with a maximum plate height of 23 mm including the 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 PRO**. Injectors are available for microplates from 6 to 384 wells.

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

2.1.4 Performance

The **INFINITE M1000 PRO** has been designed for speed and sensitivity. 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 that 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 PRO** 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, the bandwidth can also 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 PRO** 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 starting the software. 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 the i-control software).

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

Caution

If the instructions given in these 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	Windows XP/Vista (32-bit)/Windows 7 (32- or 64-bit):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	Windows XP: 512 MB RAM Windows Vista (32-bit): 1 GB RAM Windows 7 (32-bit): 1 GB RAM Windows 7 (64-bit): 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 Windows Vista: DirectX 9 graphics and 32 MB of graphics memory (for Home Basic); 128 MB of graphics memory plus WDDM support for all other versions Windows 7: 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 PRO** measurement techniques. To keep this chapter compact, a few simplifications have been made. For details, see the references.

2.2.1 Fluorescence

The **INFINITE M1000 PRO** 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 and then be referenced as HTRF (TR-FRET). Fluorescence Time Resolved (TRF) measurements should not be confused with Fluorescence Lifetime measurements.

Fluorescence Intensity

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, type 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 detect binding events of various labeled compounds that are in close proximity.

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*). An 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 of the use of 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. Stâhlberg, et.al. - American Laboratory, December 1993 page 15)* some of them having fluorescence lifetimes in excess of 100 microseconds.

The **INFINITE M1000 PRO** uses a flash lamp light source with flash duration much shorter than the fluorescence lifetime of these species. This offers the opportunity to measure fluorescence emission at the time when stray light and prompt fluorescence have already vanished (Lag Time) thus significantly lowering background fluorescence and improving sensitivity.

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

Homogeneous 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 measurements 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 homogeneous format of these assays, so-called ‘mix and measure’ protocols, satisfies 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 a multi-labeling 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 Cisbio Bioassays, which application is limited to the use of HTRF® reagents and technology, and for which Cisbio Bioassays 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'etat 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:

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

Where I_{SAMPLE} is the intensity of the light being transmitted, I_0 the light intensity not attenuated by sample. The unit is assigned with 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 PRO** provides measurement of glow type chemi- or bioluminescence. Glow type means that the luminescence assay glows much longer than a minute. Luminescence substrates are available which provide stable enough light output over hours.

As an example, luminescence can be measured to determine the activity of an enzyme labeled compound (-peroxidase, -phosphatase). Light emission results from a luminescence substrate being decomposed by the enzyme. Under excess of substrate, the luminescence signal can be assumed to be proportional to the abundance of the enzyme-labeled compound. As with enzyme-based assays, control of environmental conditions is 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 performed 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 which covers 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 BRET^{2} ™. 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
- 'Lumi Blue': wavelength range of 400 to 515 nm
- 'Lumi Orange': wavelength range of 550 to 630 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 homogeneous 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 PRO** 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 recombinants of Renilla or Firefly luciferase) can be recorded in order to define emission maxima. Also environmental influences on the spectral behavior 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 Fluorescence Measurements.

2.2.4 AlphaScreen/AlphaLISA

Caution

The AlphaScreen/AlphaLISA module uses a high-power laser light source. Do not stare into the instrument while a measurement is running.

The **INFINITE M1000 PRO** is able to measure Amplified Luminescent Proximity Homogeneous Assays (AlphaScreen and AlphaLISA). Due to their nonradioactive, homogeneous and sensitive nature, these bead-based technologies are perfectly suited for the study of biomolecular interactions.

Upon illumination with a high-energy light source, the photosensitive molecules contained in the donor beads produce high levels of oxyradicals. These oxyradicals are able to travel to the acceptor beads and trigger a cascade of reactions that ultimately lead to the generation of a strong chemiluminescent signal.

Note

AlphaScreen/AlphaLISA measurements are only possible as endpoint measurements in white or light gray microplates and cannot be performed in combination with the injector system and the heating system.

2.3 Software

The **INFINITE M1000 PRO** is delivered with the ***i-control*** software including online-help and 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 PRO**.

For robotic automation **INFINITE M1000 PRO** 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 PRO**. (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 1040.10, except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007.

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 PRO 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 PRO is a precision instrument and weighs approximately 29.5 kg. At least two people must carefully lift the instrument from the box.

Caution

The maximum load for the INFINITE M1000 PRO 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 PRO plate transport is 300 g. Overloading the plate carrier can cause instrument damage which may require service.

Plate carrier testing and wavelength calibration should be done annually with the MultiCheck-Plus Test Plate to ensure the optimal performance of the INFINITE M1000 PRO.

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 it is in operation.

3.2 Plate Carrier Transport Lock

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.

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:

1. Switch ON the computer and install the corresponding software on the computer (i-control, Magellan or EVOware).
2. Ensure that 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) on 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 PRO 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.

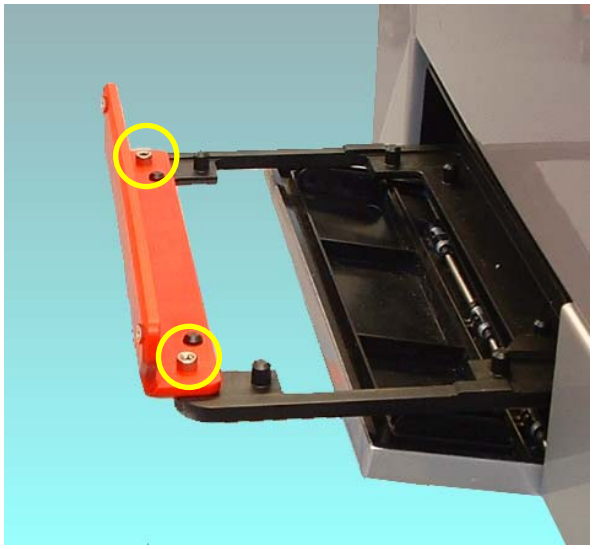


Figure 4

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**.
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 PRO 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

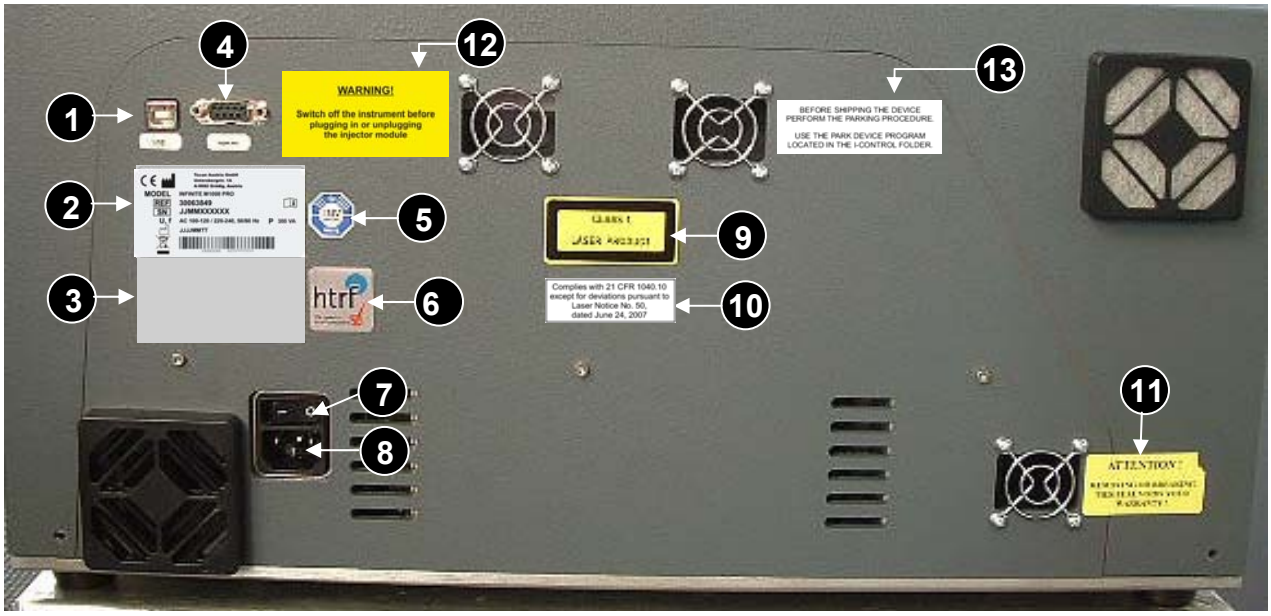


Figure 5

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 21 CFR 1040.10 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007
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.

Example Name Plate

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

For an overview of the various instruments for which these Instructions for Use are valid see the Declaration of Conformity on the last page of this document.

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 PRO for Shipping

Before shipping the **INFINITE M1000 PRO**, 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 PRO Instrument

Front View

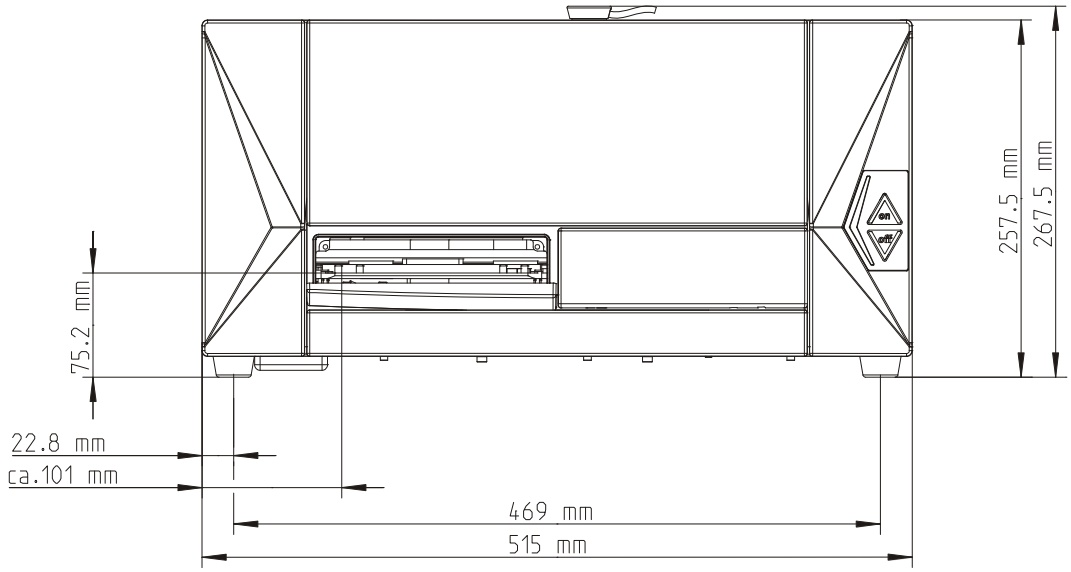


Figure 6

Side View

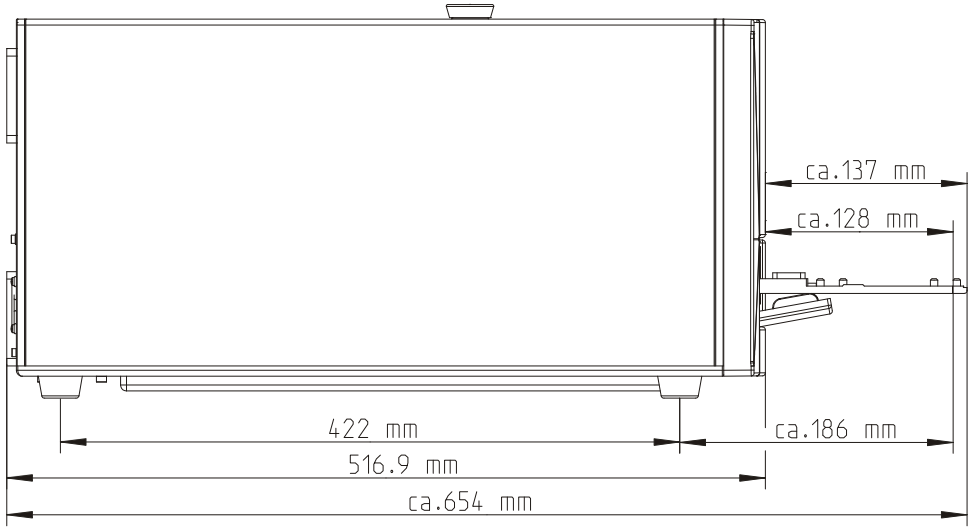


Figure 7

3.6.2 INFINITE M1000 PRO Instrument with Built-in Stacker

Front View with Built-in Stacker

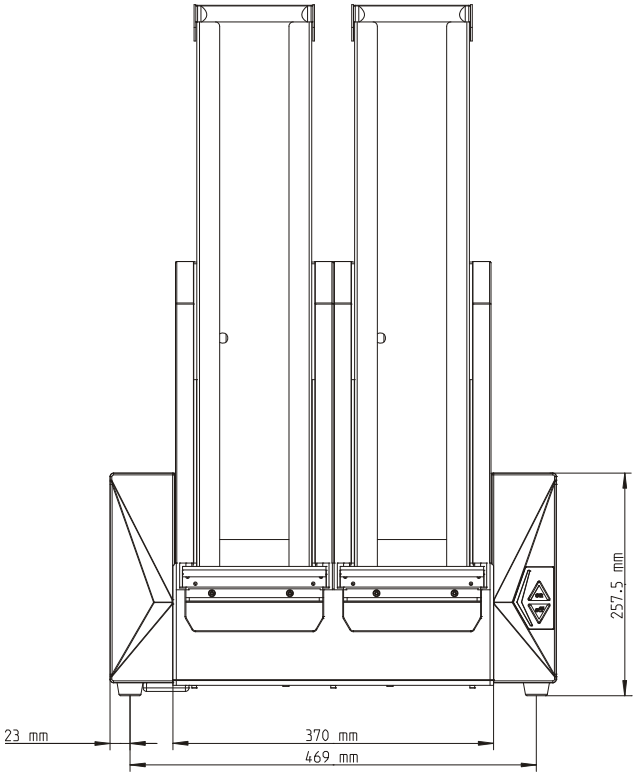


Figure 8

Side View with Built-in Stacker

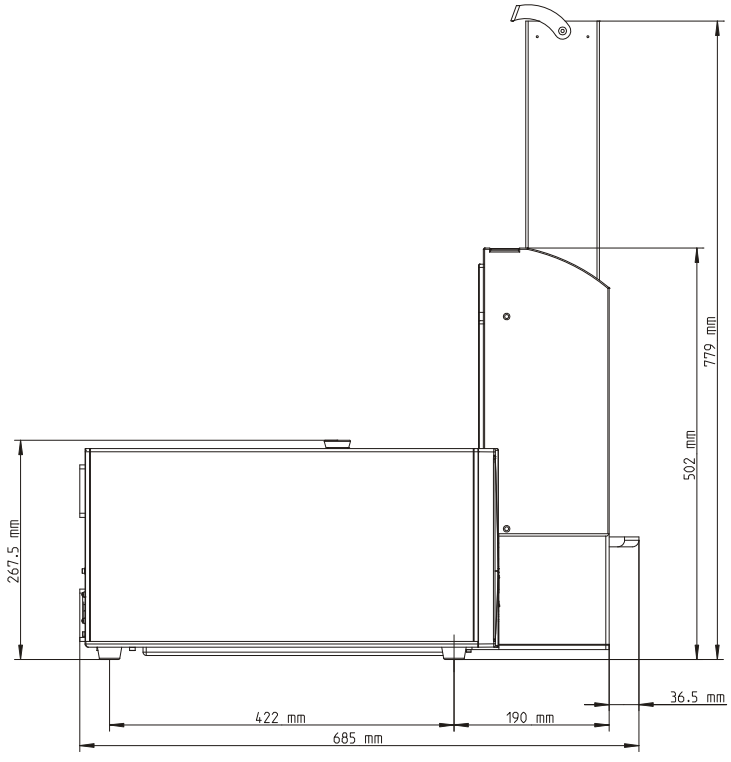


Figure 9

3.6.3 Injector Module Dimensions

Front View

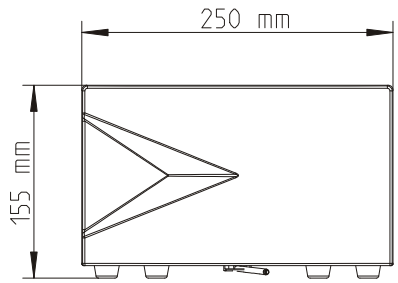


Figure 10

Side View

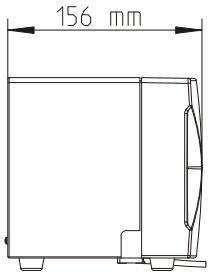


Figure 11

4. Optical System

4.1 Fluorescence Intensity System

The **INFINITE M1000 PRO** 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 is consists of:

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

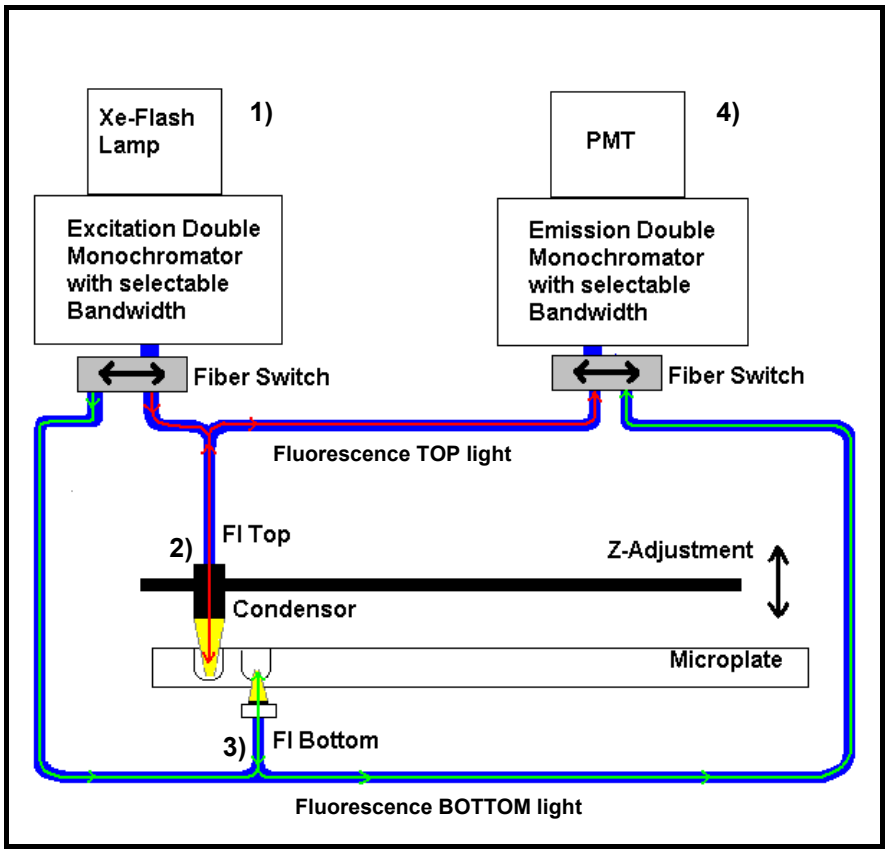


Figure 12: Optical System Fluorescence Top and Bottom

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 PRO** 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 PRO** 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 PRO** 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.

In many cases, fluorescence emission spectra do not depend on the exact excitation wavelength; therefore, for maximum total fluorescence signal, a broad excitation bandwidth should 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 a 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 the **INFINITE M1000 PRO** 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 the **INFINITE M1000 PRO**, 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 and is 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 which guides the light to the top or bottom measuring head. The light is then focused into the sample by the top/bottom lens system (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 can be adjusted. As light is refracted onto the sample liquid surface, a Z-adjustment helps to maximize signal to noise.

The fluorescence measuring Top and Bottom Optics are 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 and bottom fibers 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 PRO** 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.**

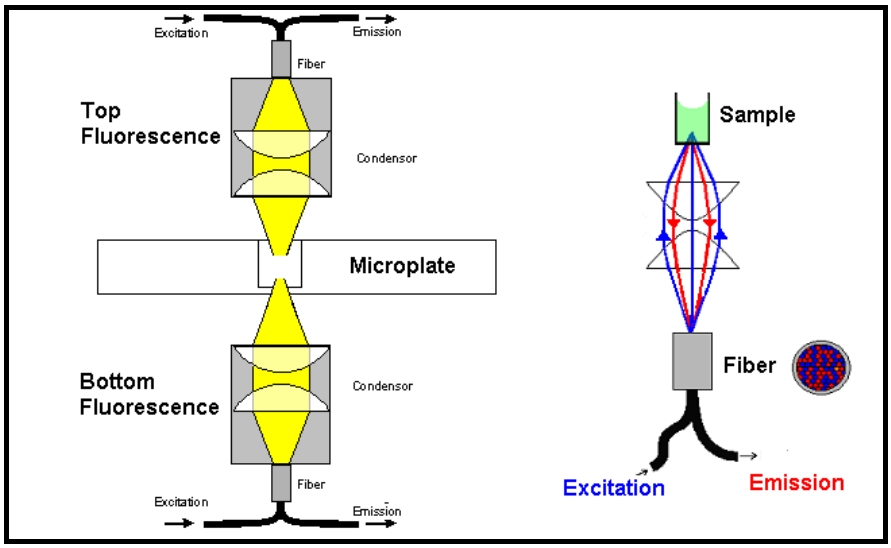


Figure 13: Fluorescence Optics used for Top/Bottom Fluorescence intensity measurement (the fiber details on the right hand side are shown for the bottom measurement which is comparable to the top optics).

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; therefore, to achieve maximum total fluorescence signal, a broad excitation bandwidth should 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 the low light levels associated with fluorescence. The dedicated fluorescence PMT of the **INFINITE M1000 PRO** 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 PRO** 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 light of the selected wavelength passes the emission monochromator, it is focused onto the detector (PMT: photo-multiplier tube). The 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.

4.2 Fluorescence Polarization System

The **INFINITE M1000 PRO** 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).

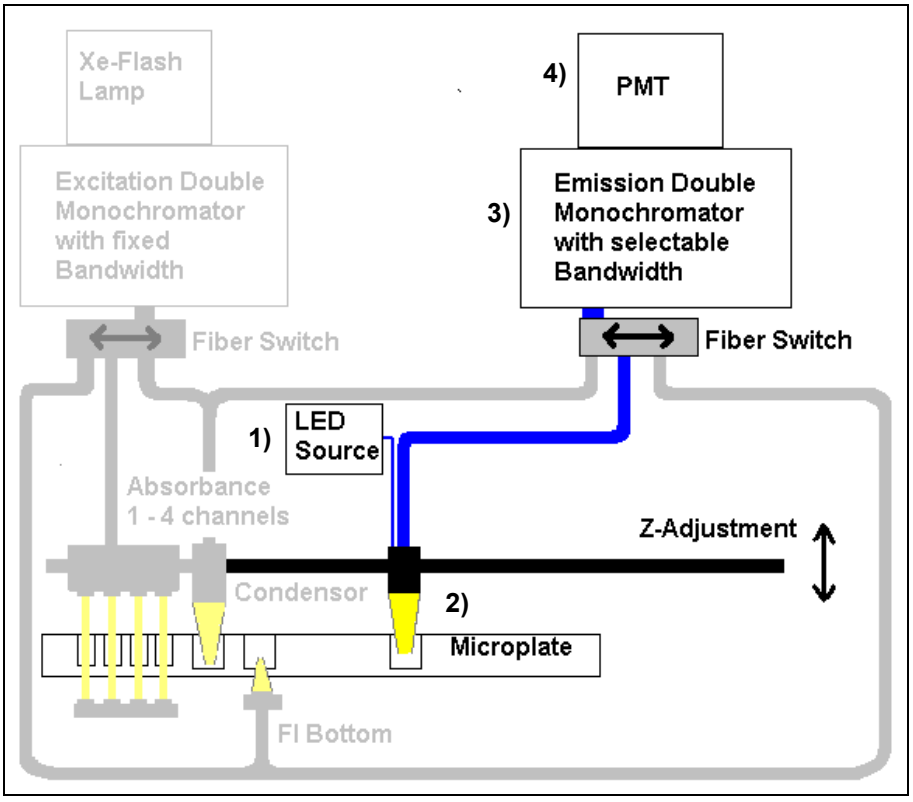


Figure 14: Optical System Fluorescence Polarization.

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 PRO** 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 wavelengths are installed in the **INFINITE M1000 PRO**: LED 1: 470 nm; LED 2: 530 nm; LED 3: 590 nm; LED 4: 635 nm.

The main benefits of LEDs are:

a) Improved excitation energy compared to monochromator system

b) No warm up time required

Lens System

The system is made of 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
- Lens 3 focuses the emission light onto the fiber optic bundle (Figure 14) and guides 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 LEDs and passes through appropriate interference filters and dichroic mirrors (Figure 14). Figure 15 shows the spectra of the resulting light which exits the sample.

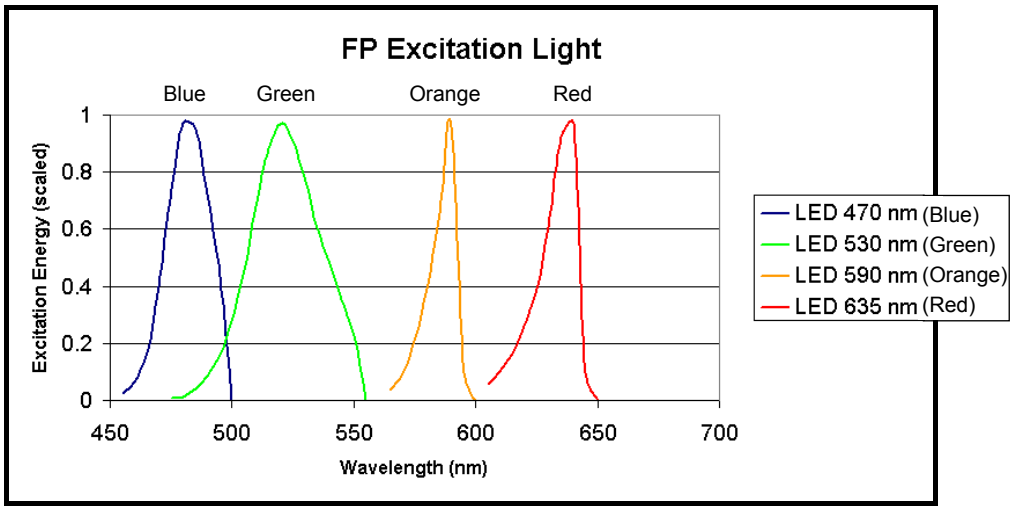


Figure 15: Fluorescence Polarization Excitation light spectra.

Z-Positioning

The Z-position of the polarization optics can be adjusted (Figure 14). As light is refracted onto the sample liquid surface, a Z-adjustment helps to maximize the signal to noise ratio.

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

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

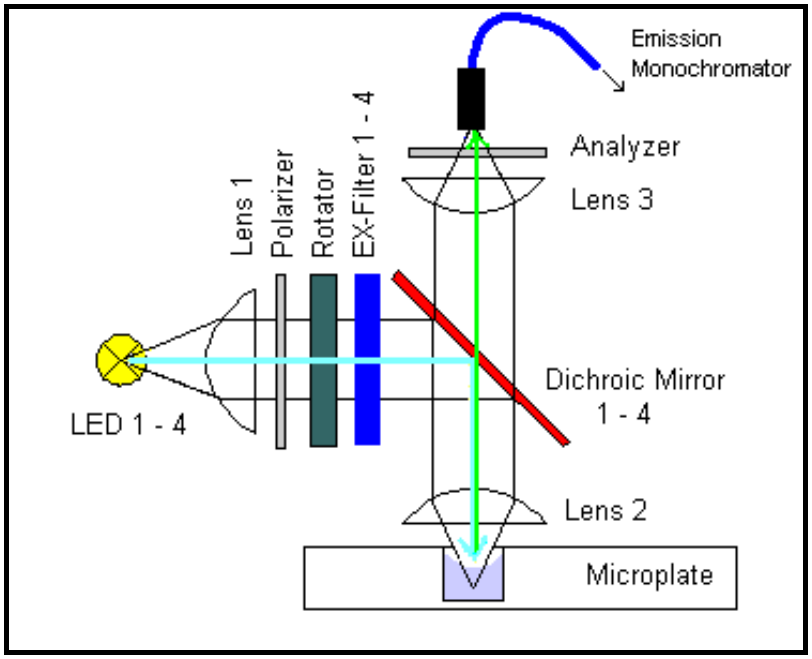


Figure 16: Fluorescence Polarization Optics.

Polarizer

A polarizer is a device for producing plane-polarized light.

Rotator

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

EX-Filter

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

Dichroic Mirror

Dichroic mirrors serve to optimize the light intensity in a certain excitation wavelength range, which provides a better signal to noise ratio when compared to a 50% mirror. For each LED in combination with an EX-filter an appropriate dichroic mirror is installed.

Analyzer

The Analyzer allows only light with a specific type of plane to pass..

4.2.3 Fluorescence Polarization Detection

A fiber bundle guides the polarized light that passes 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 entire measurement, therefore a “settle time” is not recommended for samples which bleach quickly, because the total time in which the sample is exposed to light is increased. However, when using stable samples, a ‘settle time’ can improve 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.

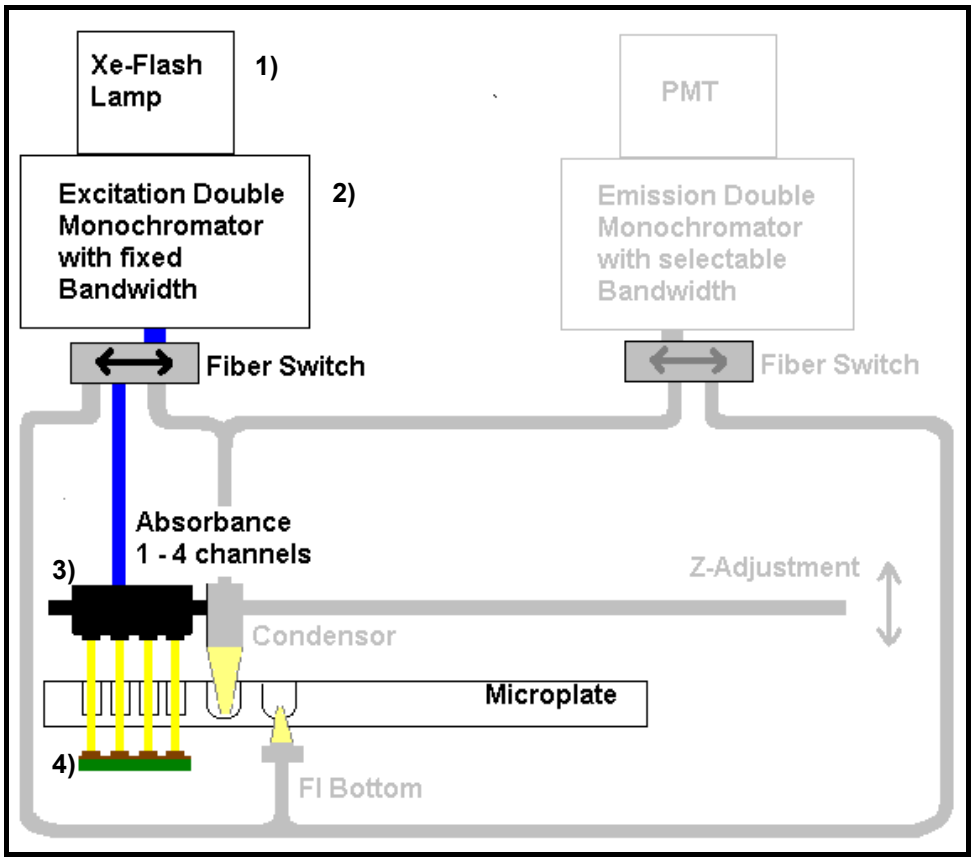


Figure 17: Optical System Absorbance.

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 consist of a pair of lenses which focus the light beam into the well of the microplate (Figure 18).

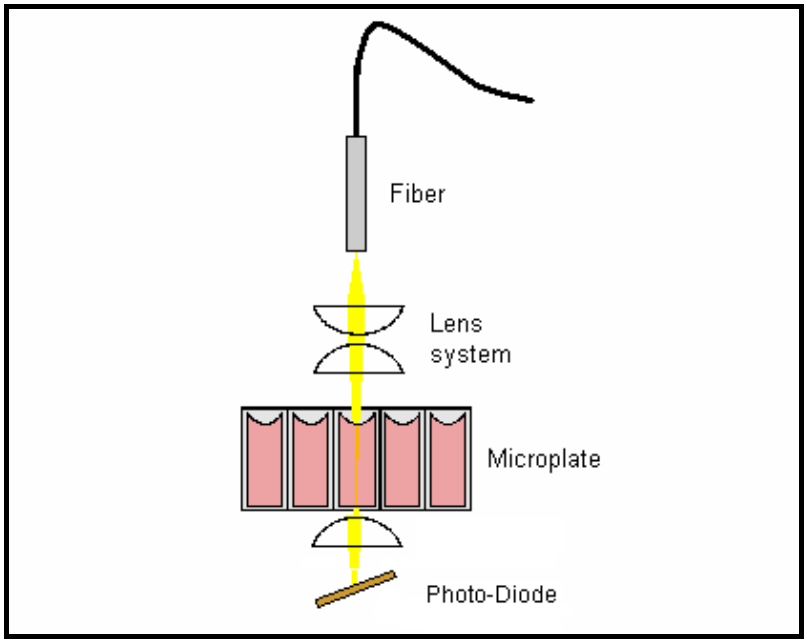


Figure 18: Absorbance Optics, one channel.

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.

The software automatically sorts the data and reports it 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.

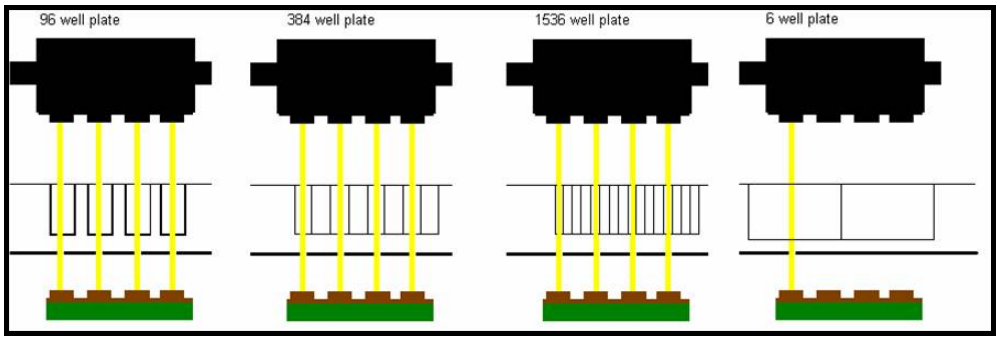


Figure 19: From left to right: Schematic view of the channel usage of an absorbance measurement of a 96, 384, 1536 and 6-well plate.

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.

4.4 Luminescence System

For uncompromising performance, the **INFINITE M1000 PRO** has a dedicated **luminescence detection module**. The luminescence optics have been designed to meet requirements different from the 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 PRO** 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 through the filter wheel to the detector. Three different fibers are available for the **INFINITE M1000 PRO**, the fibers are optimized for different plate types: 96, 384, or 1536-well.

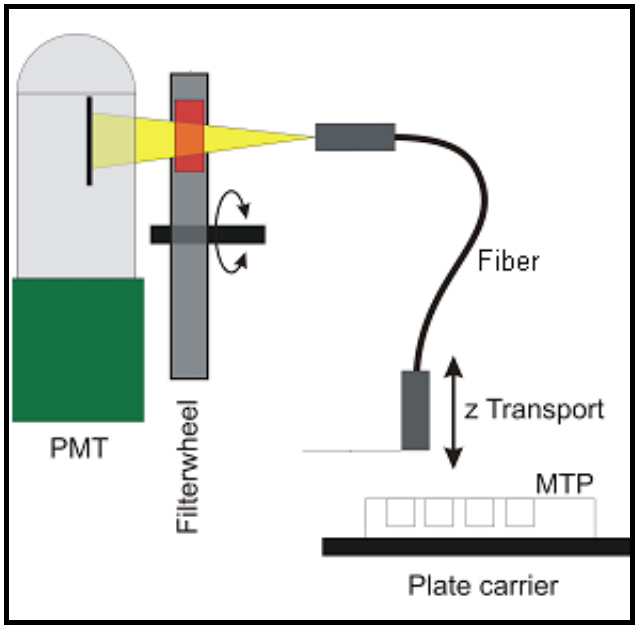


Figure 20: Optical System Luminescence.

4.4.1 Luminescence Optics

The Z-position of the luminescence fiber bundle can be adjusted. As light is refracted onto the sample liquid surface, a Z-adjustment helps to maximize signal to noise and minimize crosstalk. The software performs the adjustment automatically, once the user has selected the corresponding plate type in the software dialog box.

Fibers

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

The orifices in the ceiling of the measurement chamber are designed to receive as much light as possible from the wells of 96, 384 or 1536-well plates, thus maximizing the luminescence signal; however, the orifices do not receive substantial amounts of light from neighboring wells, thereby minimizing crosstalk.

Filter Wheel

A filter wheel in front of the PMT window is switched to the required luminescence filter channel. The sensitivity of the detection system makes it necessary to attenuate high luminescence light levels, therefore the filter wheel is also able to move a neutral density filter (OD2) across the selected fiber exit. This can be done automatically in all wells that require attenuation by using the attenuation setting “AUTOMATIC” in the instrument control software. The OD2 attenuation cannot be manually selected for all measured wells.

Installed filters

- OD2 neutral density filter
- Green (Chroma-Glo, BRET^2)
- Magenta (Chroma-Glo, BRET^2)
- Blue 1 (BRET)
- Green 1 (BRET)
- Blue (BRET3)
- Orange (BRET3)
- AlphaScreen (not selectable for luminescence measurements)
- AlphaLISA (not selectable for luminescence measurements)

Note

The AlphaScreen and AlphaLISA filters installed in the filter wheel can only be used for Alpha measurements, not for luminescence measurements.

Figure 21 to Figure 26 show the transmission spectra of the different luminescence filters.

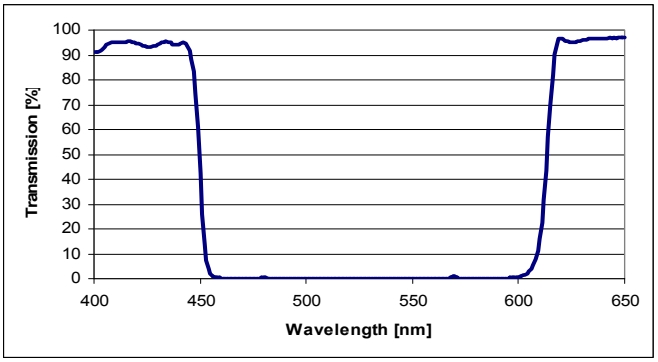


Figure 21: Transmission spectrum of filter 'Lumi Magenta'.

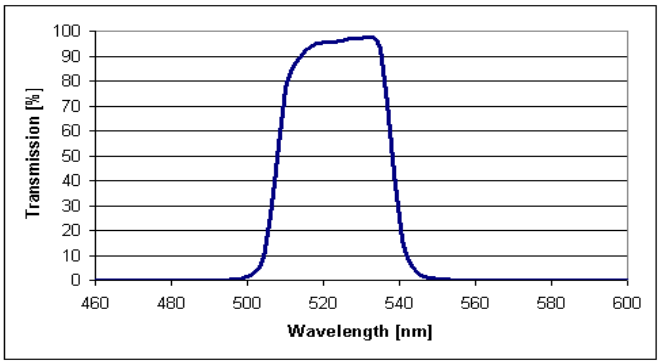


Figure 22: Transmission spectrum of filter 'Lumi Green'.

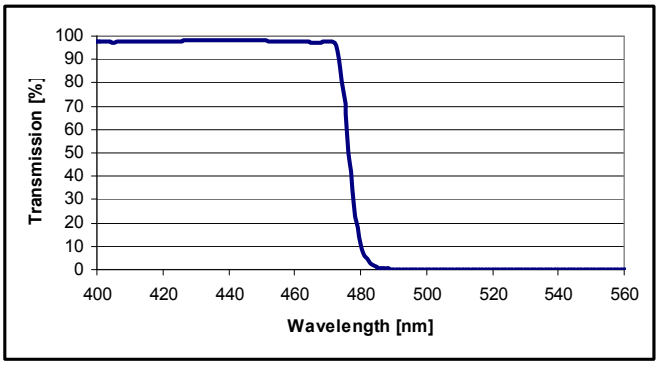


Figure 23: Transmission spectrum of filter 'Blue 1'.

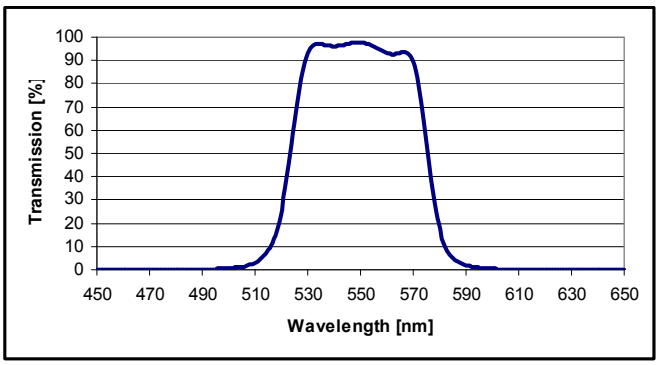


Figure 24: Transmission spectrum of filter 'Green 1'.

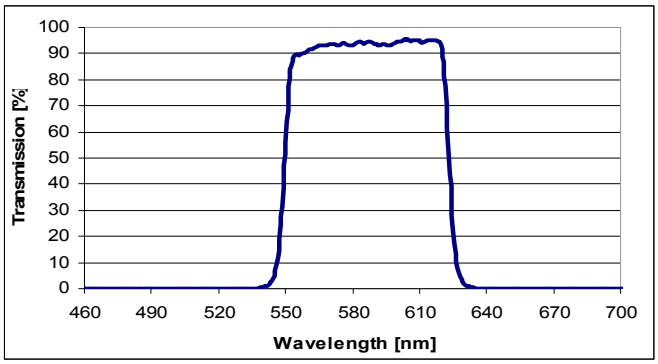


Figure 25: Transmission spectrum of filter 'Lumi Orange'.

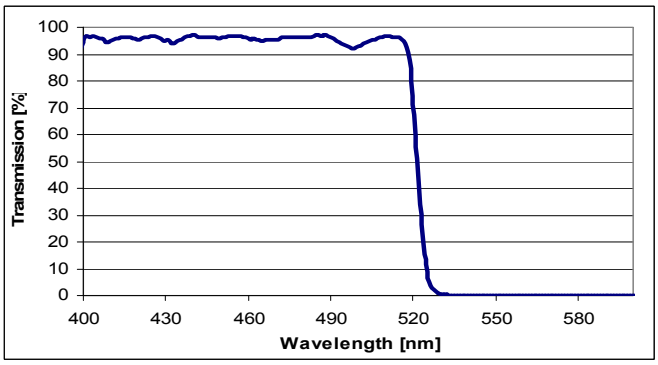


Figure 26: Transmission spectrum of filter 'Lumi Blue'.

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 exceptionally low noise and high sensitivity allow detection at 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 PRO** 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 at very low light levels.

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

Note

The results of luminescence measurements are always displayed in counts per second, regardless of the integration time used.

4.5 AlphaScreen/AlphaLISA System

For uncompromised performance, the **INFINITE M1000 PRO** uses a dedicated luminescence detection module (chapter 4.4 Luminescence System) for AlphaScreen/AlphaLISA measurements.

In addition to the Luminescence System, the **INFINITE M1000 PRO** uses a high-power laser as the excitation light source for AlphaScreen/AlphaLISA and a contactless temperature sensor to measure the temperature inside each well.

4.5.1 AlphaScreen/AlphaLISA Optics

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

Laser

The **INFINITE M1000 PRO** uses a high power laser (680 nm / 750 mW) as the excitation light source.

The **INFINITE M1000 PRO** is a LASER CLASS 1 product. The **INFINITE M1000 PRO** complies with FDA radiation performance standards, 21 CFR 1040.10, except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007.

WARNING

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

Fibers

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

The orifices in the ceiling of the measurement chamber are designed to receive as much light as possible from the wells of 96, 384 or 1536-well plates, thus maximizing the luminescence signal; however, the orifices do not receive substantial amounts of light from neighboring wells, thereby minimizing crosstalk.

Filter Wheel

Two additional filters in the luminescence filter wheel allow for measurements of AlphaScreen and AlphaLISA assays.

The transmission spectra of the AlphaScreen and AlphaLISA filters are shown in figures 27 and 28.

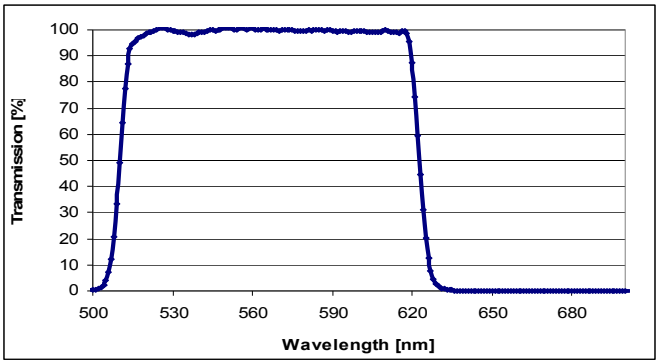


Figure 27: Transmission spectrum of filter 'AlphaScreen'.

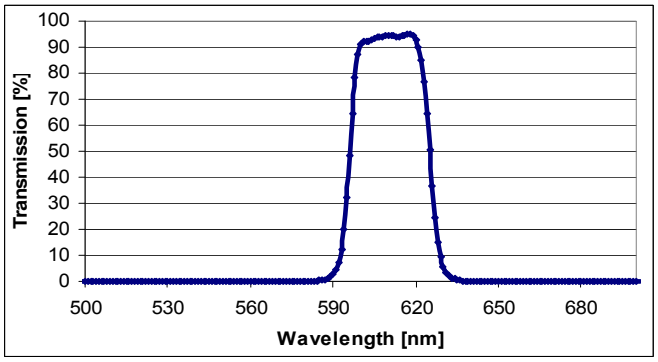


Figure 28: Transmission spectrum of filter 'AlphaLISA'.

Note

The luminescence attenuation and color filters installed in the filter wheel can only be used for luminescence measurements, not for AlphaScreen/AlphaLISA measurements.

Photon Counting Module (PCM)

The PCM, which contains a channel photomultiplier, is designed for applications in chemo- and bioluminescence. The channel photomultiplier provides a high dynamic range, which enables luminescence measurements to be made even with strong variations in light levels. The exceptionally low noise and high sensitivity allow detection at very low light levels.

4.5.2 AlphaScreen/AlphaLISA 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 PRO** 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 and is, therefore, the preferred method for performing measurements at very low light levels.

Note

AlphaScreen/AlphaLISA measurements are only possible as endpoint measurements in white or light gray microplates and cannot be performed in combination with the injector system or the heating system.

Note

The results of AlphaScreen/AlphaLISA measurements are always displayed in counts per second, regardless of the integration time used.

4.5.3 AlphaScreen/AlphaLISA Temperature Correction

To compensate for the temperature sensitive nature of AlphaScreen/AlphaLISA assays, the **INFINITE M1000 PRO** offers a unique temperature correction system (optional):

A contactless temperature sensor measures the temperature inside each well and the measured count rates are automatically normalized to a temperature of 22.5 °C.

Note

To ensure the best performance for AlphaScreen/AlphaLISA assays, the INFINITE M1000 PRO should be operated in a temperature-regulated environment (±1 °C in the range of 20–25 °C).

5. Operating the INFINITE M1000 PRO

5.1 Introduction

WARNING

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

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 PRO** 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 about how to optimize instrument parameters for specific applications.

Every effort has been made to ensure that the instrument functions correctly even if the default parameters are not appropriate for a particular application - with an important exception: the selected plate definition file must correspond to the type of plate used.

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 PRO always work according to GLP (good laboratory practice) guidelines.

Caution

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

5.2 General Operating Features

The **INFINITE M1000 PRO** has some general behavior and options, which are independent of specifically selected measurement techniques.

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 in or unplugged, the instrument and the PC should be switched off.

It is highly recommended to only use the USB interface cable provided by Tecan 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 for operation.

Note

When the software connects 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 using the MultiCheck-Plus test plate.

Loading Microplates

The **INFINITE M1000 PRO** 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 can be combined with any measurement technique.

Temperature Control

Some assays ask for an exact operating temperature. The **INFINITE M1000 PRO** can set up a specific temperature within a certain range, provide uniformity across the plate, and keep the 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

The i-control software allows a plate to be measured repeatedly in equidistant time intervals. The 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 corners evaporate faster.

Microplate Shaking

The **INFINITE M1000 PRO** is capable of plate shaking before the 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.

Multi-labeling

The *i-control* software 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 can be selected.

5.3 Optimize Fluorescence Measurements

Fluorescence measurement results may be improved by optimizing instrument parameters and selecting the appropriate materials.

5.3.1 FI Scanning (Spectral Intensity Calibration)

Due to wavelength dependence on the intensity of the excitation 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 excitation light the **INFINITE M1000 PRO** 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 the **INFINITE M1000 PRO** 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

The **INFINITE M1000 PRO** 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 can take a few seconds to 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 ±10% due to energy fluctuations of the flash lamp.

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 a 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 liquid's 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, it is recommended to 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 PRO** fluorescence detection system uses analog to digital (A/D) conversion of the PMT signal. The gain setting controls the amplification of the PMT when converting fluorescence light into electrical current. The A/D converter needs a suitable input range of PMT current to provide the proper signal to noise ratio (S/N) on the one hand, and linearity on the other hand. Therefore, the gain should be optimized based on the wells with the highest concentration give the highest possible readings. For best possible signal resolution it is recommended to use the “Optimal Gain” setting.

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 PRO** PMT is specified from 300 to 1250 V. The relationship between the gain setting of the **INFINITE M1000 PRO** and the voltage supply is described in the equation below.

The intended use of the **INFINITE M1000 PRO** 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 the **INFINITE M1000 PRO** when using gain settings below 60.

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

U	Voltage
Gain	INFINITE M1000 PRO gain
255	maximum gain on the INFINITE M1000 PRO
1250V	maximum voltage supply of PMT

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

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

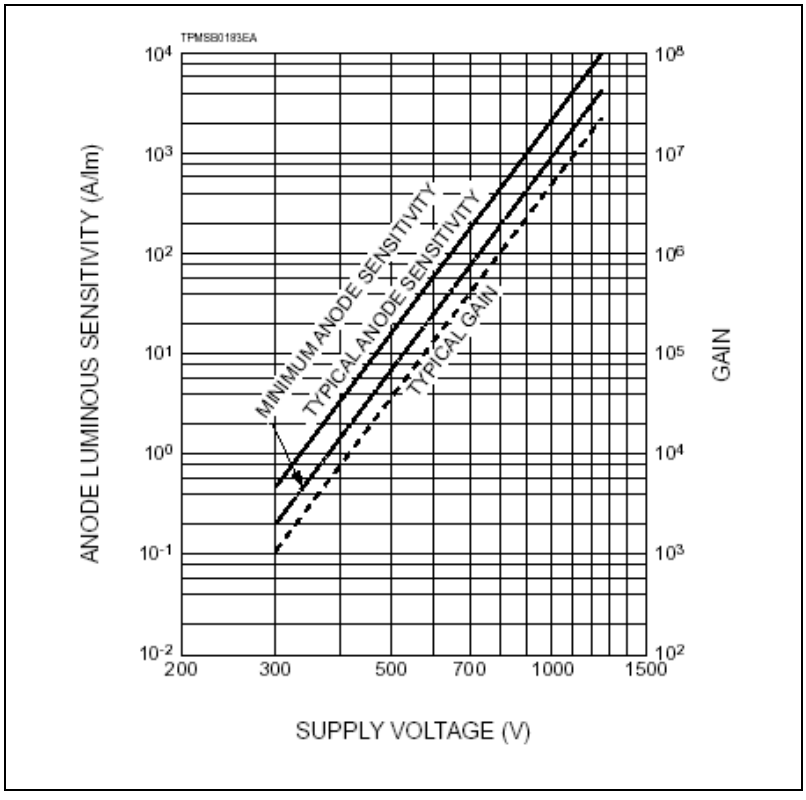


Figure 29: *Sensitivity of PMT in relation to supply voltage. Sensitivity below 300 V is not specified.*

Z-Optimization

Z-optimization is only available for FI Top and FP measurements with the **INFINITE M1000 PRO**. 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 'manual' option, 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 PRO** will automatically identify the z-position of maximum signal in the selected well for further measurements.

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

When using the 'same as' option, the **INFINITE M1000 PRO** 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-positionof 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 is to 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.

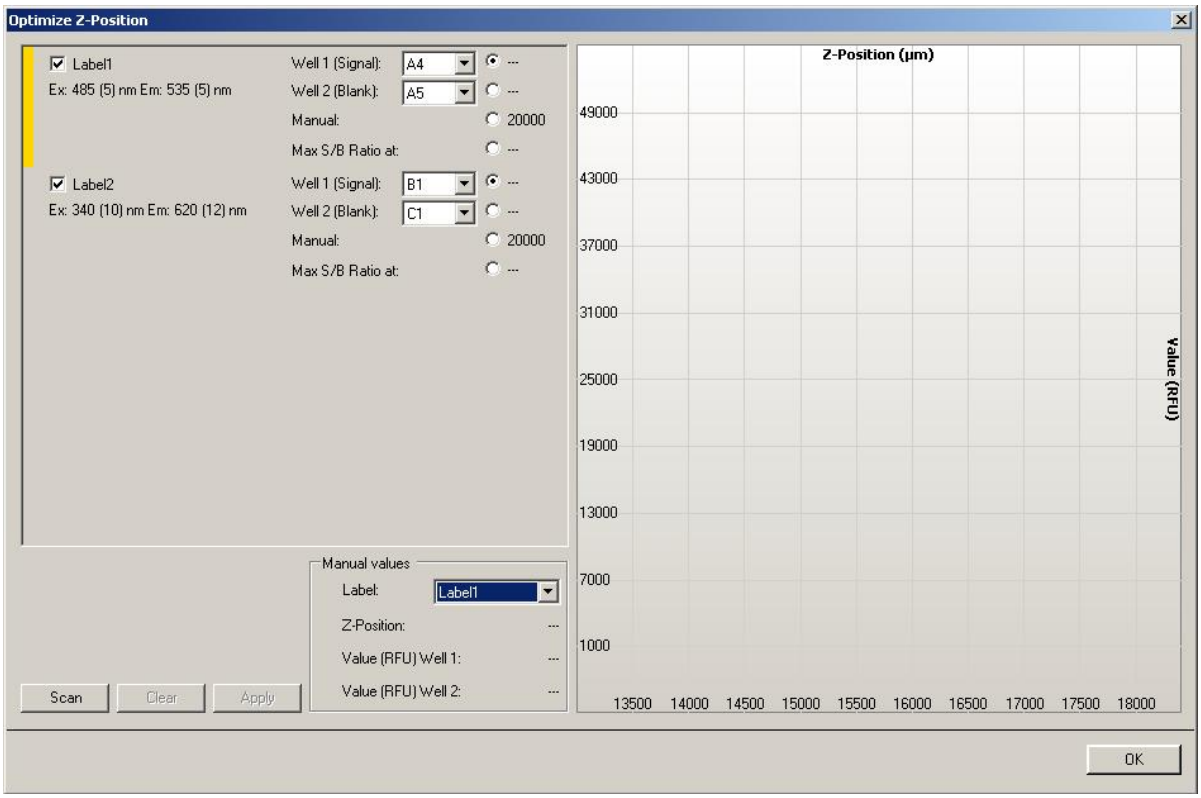


Figure 30

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 the fluorophore of interest (signal) and one filled with buffer (blank). Both wells are scanned and the resulting signal and blank curves are shown in the graph. The z-position may now be set to the maximum signal-to-blank (S/B) ratio.

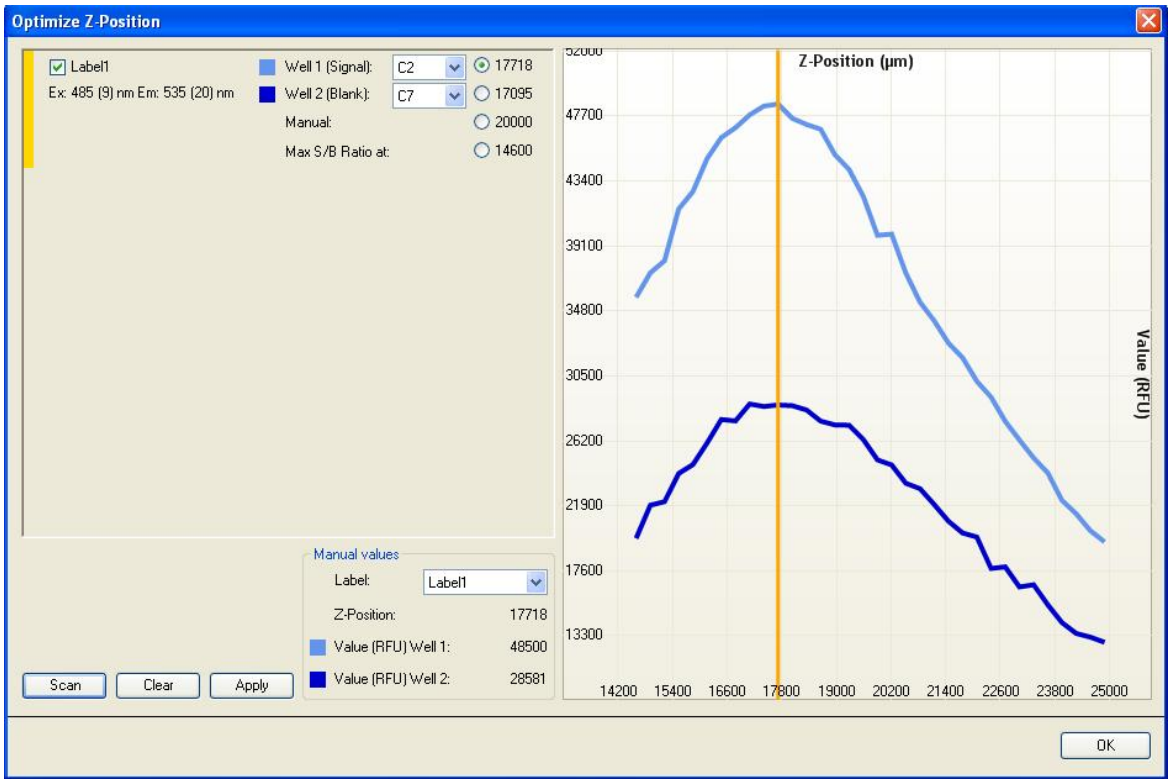


Figure 31

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 length of the reading time during which a 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 improve further, or until the measurement time per well becomes unacceptable.

Flash Frequency Mode

The **INFINITE M1000 PRO** 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, results 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 by the Lag Time of the preceding flash. TRF timing parameters may be established with the following procedure:

1) As a starting point, take, for example, the **Fluorescence Lifetime** of the label for both **Integration Time** and **Lag Time**.

2) Coarse tuning: With the Integration Time fixed, reduce the Lag Time to maximize **Signal to Background (S/B)**.

3) Fine tuning: With the Lag Time fixed, extend the Integration Time and check if S/B improves further.

4) Optional Fine-tuning: With one timing parameter fixed, vary the other one and check if S/B improves further.

A comparison of S/B with 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

When selecting more than one flash per well, a time delay between move and flash can be set. Due to the stop and go motion of the plate carrier, the meniscus of the dispensed liquid may vibrate while signal is integrated. This can give rise to fluctuations of the measured values. The effect has been observed in the wells of 96-well plates and also in larger wells. In particular, it is critical with absorbance measurements.

5.3.4 FI Ratio Mode

Ratio Mode

Up to 5 labels can 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 must 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 maximum 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 the 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 plate format used. 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.

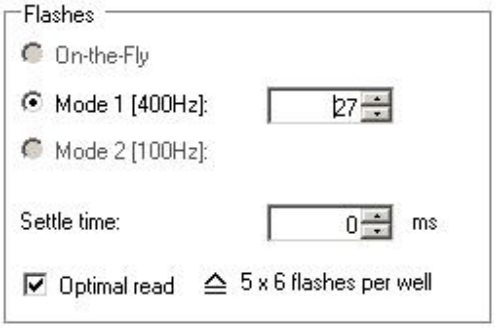


Figure 32

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 33 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 its z-Positioning capability, the **INFINITE M1000 PRO** 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.

The 400 Hz flash frequency mode is available for Absorbance measurements only. 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

When selecting more than one flash per well, a time delay between move and flash can be set (100-300 ms). Due to the stop and go motion of the plate carrier, the meniscus of the dispensed liquid may vibrate while signal is integrated. This can give rise to fluctuations of the measured values. The effect has been observed in the wells of 96-well plates and also in larger wells.

5.4.2 Absorbance Ratio Mode

Ratio Mode

When using the “Standard” tab in i-control, up to 5 labels can 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 must be performed by the user.

When 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.

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 bottoms seem superior.

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

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

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, but instead a sequence of pulses are produced, the average rate of which can be measured using a counter. The advantage of using the photon counting technique at such low light levels is that pulse height selection criteria allow much of the electronic noise to be filtered out.

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

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.

Note
If a luminescence measurement results in an INVALID in one or more wells because the measured signal was too high, the Luminescence PMT may need a certain amount of time to return to the equilibrium baseline count level.

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.’

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 values may result in excessive noise from the detector. The **INFINITE M1000 PRO** instrument offers a default gain value to keep noise levels 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 relative 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-color 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 completely 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-color 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-color luminescence measurements and luminescence scan measurements are therefore not possible.

5.6 Optimize AlphaScreen/AlphaLISA

5.6.1 Excitation Time

The excitation time defines the duration of the sample illumination by the laser. Optimizing the excitation time for AlphaScreen and AlphaLISA assays may help to minimize sample bleaching and improve the signal-to-noise ratio.

The excitation time for AlphaScreen/AlphaLISA is selectable from 10 - 1000 ms; a longer excitation time will increase the total read time of the plate.

5.6.2 Integration Time

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

At very low light levels, the measured counts per second are proportional to the light intensity. An increase in 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.

The integration time for AlphaScreen/AlphaLISA is selectable from 100 - 20,000 ms; a longer integration time will increase the total read time of the plate.

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.

Note

The results of AlphaScreen/AlphaLISA measurements are always displayed in counts per second, regardless of the integration time used.

5.7 Injectors

The **INFINITE M1000 PRO** 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 a 6-well to 384-well microplate.

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

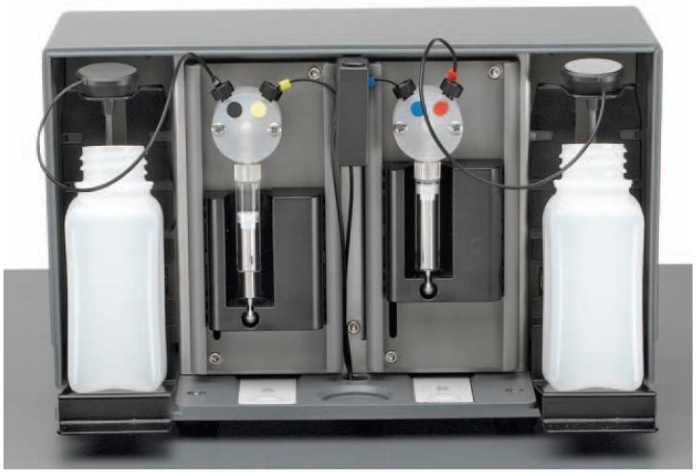


Figure 34*: View of the injector module.*

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

The **INFINITE M1000 PRO** can be equipped with one pump or two pumps (Figure 34).

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

One Injector Option (one pump): An **INFINITE M1000 PRO** 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 a 6-well to 384-well microplate.

WARNING

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

5.7.1 Measurement with Injectors

The injectors of the **INFINITE M1000 PRO** 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

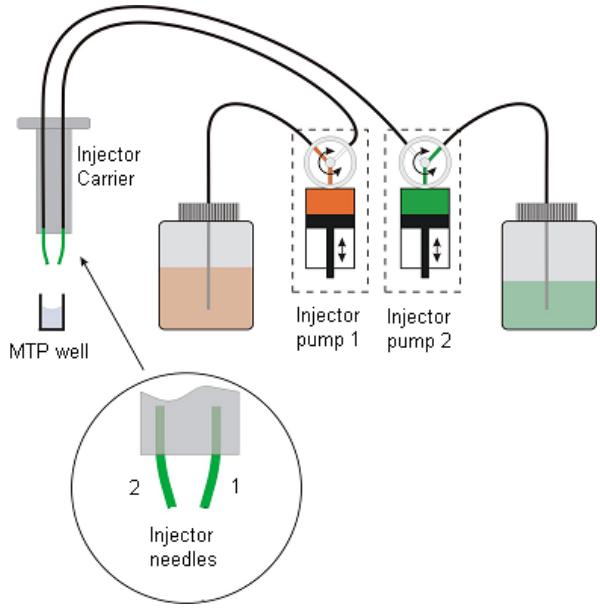


Figure 35: *Liquid path of the **INFINITE M1000 PRO** with two pumps.*

5.7.2 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.

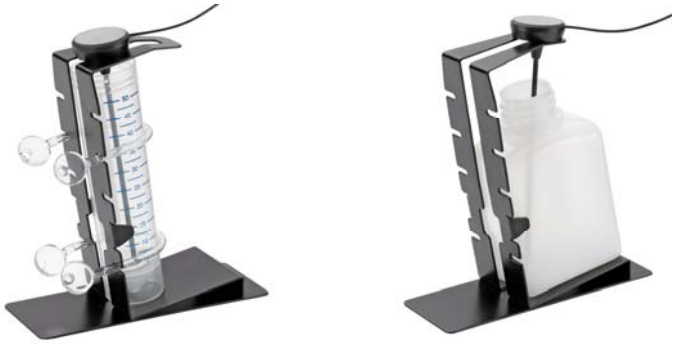


Figure 36: Bottle holders

5.7.3 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 your hear a clicking noise.

The instrument contains an injector sensor that checks the correct positioning of the injector carrier in the instrument. If the injector is not inserted correctly, the injector sensor will not recognize the inserted carrier and neither dispensing nor injection will be possible.

On the other hand, actions like washing and priming are enabled even though the injector carrier is inserted. Therefore, always make sure that the injector carrier is in the service position for washing and priming (Figure 37).

Figure 37: Injector carrier with injector in 'service position'.

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.

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 performed easily.

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 secured.
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.

5.7.4 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 in.) and 'short' = 80 cm (31.5 in.).

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

Caution

Do not touch the injector needles, 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 µl).
3. Select the ‘Prime Speed’ (100-300 µl/sec).
4. Select the ‘Refill Speed’ (100-300 µl/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 µl 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 µl/sec).
4. Select the 'Refill Speed' (100-300 µl/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 µl/sec).
4. Select the 'Refill Speed' (100-300 µl/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.

5.7.5 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:

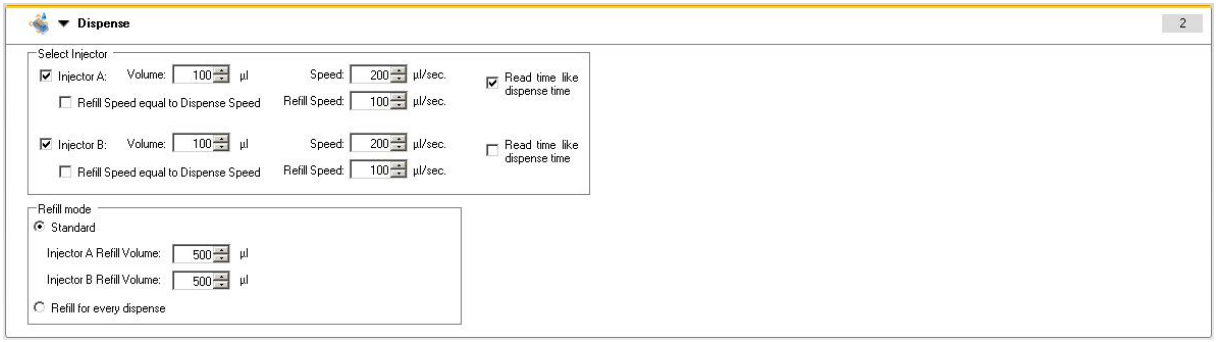


Figure 38

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:

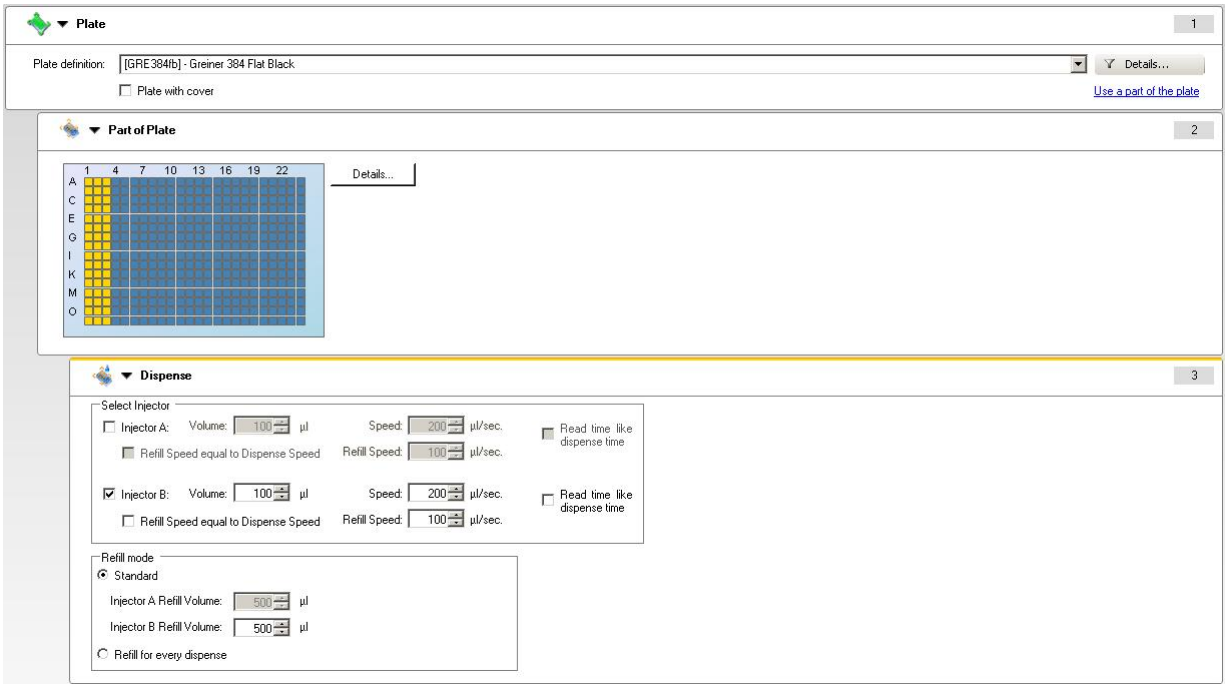


Figure 39

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:

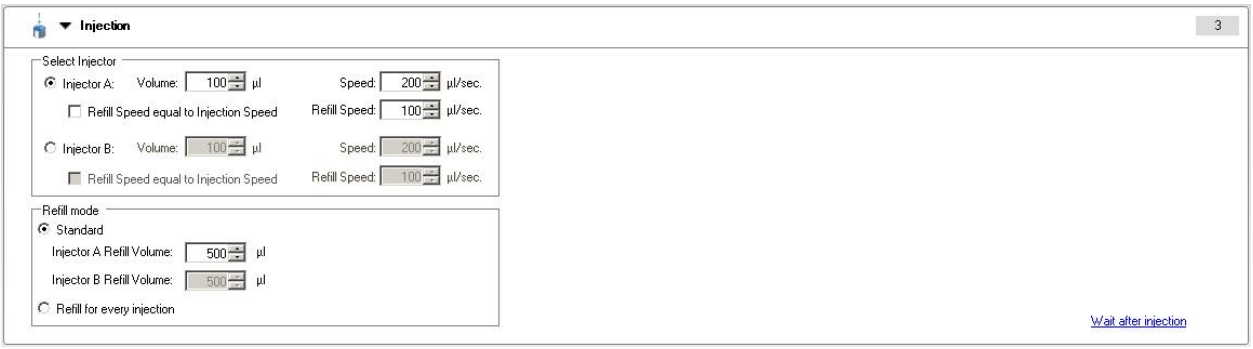


Figure 40

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 µl/sec for each injector.

Select a '**Refill speed**' from 100 – 300 µl/sec. for each injector or check the '**Refill Speed equal to Injection Speed**' box.

Select refill mode '**Standard**' if refill should be performed when syringe is empty. 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:

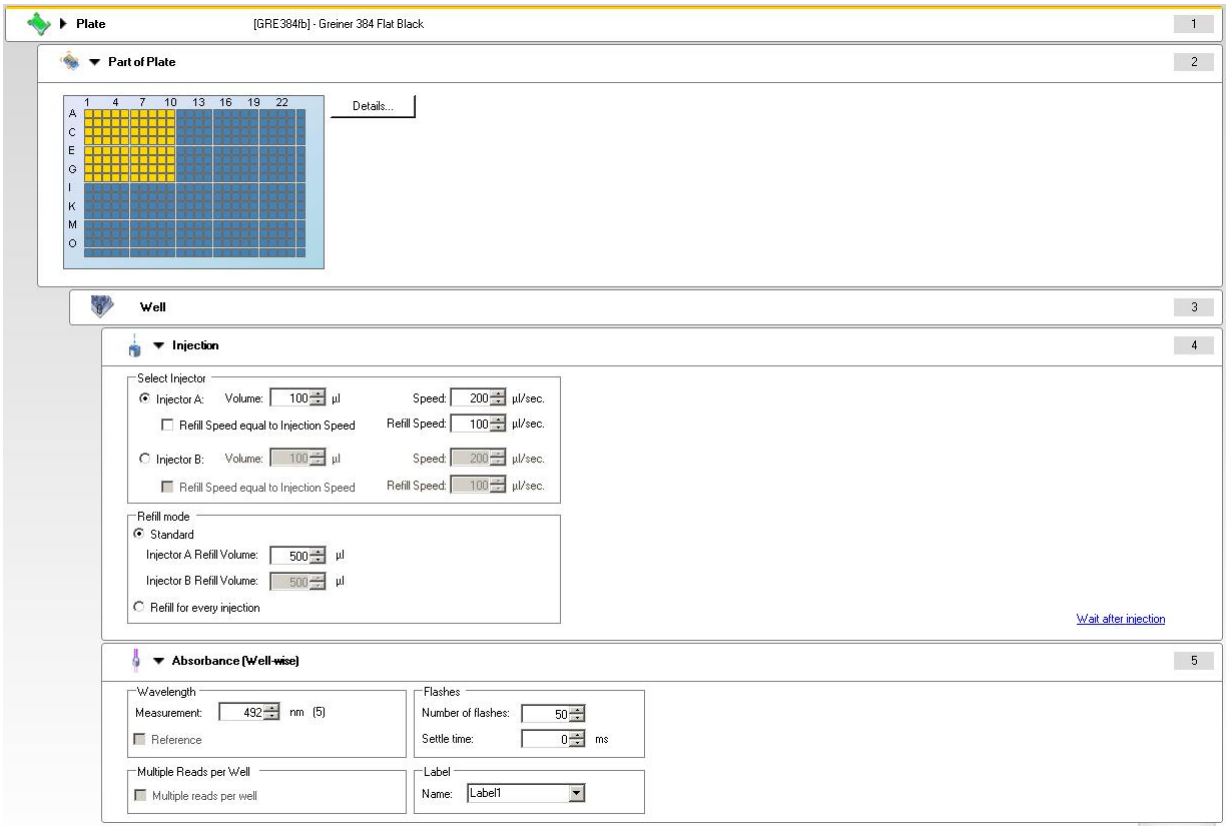


Figure 41

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.

5.7.6 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 must 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.

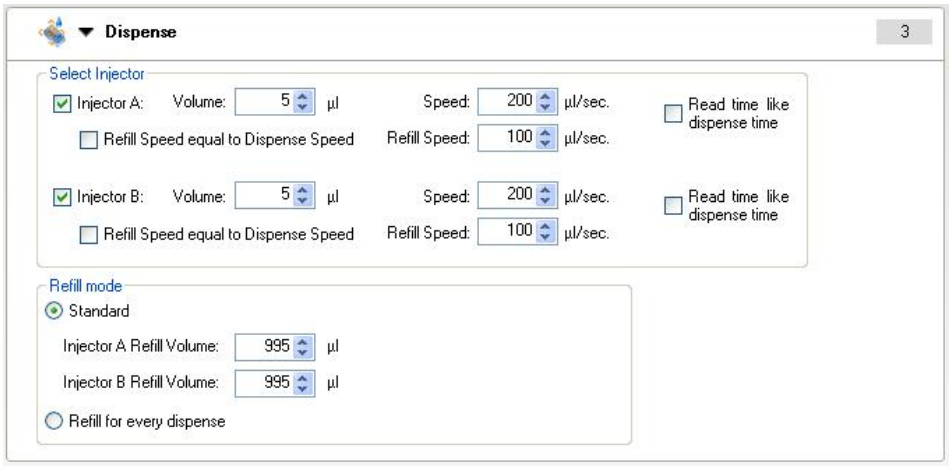
Weekly/Periodical Maintenance:

The fluid path must be cleaned weekly to remove precipitates such as salts, eliminate bacterial growth, etc.:

The following maintenance protocol is designed for two available injectors (A+B), but can be performed with only one injector (A) as well.

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

1. Depending on the user's application flush the system thoroughly with buffer or distilled water before washing with 70% EtOH.
2. Place an empty microplate in the reader.
3. Prime both injectors with 70% EtOH.
4. Set up a software script consisting of a Plate stripe (with a plate definition file corresponding to the inserted type of microplate), Part of the Plate (Well A1) and a Dispense stripe.



5. Select injectors A and B and set the dispense volume to 5 µl for each injector.
6. Set the Refill Mode to 'Standard' and the Refill Volume to the maximum possible value (e.g. 995 µl for a 1000 µl syringe).
7. Start the script and leave the syringes filled for at least 30 minutes.
8. After the 30-minute period, backflush all the fluid from the syringes and tubes into a small waste container.
9. Wash the injector system with distilled or deionized water.
10. Clean the end of the injector needles carefully with a cotton swab soaked in 70 % ethanol or isopropanol.
11. For prolonged storage, repeat steps 1-7 with distilled or deionized water and leave the fluid pathways filled for storage.

5.7.7 Injector Reagent Compatibility

The injector system of the INFINITE M1000 PRO instruments consists of the following materials:

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

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

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

Caution

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

WARNING

VARIATIONS IN CHEMICAL BEHAVIOUR DURING HANDLING DUE TO FACTORS SUCH AS TEMPERATURE, PRESSURE AND TEMPERATURE, 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.

5.8 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 PRO** may be optionally equipped with a built-in stacker for loading and un-loading standard microplates automatically (Figure 42). 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 42: 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.

5.9 Barcode Scanner

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

INFINITE M1000 PRO is a LASER CLASS 1 product. **INFINITE M1000 PRO** complies with FDA radiation performance standards, 21 CFR 1040.10, except for deviations pursuant to Laser Notice No. 50, dated June 24, 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 PRO** contains a barcode scanner, the instrument is a class I laser device.

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

Complies with 21 CFR 1040.10
except for deviations pursuant to
Laser Notice No. 50,
dated June 24, 2007

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 PRO** microplate reader:

- Fluorescence Intensity Top/Bottom (FRET)
- Time Resolved Fluorescence (TRF, TR-FRET)
- Fluorescence Polarization
- Absorbance
- Glow Type Luminescence
- Flash Type Luminescence
- Dual-color Luminescence
- AlphaScreen/AlphaLISA
- Injector option 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 not restricted to one part of the microplate, also independent well ranges can be easily 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 Fluorescence (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 µs
Lag Time	0 - 2000 µs

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 [µl]	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 [µl]	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 [µl]	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 [µl]	50
Integration Time [µs]	500
Lag Time [µs]	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 Fluorescence (TRF)

6.4.1 Fluorescein 1nM (Fluorescence Polarization)

Plate type (number of wells)	384	1536
Dispensed Volume [µl]	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 for meas. ≤ 300 nm 5 nm for meas. > 300 nm
Bandwidth Fixed Wavelength	5 nm
Measurement range	0 - 4 OD
Resolution	0.0001 OD
Accuracy (0-2 OD)	≤ ± (1 % + 6 mOD)*
Precision (0-2 OD)	≤ ± (0.5 % + 5 mOD)*
Linearity (0-2 OD)	R2 ≥ 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
Crosstalk (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

6.6.2 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

6.6.3 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

6.7 AlphaScreen/AlphaLISA

Caution

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

Parameters	Characteristics
Excitation Time: Integration Time: Plate Type: Omnibeads Detection Limit [ng/ml] (384-well)	10 - 1000 ms 100 - 20000 ms 96, 384, and 1536-well microplates (white or light gray) ≤ 12.5
Uniformity [% CV] (384-well)	≤ 8
Crosstalk [%] (384-well)	≤ 0.2

6.8 “On the Fly” Measurements

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

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

6.9 Injectors

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

6.9.1 Injector Performance

Injector Syringe Volume	500 µl	1000 µl	2500 µl
Accuracy at 10 µl	≤ 10 %	≤ 10 %	≤ 20 %
Accuracy at 100 µl	≤ 2 %	≤ 2 %	≤ 5 %
Accuracy at 450 µl	≤ 0.7 %	≤ 0.7 %	≤ 2 %
Precision at 10 µl	≤ 10 %	≤ 10 %	≤ 20 %
Precision at 100 µl	≤ 2 %	≤ 2 %	≤ 5 %
Precision at 450 µl	≤ 0.7 %	≤ 0.7 %	≤ 2 %

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

7.2 Definitions

7.2.1 Detection Limit (LOD)

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

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

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 a 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 the relationship between the concentration of fluorophore/dye and the corresponding signal probability. To evaluate 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.

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

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

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 the deviation from the true value in mOD.

7.2.5 Crosstalk

Crosstalk 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 provides an overview of the passed/failed criteria for the specification tests of the **INFINITE M1000 PRO**.

Specification	**Acceptance Criteria**	
Plate type (number of wells)	**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 Crosstalk	≤ 0.5% ATP	≤ 5% ATP
AlphaScreen Detection Limit	≤ 12.5 ng/ml Omnibeads	
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 µl of the 1 nM Fluorescein solution and 100 µl 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 µl 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 µl 1 nM Fluorescein; B…..100 µl 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:	Mode 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:	Mode 2 (100 Hz)
Number of flashes:	12
Lag time:	0 µs
Integration time:	40 µs
Plate definition file:	GRE1536fb.pdfx, 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 µl of a 1 nM Fluorescein solution (diluted in 0.01 M NaOH) into the wells of a Greiner 384-well plate (black, µClear, flat bottom) according to the plate layout.

Material:

Greiner 384-well plate, flat bottom, µClear (black with transparent bottom)
100 µl 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 µl 1 nM Fluorescein; B…..100 µl 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:	Mode 2 (100 Hz)
Number of flashes:	12
Lag time:	0 µs
Integration time:	40 µs
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:	Mode 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:	Mode 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:

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

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:	Mode 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:

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

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.pdfx
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 Crosstalk 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 crosstalk 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 the crosstalk 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 Crosstalk).

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

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 crosstalk 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:

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

$2*10^{-8}$ 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 Crosstalk 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 the crosstalk 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 Crosstalk).

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

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 crosstalk 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:

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

$2*10^{-8}$	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)
0.00001	Conversion into mol/well
$1/1e^{-15}$	Conversion into fmol/well

7.4.4.5 Crosstalk 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 Crosstalk 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 Crosstalk).

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

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

7.4.5 AlphaScreen

7.4.5.1 AlphaScreen Detection Limit:

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

Material:

AlphaScreen Omnibeads #6760626D (PerkinElmer)

Greiner 384-well plate, flat bottom, white

Phosphate-buffered saline (PBS)
10 µl pipette + tips

100 µl pipette + tips

Omnibeads dilution series:

Dilute the Omnibeads stock solution 1:500 in PBS by adding 3 µl of the stock solution (5 mg/ml) to 1497 µl PBS (yielding a solution of 10 µg/ml).

Prepare 12 further dilutions in 1:2 steps by pipetting 750 µl of the previous dilution step to 750 µl PBS. Use a new tip for each dilution step.

Plate layout:

Pipette 100 µl of each dilution into 5 replicate wells of the microplate (as shown in the Plate Layout). Use 100 µl PBS for the Blank wells.

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

< >	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		10 µg/ml																						
B		5 µg/ml																						
C		2.5 µg/ml																						
D		1.25 µg/ml																						
E		0.62 µg/ml																						
F		0.31 µg/ml																						
G		0.15 µg/ml																						
H		0.08 µg/ml																						
I		0.04 µg/ml																						
J		0.02 µg/ml																						
K		0.01 µg/ml																						
L		0.005 µg/ml																						
M		0.002 µg/ml																						
N		**Blank (PBS only)**																						
O																								
P																								

Filling volume: 100 µl/well of each Omnibeads dilution (5 replicate wells each) or blank (PBS only)

Measurement Parameters:

Before pipetting the plate, prepare instrument for measurement:

Parameters:

Measurement mode:	AlphaScreen/AlphaLISA
Excitation time:	100 ms
Integration time:	300 ms
Temperature correction:	activated
Plate definition file:	GRE384fw.pdfx
Part of the plate:	A2 – P6

Start measurement immediately after pipetting!

Evaluation:

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

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

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

y 3* stdev of the blank (wells N2-P6)

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

7.4.5.2 AlphaScreen Uniformity:

Pipette the reagents into the wells of a Greiner 384-well plate (white, flat bottom) according to the plate layout below.

Material:

AlphaScreen Omnibeads #6760626D (PerkinElmer)

Greiner 384-well plate, flat bottom, white

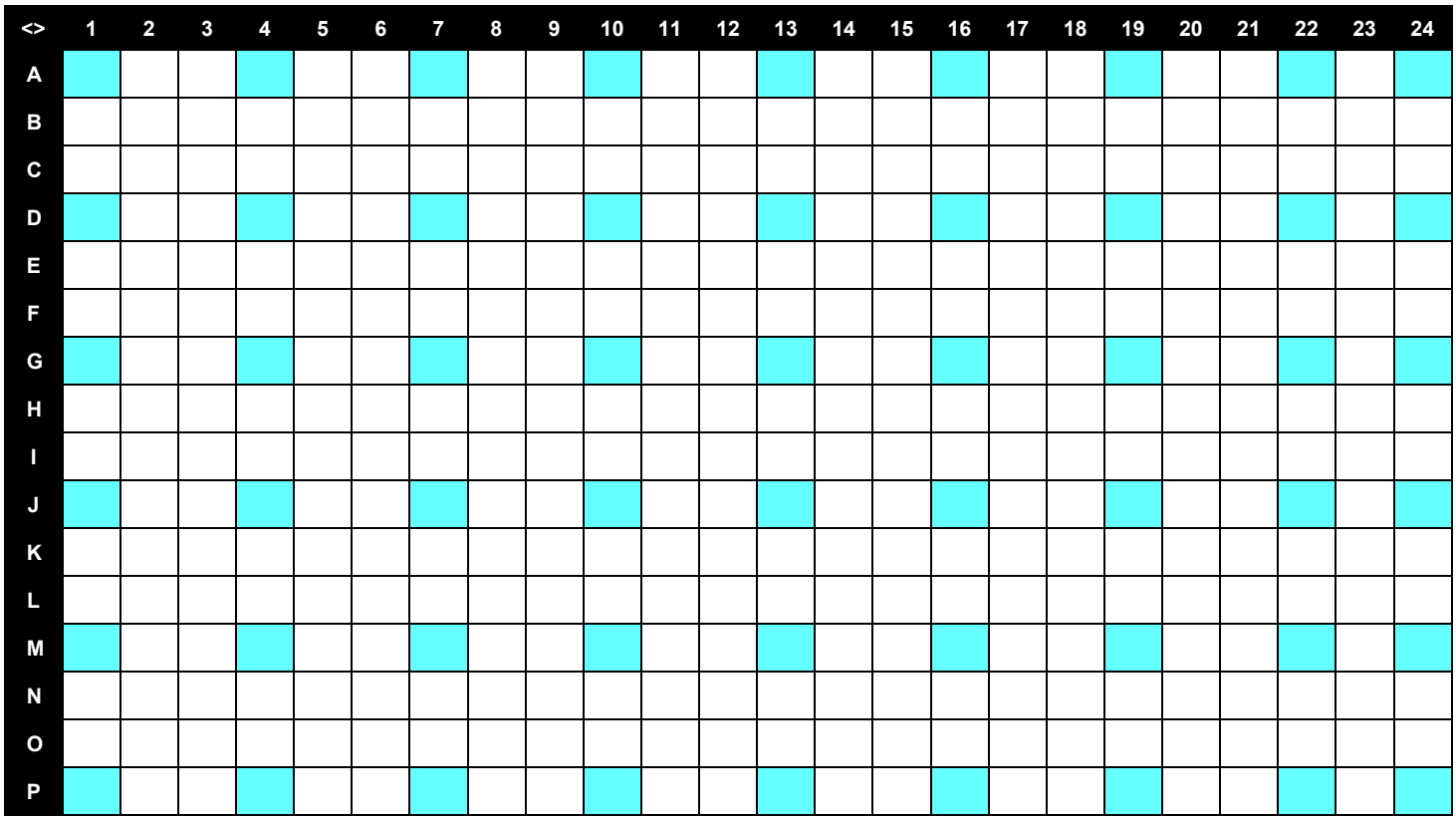
Phosphate-buffered saline (PBS)
100 µl pipette + tips

Omnibeads dilution series:

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

Plate layout:

Pipette 100 µl of the Omnibeads dilution the highlighted wells of the microplate (as shown in the plate layout).



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

Measurement Parameters:

Before pipetting the plate, prepare instrument for measurement:

Parameters:
Measurement mode: AlphaScreen/AlphaLISA
Excitation time: 100 ms
Integration time: 300 ms
Temperature correction: activated
Plate definition file: GRE384fw.pdfx, whole plate

Start measurement immediately after pipetting!

Evaluation:

Calculate the uniformity:

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

mean_{OB} average of wells filled with 2.5 µg/ml Omnibeads
stdev_{OB} standard deviation of wells filled with 2.5 µg/ml Omnibeads

7.4.5.3 AlphaScreen Crosstalk:

Pipette the reagents into the wells of a Greiner 384-well plate (white, flat bottom) according to the plate layout below.

Material:

AlphaScreen Omnibeads #6760626D (PerkinElmer)

Greiner 384-well plate, flat bottom, white

Phosphate-buffered saline (PBS)
100 µl pipette + tips

Omnibeads dilution series:

Dilute the Omnibeads stock solution 1:2000 in PBS by adding 0.5 µl of the stock solution (5 mg/ml) to 999.5 µl PBS (yielding a solution of 2.5 µg/ml).

Plate layout:

Pipette 100 µl of the Omnibeads dilution the highlighted well of the microplate (as shown in the 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																								
B		Bx	Bx	Bx		B	B	B																
C		Bx	OB	Bx		B	B	B																
D		Bx	Bx	Bx		B	B	B																
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

Filling volume: 100 µl/well Omnibeads dilution (2.5 µg/ml) or blank (PBS)

OB Omnibeads dilution (2.5 µg/ml)
B Blank wells (PBS only)
Bx Blank wells used for crosstalk calculation (PBS only)

Measurement Parameters:

Before pipetting the plate, prepare instrument for measurement:

Parameters:

Measurement mode:	AlphaScreen/AlphaLISA
Excitation time:	100 ms
Integration time:	300 ms
Temperature correction:	activated
Plate definition file:	GRE384fw.pdfx, B2-D4 and B6-D8

Start measurement immediately after pipetting!

Evaluation:

Calculate the crosstalk:

$$\text{Crosstalk}(\%) = \frac{(\text{Bx} - \text{B}) * 100}{(\text{OB} - \text{B})}$$

OB	signal of well filled with Omnibeads
B	average signal of blank wells (B)
Bx	average signal of wells surrounding the Omnibeads well (Bx)

7.4.6 Absorbance

7.4.6.1 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)

7.4.6.2 Absorbance Uniformity 384-well Plate:

Pipette 100 µl 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...100 µl 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

7.4.6.3 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).

7.4.6.4 Absorbance Precision 384-well Plate:

Use plate of chapter 7.4.6.3 Absorbance Linearity 384-well Plate:

Material:

For plate/plate layout and materials, see chapter 7.4.6.3 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):

$$\Pr ecision(mOD) = Average - Measured$$

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

7.4.6.5 Absorbance Reproducibility 384-well plate:

Use plate of chapter 7.4.6.3 Absorbance Linearity 384-well Plate:

Material:

For plate/plate layout and materials see chapter 7.4.6.3 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 biohazardous 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 biohazardous waste disposal, according to national and local regulations.

This chapter provides instructions on how to lawfully dispose of waste material that accumulates 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 PRO.

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 PRO 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.
ERR: 15	X drive init error	Hardware Failure Plate Transport Module Please report this error to your local Tecan customer support office.

Error #	Error Text	Description
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	Cannot 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.
ERR: 62	Slit steploss	Slit steploss. Switch instrument off and on, if error persists contact your local Tecan customer support office.

Error #	Error Text	Description
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.
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.

Error #	Error Text	Description
ERR: 86	ST MEAS HW: Error 10-99	Emission or excitation busy. Please report this error to your local Tecan customer support office.

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Tecan Customer Support

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Declaration of Conformity

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

Product Type: **Microplate Reader**

Model Designation: ***INFINITE M1000 PRO***

Article Numbers :

30063849

Address: Tecan Austria GmbH
Untersbergstr. 1A
A-5082 Grödig, Austria

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

These *Instructions for Use* and the included *Declaration of Conformity* are valid for all INFINITE M1000 PRO instruments with the article numbers listed above. The model designation varies depending on the specific model with different article number.

Year of CE-marking: 2011