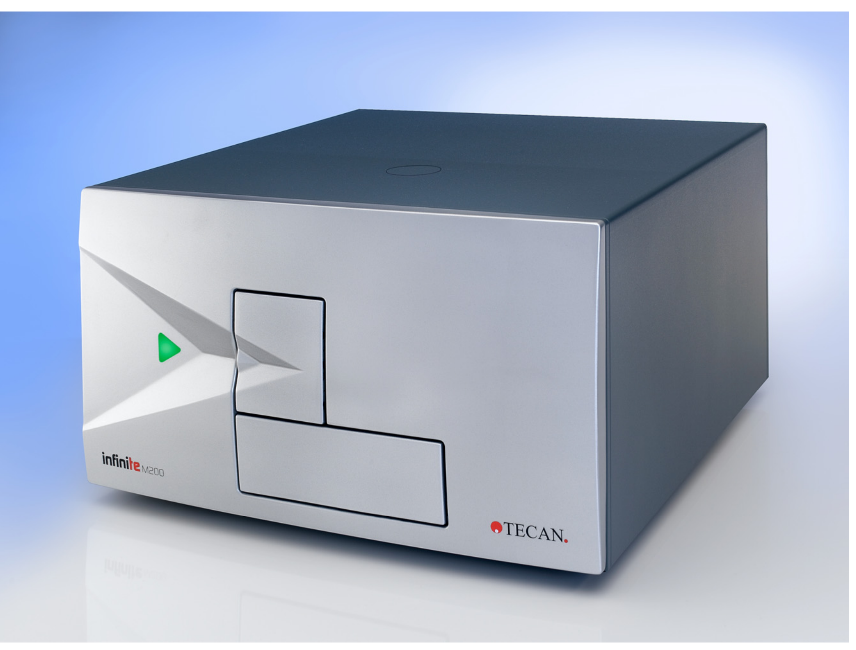
Instructions for Use for

infinite® 200



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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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Declaration for EU Certificate

Available upon request where appropriate.

About the Instructions for Use

This document describes the **infinite® 200** multifunctional microplate reader.
It is intended as reference and instructions for use.

This document instructs how to:

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

Remarks on Screenshots

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

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- Windows® and Excel® are registered trademarks of Microsoft Corporation, Redmond, WA, USA

Warnings, Cautions, and Notes

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

Note
Gives helpful information.

Caution

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

WARNING

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

WARNING

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

WARNING

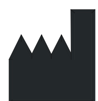
THIS SYMBOL INDICATES THE POSSIBLE PRESENCE OF FLAMMABLE MATERIALS AND A RISK OF FIRE. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

ATTENTION

NEGATIVE ENVIRONMENTAL IMPACTS ASSOCIATED WITH THE TREATMENT OF WASTE.

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

Symbols



Manufactured by

Indicates the possible presence of biologically hazardous material.

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1. Safety

1.1 Instrument Safety

1. Always follow basic safety precautions when using this product to reduce the risk of injury, fire, or electrical shock.
2. Read and understand all information in the Instructions for Use. Failure to read, understand, and follow the instructions in this document may result in damage to the product, injury to operating personnel or poor instrument performance.
3. Observe all WARNING and CAUTION statements in this document.
4. Never open the housing of the **infinite® 200** while the instrument is plugged into a power source.
5. Never force a microplate into the instrument.
6. **infinite® 200** is intended as a general purpose laboratory instrument for professional use. Observe proper laboratory safety precautions, such as wearing protective clothing and using approved laboratory safety procedures.

Caution

Tecan Austria GmbH have taken great care when creating the stored Plate Definition Files that are received with the instrument software.
We take every precaution to ensure that the plate heights and well depths are correct according to the defined plate type. This parameter is used to determine the minimum distance between the top of the plate and the ceiling of the measurement chamber. Additionally, Tecan Austria adds a very small safety gap to prevent any damage occurring to the measurement chamber as a result of small changes in plate height. This does not affect the performance of the instrument.

Users MUST ensure that the plate definition file selected corresponds to the actual plate being used.

Users should also take care that no potential fluorescent or luminescent contamination lies on top of the plate. Be aware that some plate sealers leave behind a sticky residue that must be completely removed before starting measurements.

Caution

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

Caution

To insure the optimal working of Tecan instruments we recommend a service interval of 6 months.

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

Adhere to the following laws and guidelines:

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

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE infinite® 200 MAY COME IN CONTACT WITH BIOHAZARDOUS/INFECTIOUS MATERIAL. MAKE SURE THAT ONLY QUALIFIED PERSONNEL OPERATE THE INSTRUMENT. IN CASE OF SERVICE OR WHEN RELOCATING OR DISPOSING OF THE INSTRUMENT, ALWAYS DISINFECT THE INSTRUMENT ACCORDING TO THE INSTRUCTIONS GIVEN IN THIS MANUAL.

2. General Description

2.1 Instrument

The Tecan **infinite**® **200** is a multifunctional microplate reader with injector option. The **infinite**® **200** provides high performance for the vast majority of today's microplate applications and research and is robotic compatible.

2.1.1 Intended Use

The **infinite**® **200** has been designed as a general purpose laboratory instrument for professional use, supporting common 6 to 384-well microplates conforming to the ANSI/SBS standards (see 2.1.2 Multifunctionality for further details).

Note
System Validation by Operating Authority is Required

The infinite® 200 has been validated on a selected set of assays only.

It is the responsibility of any operating authority to ensure that the infinite® 200 has been validated for every specific assay used on the instrument.

2.1.2 Multifunctionality

Depending on the type of wavelengths selection, the **infinite® 200** is available in two different versions:

- **infinite® M200** (monochromator-based instrument)
- **infinite® F200** (filter-based instrument)

The following measurement techniques are supported by the **infinite® M200**:

- Fluorescence Intensity (FI) Top
- Fluorescence Intensity (FI) Bottom
- Fluorescence Time Resolved (TRF)
- Flash Fluorescence
- Absorbance
- Absorbance with injectors
- Absorbance with cuvette
- Glow Type Chemi- or Bioluminescence
- Bioluminescence Resonance Energy Transfer (BRET ™)
- Flash Luminescence

The fully equipped **infinite® F200** provides the following measurement techniques:

- Fluorescence Intensity (FI) Top
- Fluorescence Intensity (FI) Bottom
- Fluorescence Time Resolved (TRF)
- Flash Fluorescence
- Fluorescence Polarization (FP)
- Absorbance
- Absorbance with injectors
- Glow Type Chemi- or Bioluminescence
- Bioluminescence Resonance Energy Transfer (BRET ™)
- Flash Luminescence

Any common microplate ranging from 6 to 384 well formats conforming to the ANSI/SBS standards (ANSI/SBS 1-2004; ANSI/SBS 2-2004, ANSI/SBS 3-2004 and ANSI/SBS 4-2004) may be measured with any of the above measurement techniques. Switching between measurement techniques or plate formats is fully automated via software. It is not necessary to manually reconfigure the optics in order to switch between the reading modes supported by the **infinite® 200**.

Both instrument versions, the filter-based (**infinite® F200**) and the monochromator-based (**infinite® M200**), may be equipped with up to two injectors.

2.1.3 Performance

The **infinite**® **200** has been designed to meet the requirements of a general-purpose laboratory instrument.

The **infinite**® **200** provides a range of parameters for optimizing the measurement results according to: the assay type (cell-based or homogeneous), the microplate type, and the dispensed volumes per well and dispensing speeds.

2.1.4 User Friendliness

The **infinite**® **M200** offers unparalleled flexibility in wavelength selection for fluorescence intensity and absorbance measurements. Via software any wavelength can be easily adjusted within the specified wavelength range. In addition to single wavelength measurements, absorbance and fluorescence spectra can be recorded. When running a spectrum there is no restriction due to cut-off filters.

The **infinite**® **F200** offers high flexibility for the customization of fluorescence and absorbance measurements; slides containing fluorescence and absorbance interference filters are easily accessible to the user.

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

2.1.5 System Requirements

Minimum

- Pentium PIII 1 GHz
- 20 GB HDD
- 256 MB RAM
- 1 x USB 2.0
- CD ROM Drive
- Screen Resolution: 1024 x 768

Recommended

- Pentium P4 2 GHz
- 40 GB HDD
- 512 MB RAM
- 2 x USB 2.0, 1 x RS232
- CD ROM Drive
- Screen Resolution: 1280 x 1024

Operating System Requirements

- Windows XP Professional (English), Minimum Service Pack 1
- Microsoft Excel® 2000 (English) or above (for i-Control)

Infinite 200 and i-control are also compatible with Windows Vista (32 Bit) and Excel® 2007

2.2 Measurement Techniques

The following sections provide an introduction to the **infinite® 200** measurement techniques when fully equipped. To keep this compact, a few simplifications have been made. For details see the references.

2.2.1 Fluorescence

The **infinite® 200** offers the basic fluorescence measurement technique and some even more sophisticated variants:

A. Fluorescence Intensity (FI) (or simply Fluorescence)
B. Fluorescence Resonance Energy Transfer (FRET)
C. Fluorescence Time Resolved (TRF)
D. 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 FP, FRET requires both binding partners to be labeled in a suitable way. On the other hand, FRET may utilize TRF labels for increased sensitivity, then being referenced as HTRF (Homogeneous TRF).

TRF should not be confused with Fluorescence Lifetime Measurements.

Fluorescence (An Abstract)

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

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

For a detailed treatise on fluorescence techniques and applications see:

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

A) Fluorescence Intensity (FI)

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

B) Fluorescence Resonance Energy Transfer (FRET)

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

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

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

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

C) 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® 200** uses a Flash lamp light source with flash duration much shorter than fluorescence lifetime of these species. This offers the opportunity to measure fluorescence emission at some time, when stray light and prompt fluorescence have already vanished (Lag Time). Thus, background can be significantly lowered while sensitivity is improved.

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

D) Fluorescence Polarization (FP)

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

Fluorescence polarization measurements are based on the detection of the depolarization of fluorescence emission after excitation of a fluorescent molecule by polarized light. A fluorescent molecule can be visualized as an antenna. Such a molecule can absorb energy if and only if the polarization of the excitation light matches the orientation of the antenna. During the fluorescence lifetime, i.e. the time a molecule remains in the excited state, small molecules diffuse rotationally relatively rapidly. Hence they re-orient before they emit their photon. As a result and due to the random character of diffusion, a linearly polarized excitation light will be translated into a less polarized emission light. Thus, a high resultant mP value denotes the slow rotation of the labeled molecule, indicating that binding probably did occur. A resultant low mP value denotes a fast rotation of a molecule, indicating that binding probably did not occur.

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

2.2.2 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 Optical Density (O.D.)

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 (79.4% transmission).

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

2.2.3 Luminescence

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Glow Type Chemi- or Bioluminescence

The **infinite® 200** provides measurement of glow type chemi- or bioluminescence. Glow type means that the luminescence assay glows much longer than a minute. Luminescence substrates are available, which provide stable enough light output over hours.

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

For practical aspects of luminescence assays see the following example:

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

Bioluminescence Resonance Energy Transfer (BRET ™)

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

Flash Luminescence

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

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

* For Flash reactions with the **infinite 200**, see also 2.3.1 Measurement with Injectors.

Flash Type Luminescence with Injectors

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

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

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

2.3 Injectors

The **infinite**® **200** can be optionally equipped with an injector module consisting of one or two syringe pumps (XE-1000, Tecan Systems) located in a separate box, which feed one or two injector needles. The injector needles are designed to inject liquid in any SBS-conform microplate well types, in which the well-size is equal to or larger than an SBS standard 384-well plate.

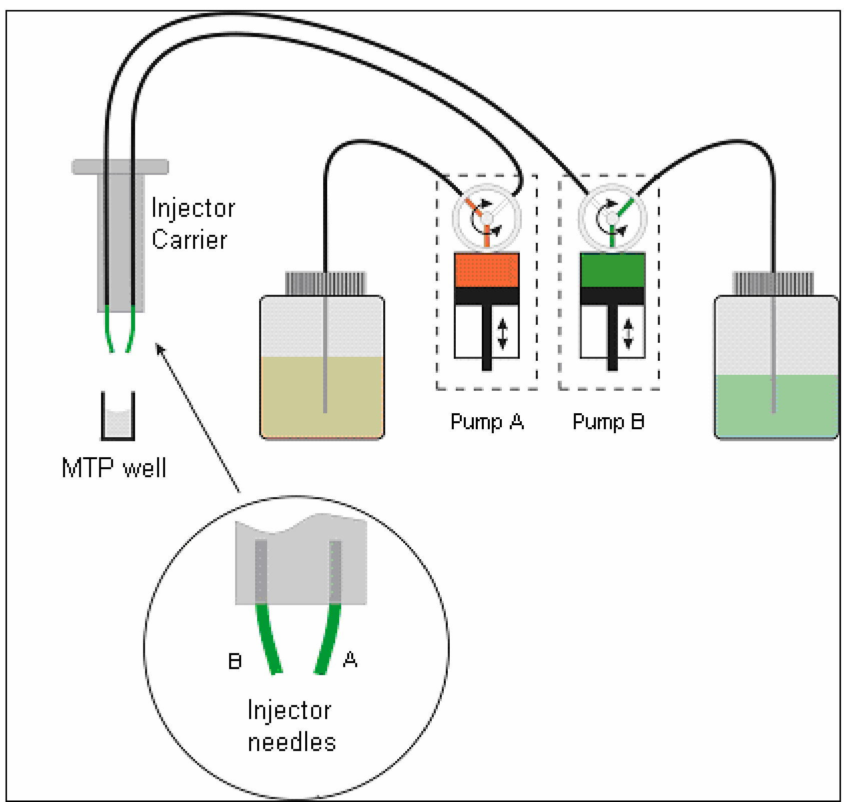


Figure 2-1: Schematic view of the injector module

There are up to two pumps available for the **infinite**® **200** (see *Figure 2-1* above):

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

The **infinite**® **200** can be equipped with one pump (pump A) or two pumps (pumps A and B).

One Injector Option (one pump): An **infinite**® **200** equipped with one pump allows injections in any SBS-conform microplate well types, in which the well-size is equal to or larger than an SBS standard 384-well plate.

Two Injector Option (two pumps): Some applications, such as flash luminescence reactions or dual reporter gene assays require the injection of two *independent* liquids into the same well; therefore, Tecan Austria offers a two-injector option.

2.3.1 Measurement with Injectors

The injectors of the **infinite**® **200** 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. As the measurement position is not the same as the injector position, a short time delay (approx. < 0.5 s) between injection and reading occurs.

For details on how to set up a measurement with injectors please refer to chapter 2.4.1 i-Control and Injectors.

2.3.2 Storage Bottles

The injector box may hold up to two 125 ml bottles. An adapter for smaller tubes allows using tubes of different size (1.5 ml, 15 ml, 50 ml tubes etc.)

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

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

Figure 2-2: Storage bottles and adapter for smaller tubes

2.3.3 Injector/Injector Carrier

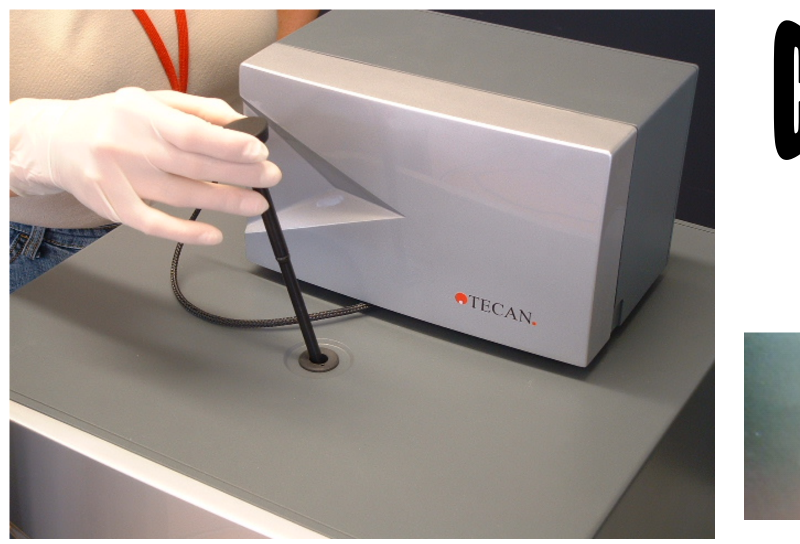
The carrier, which includes the injector needles, can be easily removed from the instrument for priming or washing the system and for optimizing the injection speed.

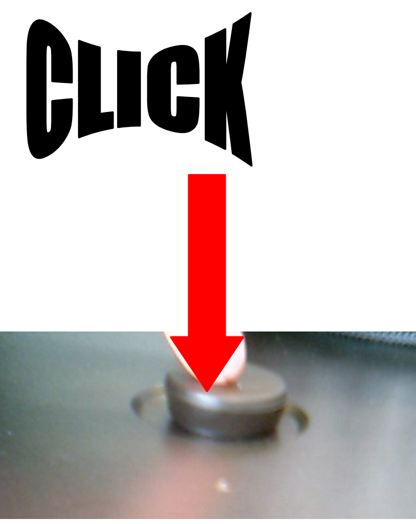
Figure 2-3: Injector carrier

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

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

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





Picture 2-1: Inserting the injector carrier into the injector port

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 ‘**backflush**’ for each syringe. The function of ***backflush*** is to return any unused reagent to the reservoir bottles. The injection speed can be adjusted via the software to allow for good mixing of reagents. The optimum injection speed depends on the assay parameters, such as viscosity of fluids, the plate format and the measuring behavior of the liquids.

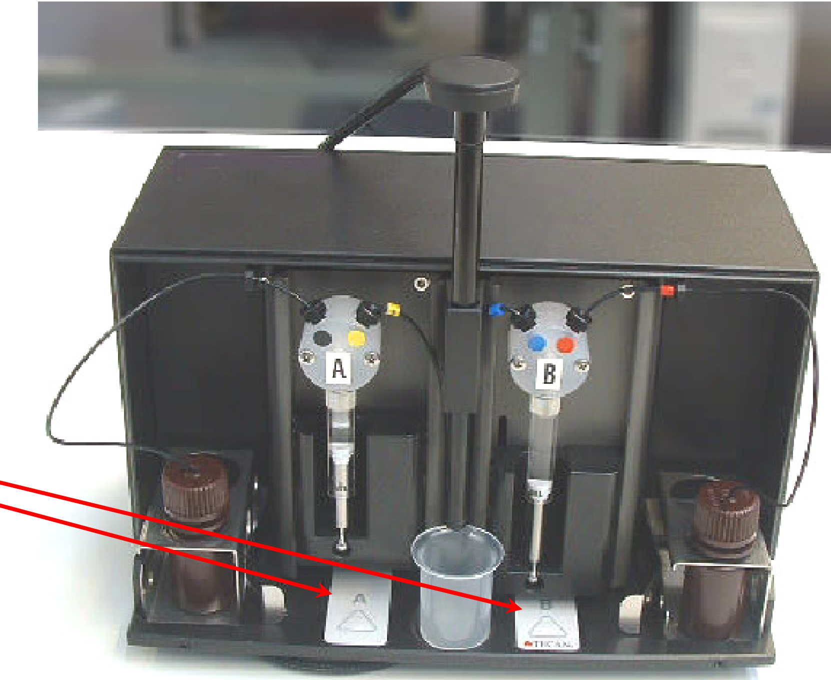


Figure 2-4: Injector-box with injector in ‘service position’

Before starting a measurement make sure that:

- The tubes are clean. If not please refer to chapter 2.3.4 Priming and Washing of the **infinite**® **200** for details how to clean the injector system.
- The injector tubes are correctly inserted into the storage bottles and fixed.
- The injector system is primed. It is not possible to start a measurement without priming the system.

When priming the system:

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.

2.3.4 Priming and Washing of the infinite® 200

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, and ‘short’: 80 cm.

For the initial filling step of the injector system (priming) it is recommended to use at least 2000 µl to remove all air bubbles from the injection system. The minimum prime volume is therefore 2 ml. To save precious reagents, this initial filling step can be performed with distilled water. To replace the water with the required reagent, a second priming step is needed. For this second priming step, the priming volume can be reduced to approx. 1500 µl.

Caution
A prime volume below 2 ml in an empty system may result in incomplete filling of the system, and therefore may negatively affect assay performance.

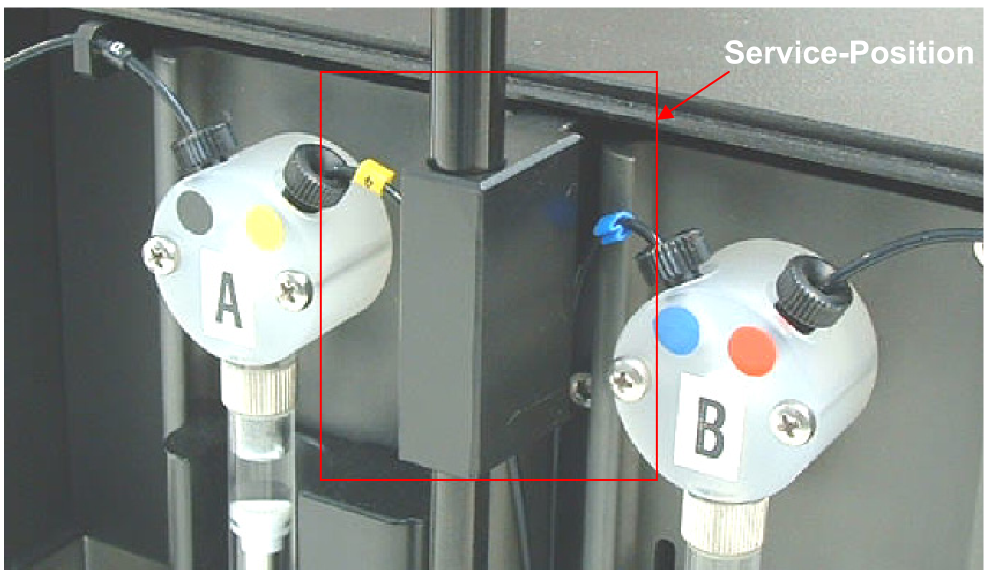


Figure 2-5 'Service Position' of the injectors. The injectors are removed from the carrier slot and inserted into the holder of the injector carrier system.

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

If the injector carrier is not inserted correctly in the injector port, the injector sensor does not detect the inserted injector and therefore washing and priming is enabled which can damage the instrument. In addition to this, the actions 'dispense' and 'inject' will not be possible.

Priming

Before the injection system can be used, an initial filling step (priming) is needed to remove all air and to completely fill the system with liquid.

It is recommended to perform a washing step before priming.

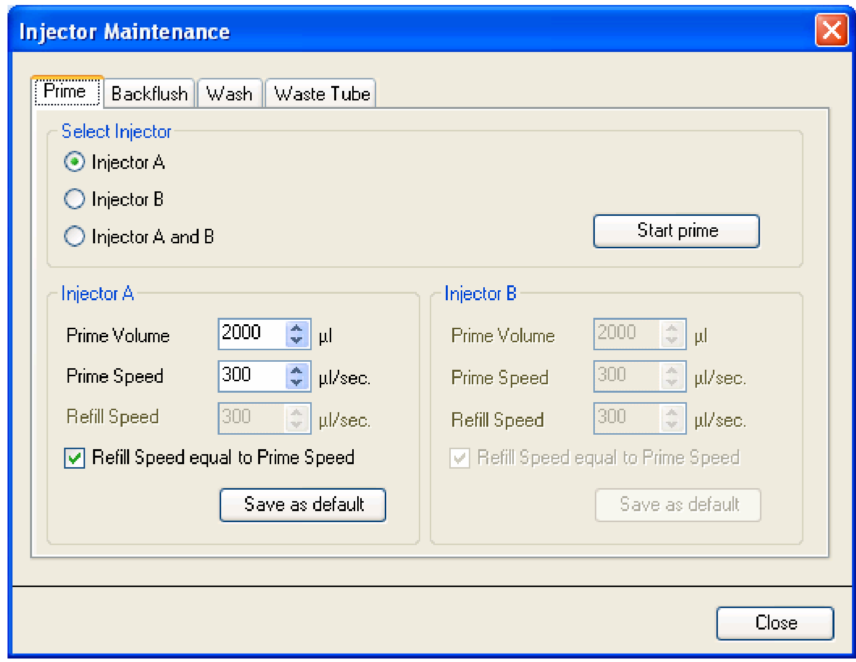
Priming can be performed by using the i-Control software or by using the hardware buttons on the injector box:

Priming procedure (general):

1. Fill the storage bottles with the necessary reagents and insert the feeding tube(s). Make sure, that the tube(s) reaches the bottom of the bottle.
2. Remove the injector from the carrier slot and insert it into the service position of the injector box.
3. Put an empty container under the injector.

Priming procedure (i-Control):

1. Adjust parameters at the prime tab of the injector maintenance dialog box in the settings menu
2. Activate the priming procedure by clicking the 'Start prime' button in the injector maintenance dialog box.
3. Visually inspect the syringes for air bubbles. Any bubbles should be removed after priming to ensure good injection performance.



Prime

Select one of the injectors **Injector A** or **Injector B** or **Injector A and B**.

Select the '**Prime Volume**' (5 -60000 µl)

Select the '**Prime Speed**' (100 - 300 µl/sec).

Select the '**Refill Speed**' (100 – 300 µl/sec.) or select '**Refill Speed equal to Prime Speed**'.

Start prime by clicking the '**Start prime**' button.

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.

Select '**Close**' to exit the dialog box

Priming Procedure (hardware button):

Priming can also be performed without using the software. Priming parameters can be stored on the injector by clicking **Save as Default** on the *Prime* tab of the *Injector Maintenance* dialog box of the i-Control software (in the *Settings* menu, click **Injectors...** and the *Injector Maintenance* dialog box appears). Press the Prime/Wash button on the injector box to start the priming sequence using the default parameters, (see Figure 2-4: Injector-box with injector in 'service position', page 24). The injector must be connected the instrument and the instrument must be switched on. Start the prime procedure by pressing the Prime/Wash button for less than 3 seconds.

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

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

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

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

Priming Example – Operational Sequence of Pump System

The following example describes the operational sequence of the pump system when performing a prime step with 500 µl.

- The system is already washed (syringe is empty; piston in upper position):
  - The first action is always that the syringe has to be filled completely with liquid. The piston therefore moves down to the lowest position to fill syringe completely (volume 1 ml).
  - Now the system prepares for the priming step. The piston makes space for the selected prime volume: The selected prime volume is ejected: 500 µl – the piston moves up.
  - The piston moves down again to prime the syringe with the selected volume. The syringe is now completely filled.
  - After finishing priming the last action is to eject 5 ml. This step makes sure that the injection/dispense conditions are equal for each well (for details please refer to 'Waste tub')

  Be aware that for selected prime volume of 500 µl, 1500 µl liquid are needed due to the initial filling step of the syringes.
- The system is not washed (the syringe is partly filled with liquid, the piston is not in the upper position):
  - The first action is always that the syringe is emptied. The piston therefore moves up to the highest position to empty the syringe.
  - The next actions are similar to case 1.

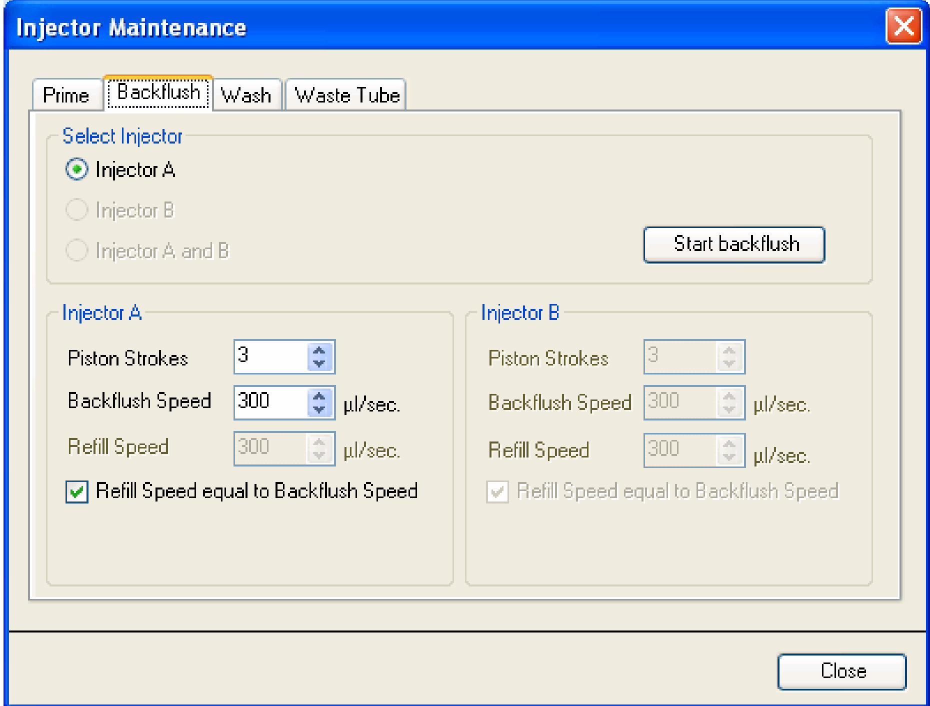
Note
For a selected prime volume of 500 µl, a minimum volume of 1500 µl of liquid is required to perform a complete priming step.

Reagent Backflush

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

Reagent backflush procedure:

1. Remove the injector carrier from the instrument and insert the injector carrier into the service position of the injector box.
2. Insert 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. Start the reagent backflush procedure by clicking **Start backflush**.



Backflush Select one of the injectors **Injector A** or **Injector B or Injector A and B** (only 'primed' injectors are available for 'backflush').

Select the **Piston Strokes** (1 – 60; 1 stroke equals 1 ml)

Select the **Backflush Speed** (100 - 300 µl/sec).

Select the '**Refill Speed**' (100 – 300 µl/sec.) or select the **Refill Speed equal to Backflush Speed** check box.

Click **Start backflush** to start the reagent backflush procedure.

Click **Close** to exit the dialog box.

Caution
The injector carrier must be in the service position for the action 'backflush'.

Do not perform backflush when the injector is in the instrument!

Washing

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

Wash procedure:

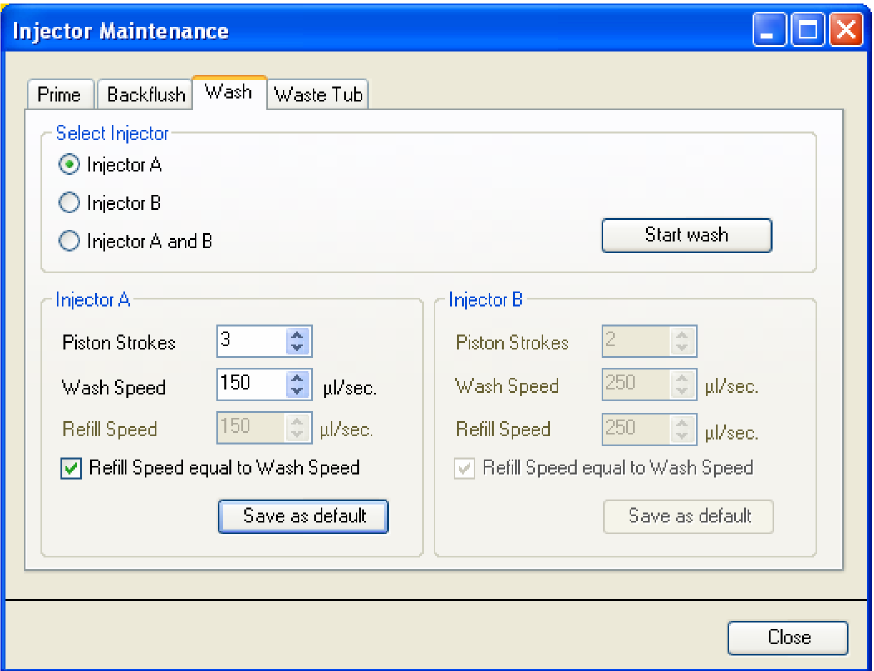
Washing can be performed by using the i-Control software or by using the hardware buttons on the injector box.

Washing (general procedure):

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

Washing (i-Control):

1. Adjust the parameters on the **Wash** tab of the **Injector Maintenance** dialog box in the **Settings** menu
2. Start the washing procedure by clicking the **Start wash**.



Wash

Select one of the injectors **Injector A** or **Injector B or Injector A and B**.

Select the **Piston Strokes** (1 – 60; 1 stroke equals 1 ml)

Select the **Wash Speed** (100 - 300 µl/sec).

Select the **Refill Speed** (100 – 300 µl/sec.) or select **Refill Speed equal to Wash Speed**.

Click **Start wash** to start the wash procedure.

Click **Close** to exit the dialog box.

Washing (hardware buttons):

Washing can also be performed without using the software. Washing parameters can be stored on the injector by clicking **Save as Default** on the *Wash* tab of the *Injector Maintenance* dialog box (in the *Settings* menu, click **Injectors...** and the *Injector Maintenance* dialog box appears) of the i-Control software. Press the Prime/Wash button on the injector box to start the washing sequence using the default parameters. (see Figure 2-4: Injector-box with injector in 'service position', page 24). The injector must be connected the instrument and the instrument must be switched on. Start the wash procedure by pressing and holding the Prime/Wash button for more than 3 seconds.

Caution
The injector carrier must be in the service position for the action 'wash'.
Do not perform washing when the injector is in the instrument!

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
Please see the corresponding reagent kit for advice on how to remove the substrate completely from the tubing system.

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 carrier together with the corresponding tubing.

Important
The button(s) on the injector box include two functions:

- **Press the button for less than 3 seconds to start PRIME.**
- **Press the button for more than 3 seconds to start WASH.**

The parameters have to be set in the i-Control software.

Waste Tub

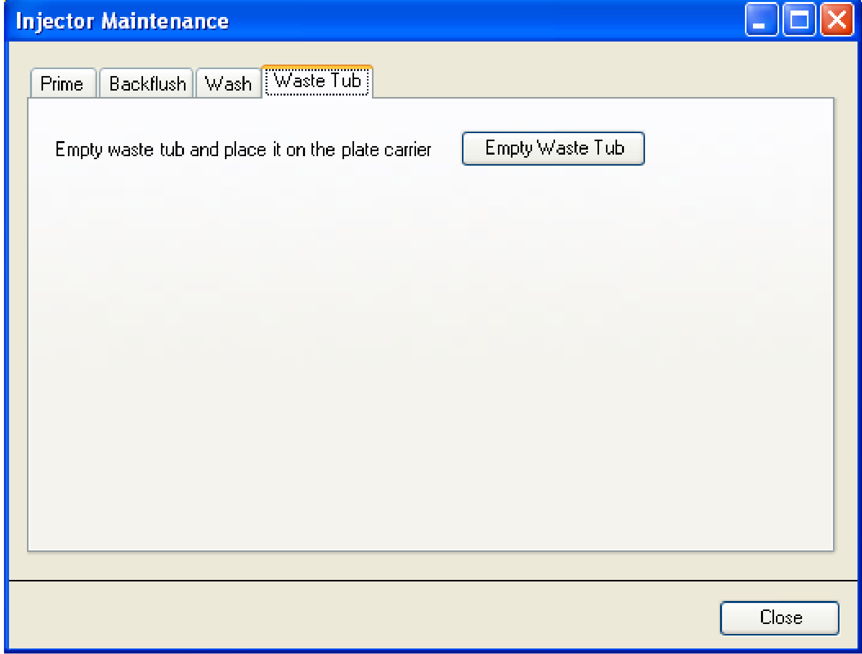
When starting a measurement with the actions ‘injection’ or ‘dispense’, 5 µl of liquid are dispensed into a disposable container on the plate carrier before starting ‘injection’ or ‘dispense’.

This initial dispense step makes sure that the injection/dispense conditions are equal for each well. This special dispense step depends on the selected refill mode selected on the injector or dispense strip (see chapter 2.4.1 i-Control and Injectors for details).

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

Picture 2-2: Waste tub on plate carrier



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. The i-Control software will alert you when the waste tub needs to be emptied again.

Caution
Place the waste tub on the plate transport before starting a measurement with the actions 'injection' and/or 'dispense'.

Caution
It is recommended to empty the waste tub before starting a measurement and to empty it at least once a day.

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF THE PROCESSES RUN ON THE infinite® 200.

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.

2.3.5 *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, the following tasks must be performed at least daily:

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

Caution
Do not allow the pump(s) to run dry for more than a few cycles.

Weekly/Periodical Maintenance:

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

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

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

WARNING

RISK OF FIRE AND EXPLOSION!

ETHANOL IS FLAMMABLE AND WHEN IMPROPERLY HANDLED CAN LEAD TO EXPLOSIONS. PROPER LABORATORY SAFETY PRECAUTIONS MUST BE OBSERVED.

2.3.6 Injector Reagent Compatibility

The injector system of the infinite® F/M200 consist 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. The column 'Rating' indicates the compatibility with the reagents listed in the 'Chemical' column. 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.

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

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.

Caution
Variations in chemical behaviour during handling due to factors such as temperature, pressure and temperature, pressure, and concentration can cause equipment to fail, even though it passed an initial test. SERIOUS INJURY MAY RESULT. Use suitable guards and/or personal protection when handling chemicals.

2.4 Software

The **infinite**® **200** is delivered with the *i-Control* software, for operating the instrument and includes an online-help file and a printed Instructions for Use. The software is formatted as a self-extracting archive on CD-ROM.

For advanced data reduction and full regulatory compliance with CFR 2 part 11 guidelines, The ***Magellan*** software can be used to control the **infinite**® **200**. (For more information, contact your local Tecan representative).

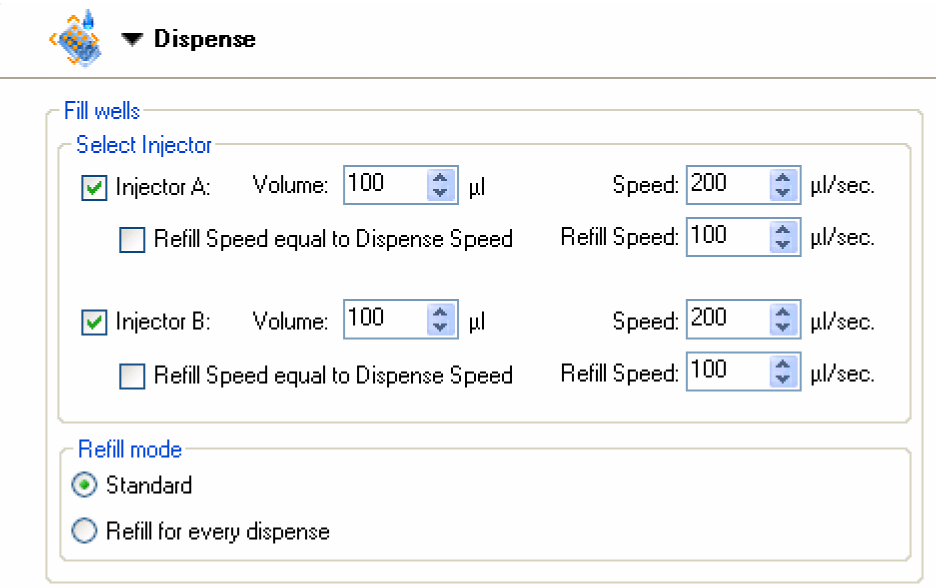
2.4.1 i-Control and Injectors

When using the injector, two modes are available:

- **Dispense**: The dispense mode allows liquid to be dispensed plate-wise into the selected wells
- **Injection**: This mode must be used in combination with a measurement strip. The injection is performed in a well-wise mode.

Dispense Mode

The dispense settings can be adjusted via the software:



Dispense

Select Injector: Injector A and/or Injector B can be selected.

Speed: The injection speed is selectable from 100 – 300 µl/sec for each injector.

Select '**Refill speed**' from 100 – 300 µl/sec. for each injector or select '**Refill Speed equal to Dispense Speed**'.

Select refill mode '**Standard**', if refill should be performed when syringe is empty (multiple dispense steps are performed before refilling, refill occurs after dispensing approx. 800 µl).

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

Using the Dispense Strip:

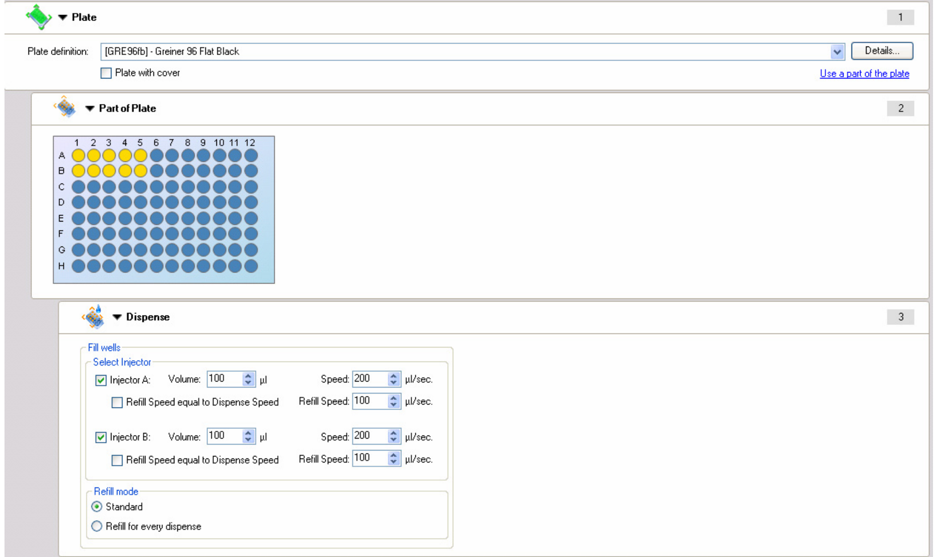
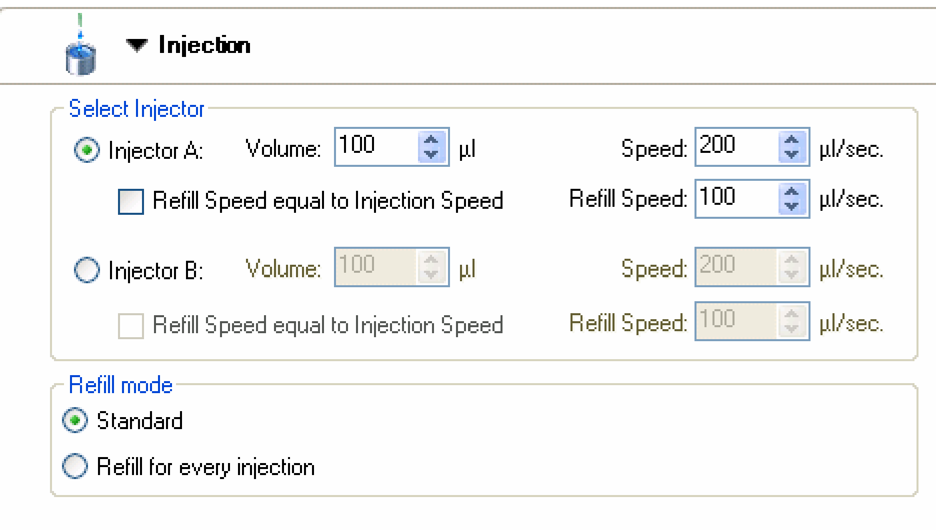


Plate	Select an appropriate plate type
Part of the plate	Optional; Select the wells to be dispensed
Dispense	Set up the dispense parameters. If both injectors are selected, all wells are first dispensed with injector A and then with injector B. The dispense strip does not require an additional measurement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

Injection Mode

The injection settings can be adjusted via the software:



Injection

Select Injector:

Injector A or Injector B can be selected. It is not possible to select both injectors on one strip. If a measurement with two injectors is to be performed, two injector strips are necessary.

Speed: The injection speed is selectable from 100 – 300 µl/sec for each injector.

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

Select refill mode '**Standard**' if refill should be performed when syringe is empty (multiple injection steps are performed before refilling, refill occurs after dispensing approx. 800 µl). Select '**Refill for every injection**' if refill should be performed for every injection step.

Injection volume

The injection volume depends on the microplate type. The plate definition files include a so-called working volume. This working volume defines the maximum volume to be injected into the selected microplate. Therefore, always make sure that the selected plate definition file contains a correct setting for the working volume. The maximum injection volume is 800 µl/injection strip. If volumes greater than 800 µl are to be injected (e.g. into 6-well plates), more than one injection strip has to be used.

Using the Injection Strip:

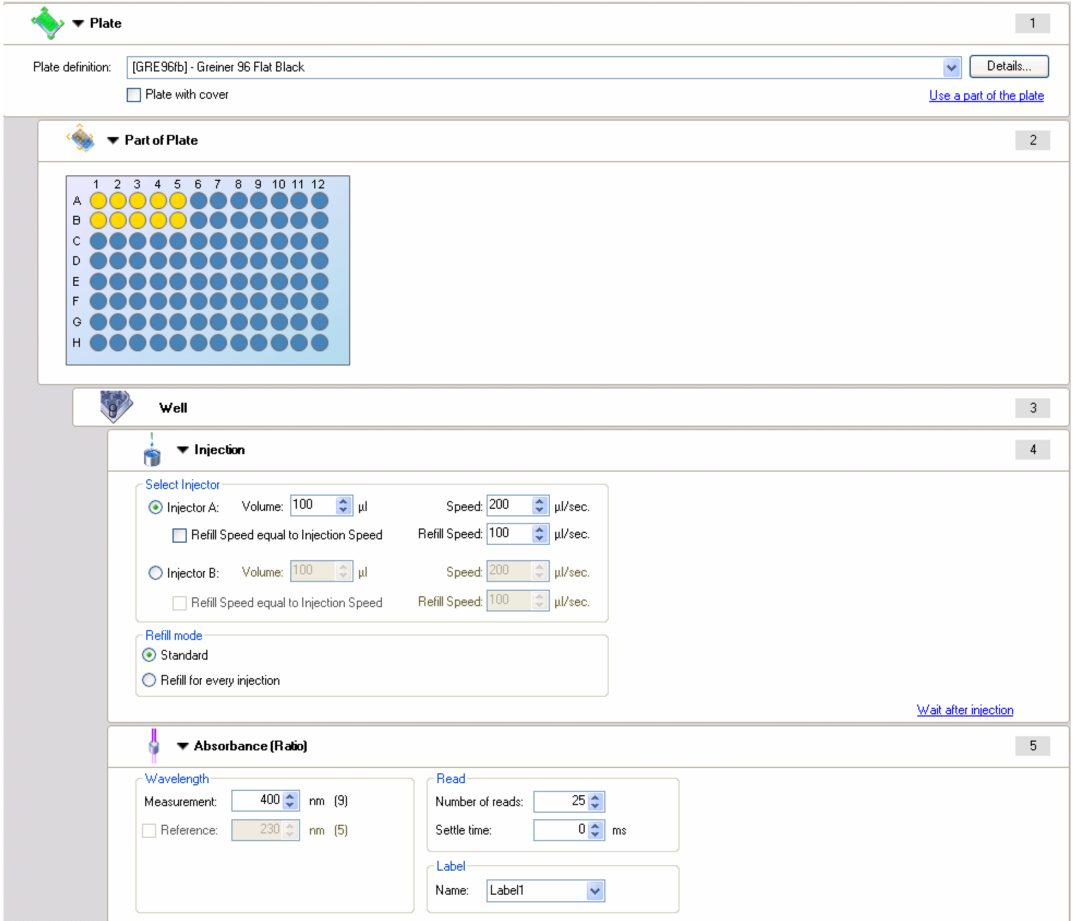


Plate Select an appropriate plate type.

Part of the plate Optional;
Select the wells to be dispensed

Well The well strip is mandatory.
Injection is only possible with a 'well' strip. This strip ensures that the following indented strips are performed well-wise.

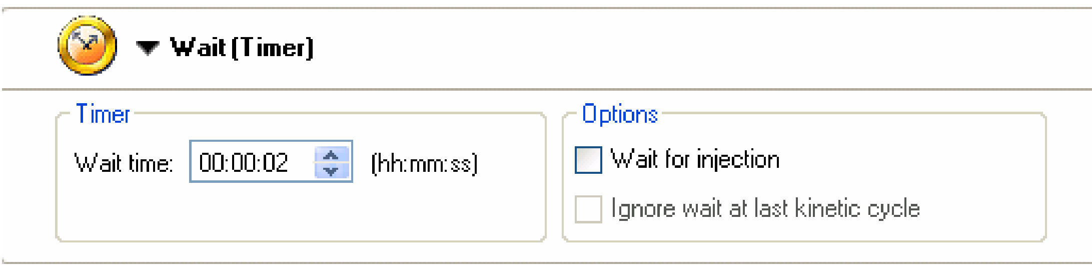
Injection Set up the injection parameters.
Only one injector can be selected per strip. If both injectors are required or one injector will perform two injections, an additional injection strip has to be inserted.

Measurement strip (Example Absorbance) It is mandatory to use at least one measurement strip in combination with the injection strip. The position of the measurement strip(s) (before and/or after the injection strip) depends on the application and is therefore user-selectable.

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

Wait Strip

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



Wait time Select a time in hh:mm:ss from 00:00:01 up to 23:59:59

Options If '**Wait for injection**' is selected, the wait time includes the injection time.

If '**Wait for injection**' is NOT selected, the wait time is added to the injection time.

2.4.2 *i-Control Examples*

Example 1: Dual Luciferase Assay™ (Promega Corp.)

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

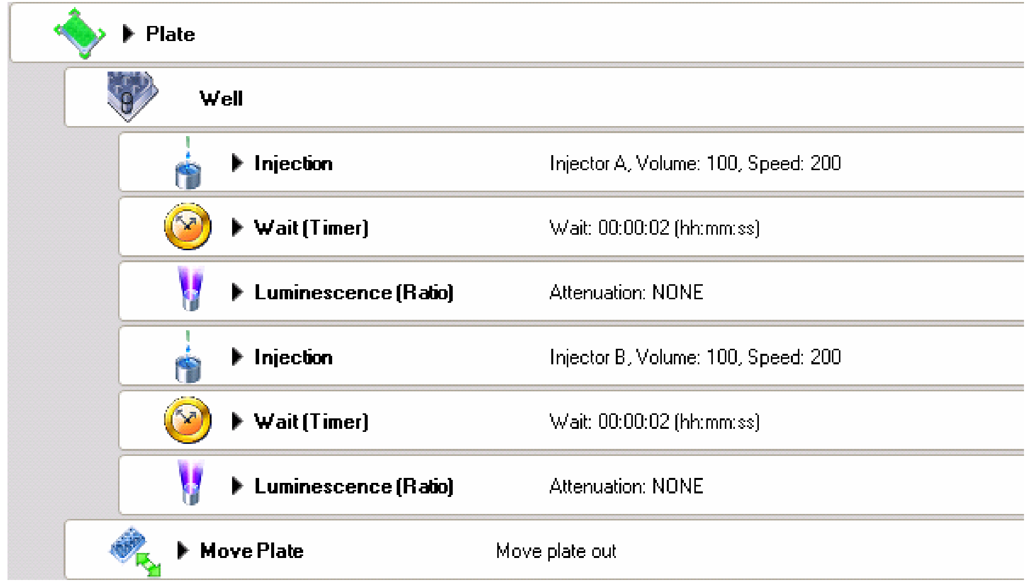


Plate	Select an appropriate plate type. For luminescence measurements, white microplates are recommended. For this example, a white 96-well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate is to be processed.
Well	Mandatory for measurements with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

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

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

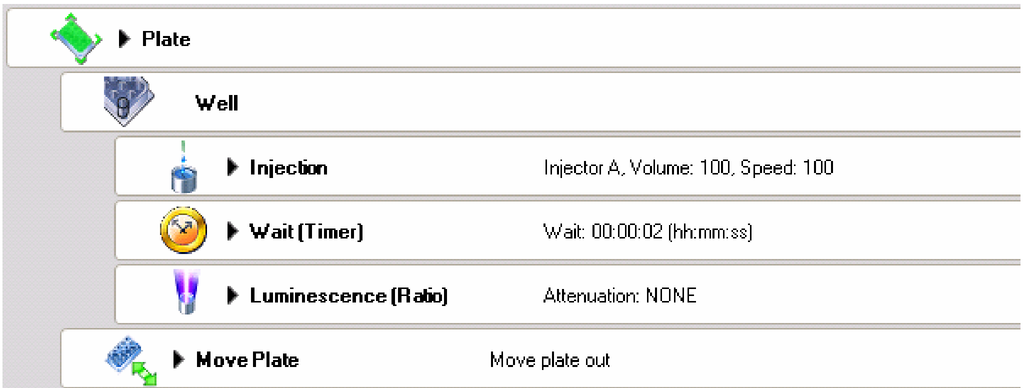


Plate	Select an appropriate plate type. For luminescence measurements, white microplates are recommended. For this example, a white 96 well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with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

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

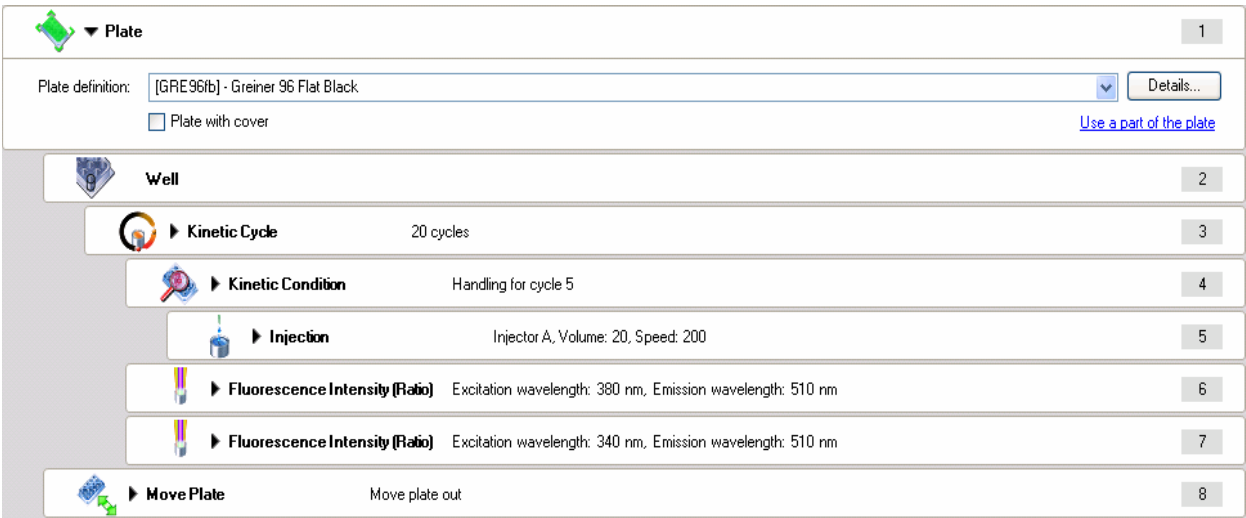


Plate	Select an appropriate plate type. For fluorescence measurements, black microplates are recommended. For this example, a black 96 well plate was selected.
Part of the plate	(Not shown), can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with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

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

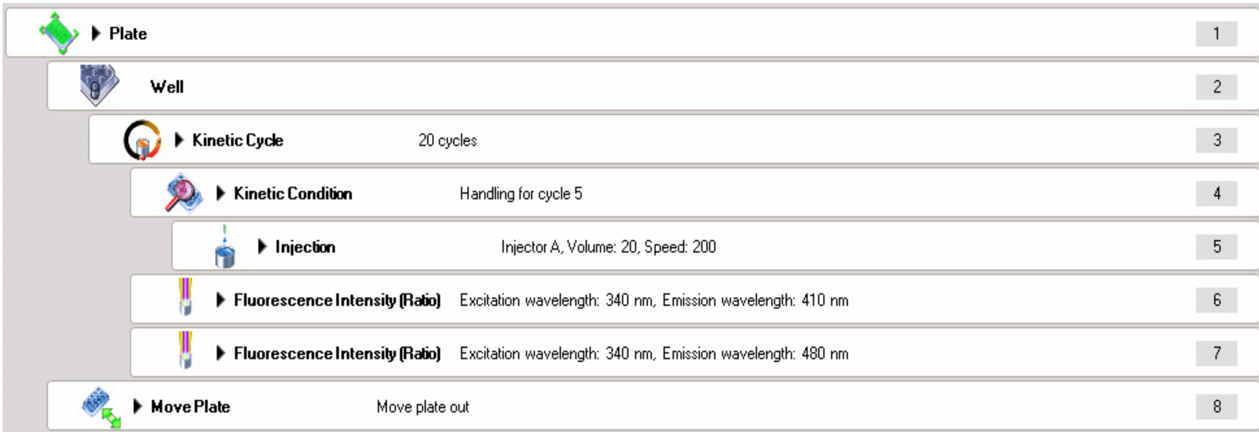


Plate	Select an appropriate plate type. For fluorescence measurements, black microplates are recommended. For this example, a black 96 well plate was selected.
Part of the plate	(Not shown); can be optionally selected if only part of the plate should be processed
Well	Mandatory for measurements with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

3. Installation

3.1 Unpacking and Inspection

The delivered packaging includes the following items:

- CABLE USB 2.0 A/B 1.8 M Black with housing receptacle ferrite
- CDROM **infinite® F200/infinite® M200**
- OOB Quality Report
- Transport lock (mounted)
- Instructions for Use
- Final test protocol

The **infinite® F200** packaging includes additionally the following items:

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

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

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

The second injector comes with the following items:

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

Caution
The reader has been tested with the supplied USB cable. If another USB cable is used, Tecan Austria cannot guarantee the correct performance of the instrument.

3.1.1 Unpacking Procedure

1. Visually inspect the container for damage before it is opened.
   Report any damage immediately.
2. Select a location to place the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors. Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment. Ensure that the plate carrier cannot be accidentally hit when moved out. Ensure that the main switch and the main cable can be reached at all times and are in no way obstructed.
3. Place the carton in an upright position and open it.
4. Lift the instrument out of the carton and place it in the selected location. Take care when lifting the instrument and ensure that it is held on both sides.
5. Visually inspect the instrument for loose, bent or broken parts.
   Report any damage immediately.
6. Compare the serial number on the rear panel of the instrument with the serial number on the packing slip.
   Report any discrepancy immediately.
7. Check the instrument accessories against the packing list.
8. Save packing materials and transport locks (see next section) for further transportation purposes.

WARNING

THE infinite® 200 IS A PRECISION INSTRUMENT AND WEIGHS FULLY EQUIPPED APPROX. 16 KG.

Caution

The maximum load for the infinite® 200 cover is 16 kg, however the load must be distributed evenly across the entire surface of the cover.

Caution

The maximum load for the infinite® 200 plate transport is 100 g.

Caution

Allow at least 10 cm distance between the back of the instrument and the wall or any other equipment.

Caution

The instrument must be placed in a location away from direct sunlight. Illumination > 500 lux can negatively influence luminescence measurements.

3.2 Removal of the Transport Locks

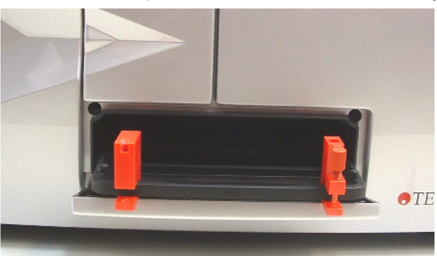
Caution
Remove the transport lock before operating the instrument.

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

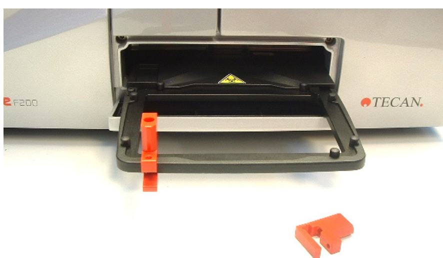
1. Ensure that the instrument is disconnected from the main power supply.
2. Open the plate carrier door and filter carrier/cuvette carrier door.

3. Remove the screw from the transport lock, which extends into the filter carrier/cuvette carrier compartment from the plate carrier compartment (left transport lock in picture above).

4. Loosen the screw from other transport lock, which is only in the plate carrier compartment (right transport lock in picture above).
5. Pull the plate carrier out manually.



6. Remove the screw from the transport lock, which is only in the plate carrier compartment (right transport lock in picture above).
7. Slide the transport locks off of the pins and remove them from the plate carrier.



8. Save the transport locks for further transportation purposes.

Caution

Save packing materials and transport locks for further transportation purposes. The infinite® 200 must be shipped only with the original packaging and installed transport locks.

3.3 Transport and Storage

3.3.1 Transport

The **infinite**® **200** must be shipped using the original packing and installed transport locks. Before shipping the instrument, it must be thoroughly disinfected (see 9.3 Instrument Disinfection).

3.3.2 Storage

Before storing the instrument the injectors must be rinsed using a wash procedure (see 2.3.4 Priming and Washing of the **infinite**® **200**). Select a location to store the instrument that is flat, level, vibration free, away from direct sunlight, and free from dust, solvents and acid vapors

Storage Specifications

Temperature	- 20 °C to + 60 °C	-4 °F to + 140 °F
Relative Humidity	< 90 % non condensing	

3.4 Power Requirements

The instrument is auto sensing and it is therefore not necessary to make any changes to the voltage range. Check the voltage specifications on the rear panel of the instrument and ensure that the voltage supplied to the instrument is correct to this specification.

The voltage range is 100-120/220-240V.

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

Caution

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

WARNING

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

3.5 Switching the Instrument On

Caution

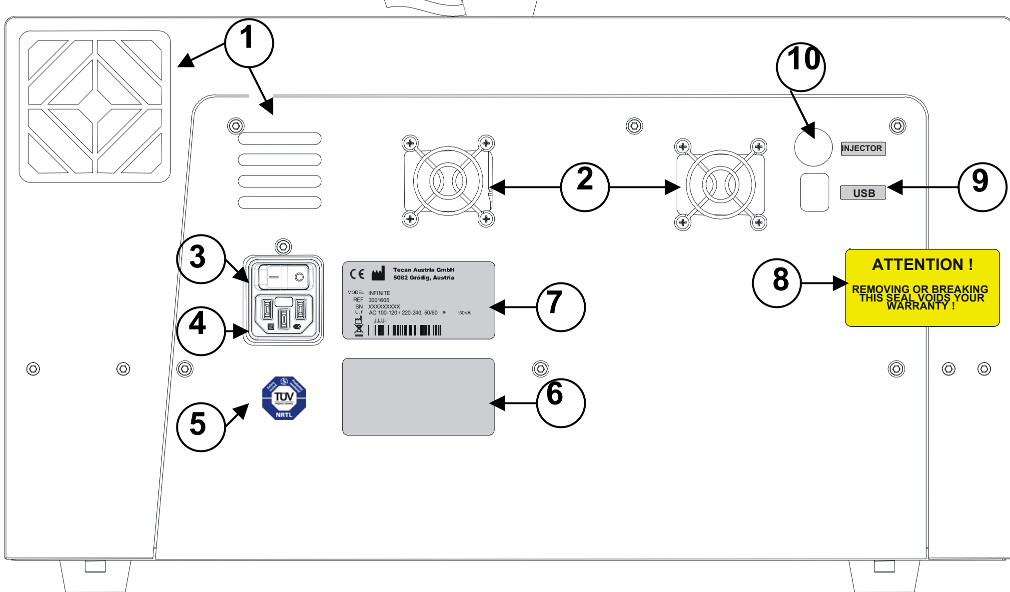
Before the instrument is switched on for the first time after installation, it should be left to stand for at least 3 hours, so there is no possibility of condensation causing a short circuit.

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

Caution

The reader has been tested with the supplied USB cable. If another USB cable is used, Tecan Austria cannot guarantee the correct performance of the instrument.

Rear View



1	Instrument Fan
2	Power Supply Fan
3	Main Power Switch
4	Main Power Socket
5	Label – Technical Inspection Agency
6	Label – Options/Configuration
7	Name Plate
8	Warranty Label
9	USB Connection
10	Injector Connection

Caution

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

4. Defining Filter Slides (infinite® F200 only)

4.1 About Filters

4.1.1 Fluorescence Filters

The optical filters (bandpass style) in a filter slide are specially designed for fluorescence measurements. The spectral rejection and the bandwidth of the fluorescence filters are optimized for achieving excellent sensitivity.

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

4.1.2 Absorbance Filters

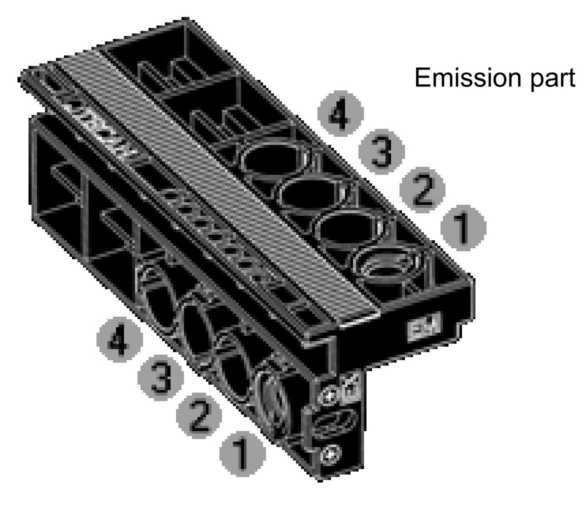
Bandpass filters, which are commonly used in microplate readers for absorbance measurements, usually have a bandwidth of 10 nm. Therefore it is not recommended to use fluorescence filters for absorbance measurements because the bandwidth (FWHM) is usually larger than 10 nm. This could cause a bright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

4.2 Filter Slide and Filter Orientation

4.2.1 Filter Slide

The **infinite**® **F200** filter slide consists of an excitation and an emission part. The filter slide enables the user to work with four independent and non-interchangeable excitation/emission filter pairs, which can be defined on positions 1 to 4. The information about the inserted filters is saved on the integrated microchip.

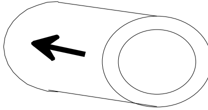
infinite® F200 : Filter slide



4.2.2 Filter Types

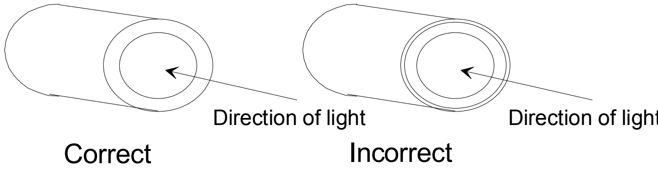
Caution
There are two types of filters. It is important that light travels through both types of filter in the correct direction. Before inserting a new filter carefully consider the filter and the direction of light through the filter slide.

1. Filters with an arrow on the side:



Light must travel in the direction of the arrow.

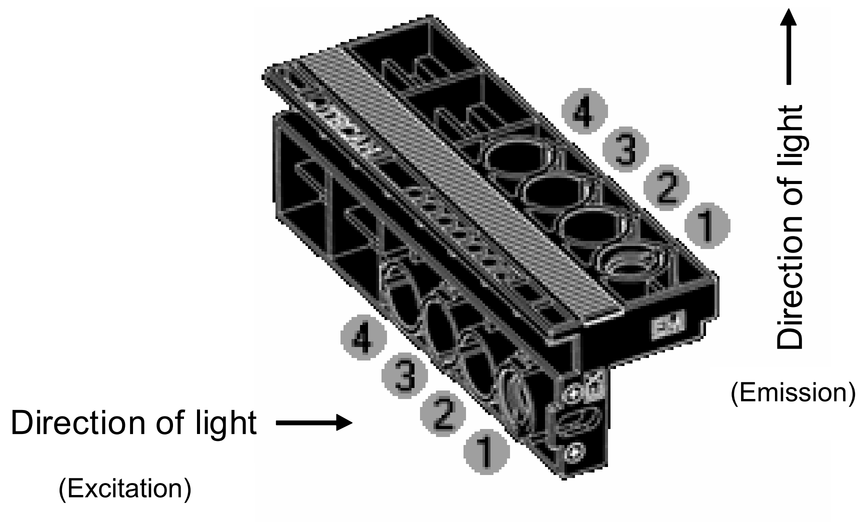
2. Filters without an arrow on the side:



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

infinite® F200:

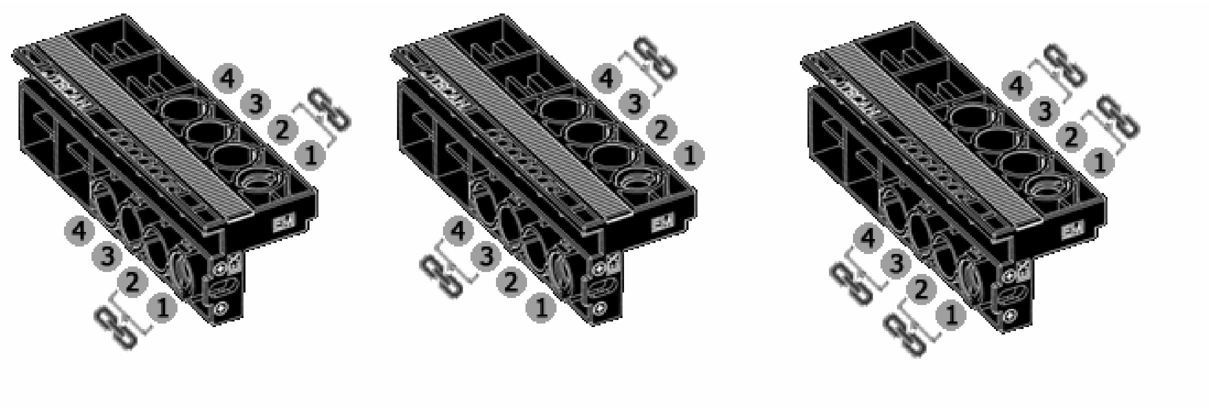
Filter Slide - Direction of Light



4.2.3 Position of Polarization Filters

Note
Fluorescence polarization measurements on Infinite® F200 require two identical excitation and emission filters placed together with the polarizers either on the positions 1 and 2 or 3 and 4.

The **infinite®** **F200** filter slide can be equipped with maximal two different fluorescence polarization filter pairs as each fluorescence polarization measurement requires two identical excitation and emission filters, which are placed together with the polarizers either on the position 1 and 2 or 3 and 4.



infinite® **F200**: Filter slide with the indicated positions for fluorescence polarization filters and polarizers.

4.3 Installing a Custom Filter

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

4.3.1 Removing a Filter

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

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

4.3.2 Mounting a Custom Filter

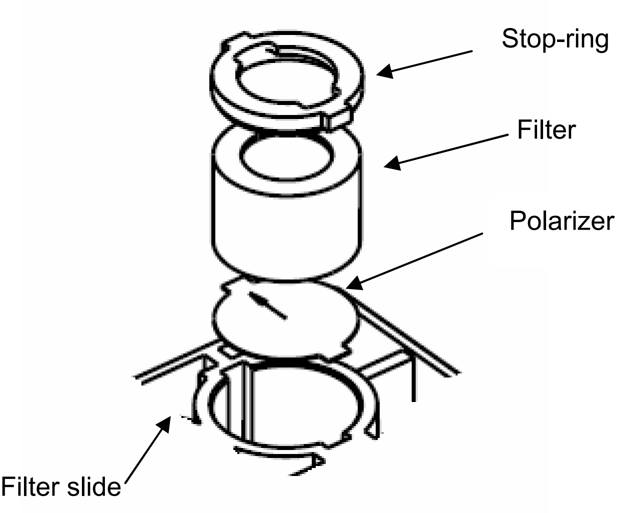
A new filter (and polarizer) must be inserted into the slide as shown below.

Note

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

Caution

Take care to insert the polarizers and the filters into the filter slide when working with fluorescence polarization.



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

In order to install a custom filter do the following:

1. If required, carefully insert a polarizer at the excitation and emission half of the filter slide using tweezers, taking care not to scratch it or get fingerprints on it.
2. Carefully insert the filter into the opening, taking care not to scratch or get fingerprints on the filter.
3. Place the stop-ring on the end of the filter assembly tool and turn it so it cannot slip off.

Filter assembly tool with stop-ring

4. Using the filter assembly tool, push the stop-ring into the filter slot and press firmly into place.
5. Rotate the tool until the notch in the stop-ring is aligned with the end of the filter assembly tool and remove the tool.
6. If there are unused openings remaining after the required filters have been inserted (e.g. the emission part of an absorbance filter), filter dummies should be mounted in the holes that are still open.

4.4 Defining the Filters

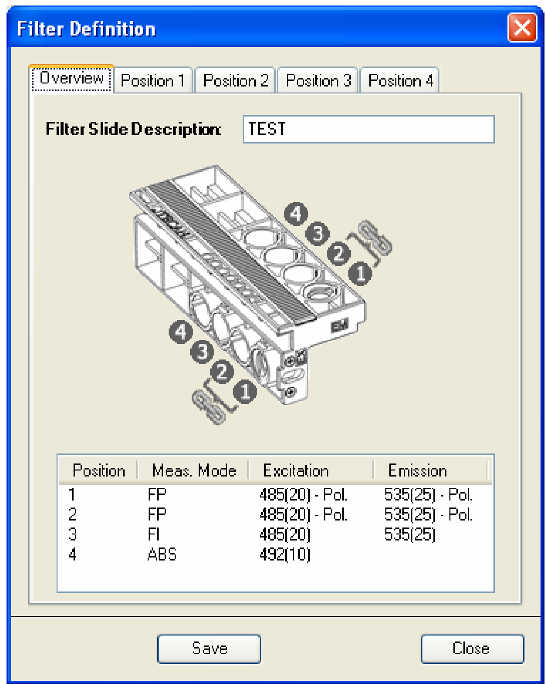
Caution

Any changes to the filters in the filter slide are to be carried out by trained personnel! The instrument is able to recognize predefined filter slides and you should not attempt to change the filter values.
However, if the filters in the filter slide have been changed (by a service engineer) or if a new undefined customized filter slide is to be used, the filter slides need to be defined.*

*** Depending on the frequency of use and environmental conditions, optical filters may deteriorate over time and therefore have a limited lifetime.**

Define a filter (pair) as follows:

1. Select **Filter Definitions** from the **Settings** menu.
2. The following dialog box is displayed showing an overview tab and four filter definition tabs:



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

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

Note

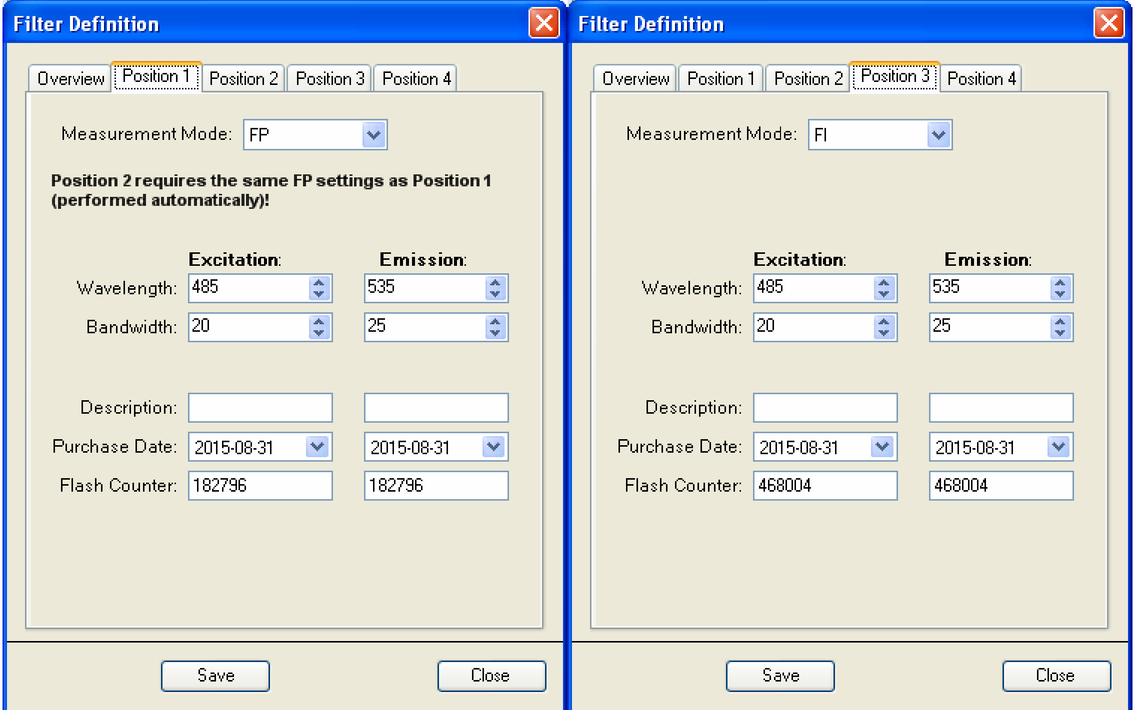
No special characters (blank, ?, $, %, ., /, etc.) except '_' are allowed for the filter slide description.

Caution
The filter slide description is part of the G-Factor key value. If manually entered, avoid using the same description for the different filter slides.

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

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

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



Note

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

Caution

Make sure that the filter slide contains polarizers together with the filters defined for fluorescence polarization.

Wavelength: Enter the filter wavelength within the following range:

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

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

(3) Absorbance mode: 230 to 1000 nm

Bandwidth: Enter the bandwidth (nm) of the filter

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

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

Note

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

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

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

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

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

Caution

It is recommended to manually document the last flash counter number before replacing the filter; otherwise this information will be lost.

5. Optical System

5.1 Fluorescence Intensity System — infinite® M200

The optical system of the fluorescence top and bottom system of the **infinite® M200** is sketched below. The system consists of the light source system (1) including the excitation **double** monochromator (2), the fluorescence top optics (3), the emission **double** monochromator (4) and the fluorescence detection (5). The solid arrows indicate the light path of the excitation light; the dashed arrows indicate the emission light path. To simplify the system, the 'Flash Monitor' (see 5.1.1; Flash Monitor) is not shown. Each monochromator unit, (2) and (4), is built of two gratings and a schematic view is displayed in more detail in *Figure 5-3*.

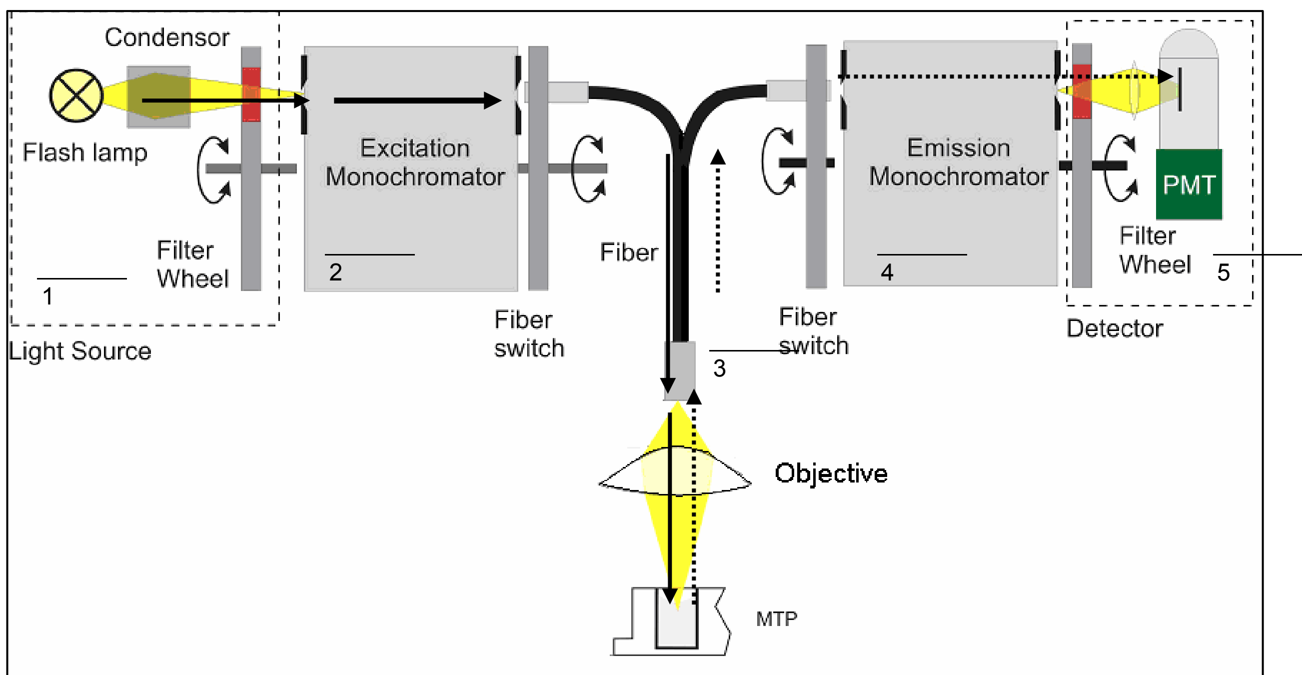


Figure 5-1 Optical System Fluorescence Top

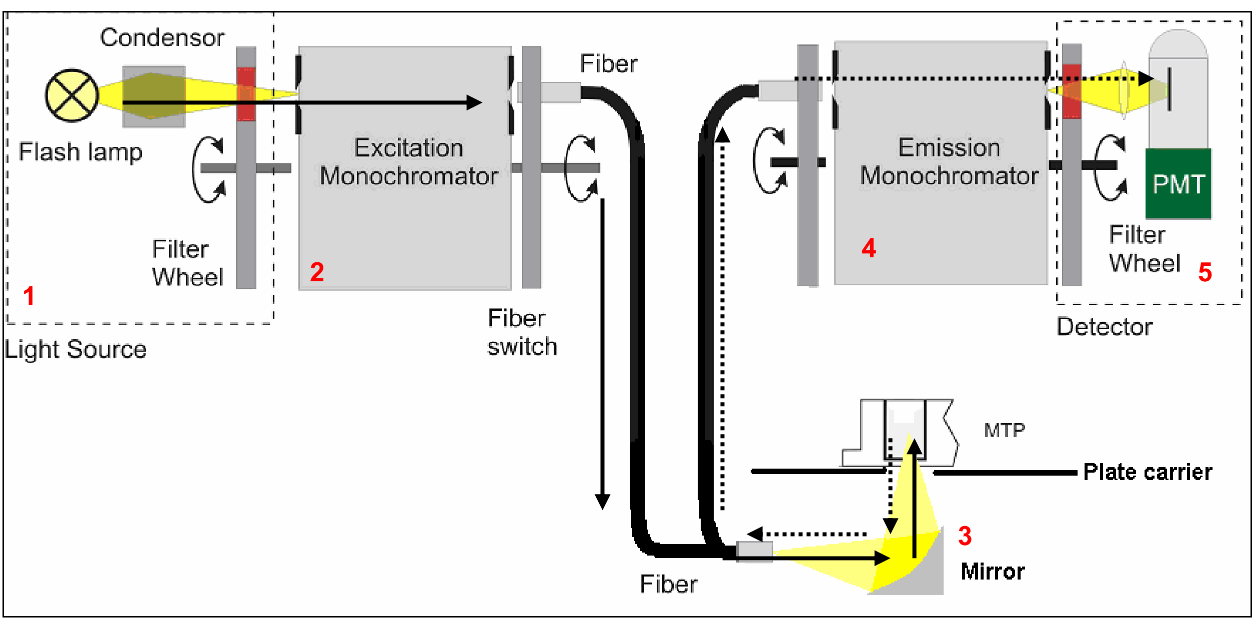


Figure 5-2: Optical System Fluorescence Bottom

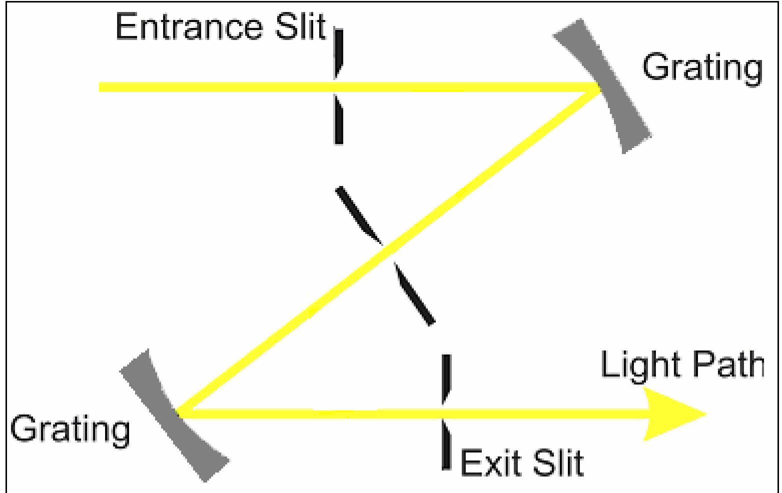


Figure 5-3: Detailed view of excitation and emission double monochromator unit

5.1.1 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® M200** light source system is built from the following components:

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

Flash Lamp

The **infinite® M200** utilizes a high energy Xenon arc discharge lamp (flash lamp). The flash sparks across a small gap between two electrodes. The lamp bulb contains a high pressure Xenon atmosphere. The flash decays within a few microseconds. The flash frequency is 40 Hz.

The **infinite® M200** 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:

1. High intensity from the deep UV to the near IR
2. Very long lifetime
3. Many applications - only one kind of lamp
4. No warm up time required

Condenser

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

Filter Wheel

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

Excitation Double Monochromator

In both fluorescence and absorbance applications, the Excitation Double Monochromator is used to select any desired wavelengths from the flash lamp spectrum in the range from 230 nm to 600 nm (standard version) or 230 to 850 nm (spectrally enhanced version) for fluorescence intensity and from 230 nm to 1000 nm for absorbance applications.

Fluorescence emission spectra in many cases do not depend on the exact excitation wavelength. For a maximum total fluorescence signal; therefore, rather broad excitation bandwidth may be used. The bandwidth of the **infinite® M200** monochromator system is < 9 nm for wavelengths > 315 nm and < 5 nm for wavelengths ≤ 315 nm.
For a more detailed description of how a monochromator works see below.

Description of how a Monochromator Works

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

A monochromator consists of an entrance slit, a dispersive element and an exit slit. The dispersive element diffracts the light into the optical spectrum and projects it onto the exit slit. A dispersive element can be realized by using a glass prism or an optical grating. Modern monochromators such as those used in the **infinite® M200** are designed with optical gratings.

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

Monochromators block undesired wavelengths, typically amounting to 10^3. This means when the monochromator is set for light with a wavelength of 500 nm and the detector detects a signal of 10,000 counts, light with different wavelengths creates a signal of only 10 counts. For applications in the fluorescence range, this blocking is often not sufficient, since the fluorescence light to be detected is usually much weaker than the excitation light. To achieve a higher level of blocking, two monochromators are connected in series, i.e. the exit slit of the first monochromator acts as the entrance slit of the second monochromator simultaneously. This is known as a double monochromator. In this case, the blocking count reaches a factor of 10^6, a value typically achieved by Interference filters.

In the **infinite® M200**, a double monochromator is installed on both the excitation and detection side. This opens the opportunity for easy selection of excitation and fluorescence wavelengths with no limitations by cut off filters.

Fiber Optic Bundle

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 (see 5.1). The lower end of each fiber bundle acts as a color specific light source. In both cases a small portion of the light is always guided to the flash lamp monitor diode.

Flash Monitor

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

5.1.2 Fluorescence Top/Bottom Optics

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

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

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

- Fluorescence Intensity Lens System Top
- Fluorescence Fiber Bundle

The bottom optics consists of the following components:

- Fluorescence Bottom Mirror
- Fluorescence Fiber Bundle

Fluorescence Intensity Lens System Top

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

Excitation Spot Size

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

Fluorescence Fiber Bundle Top and Bottom

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

Fluorescence Bottom Mirror

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

5.1.3 *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). A filter wheel is located between the monochromator and the PMT.

The Fluorescence Detection system is built from the following components:

- Emission Double Monochromator
- Filter Wheel PMT
- PMT Detector

Emission Double Monochromator

Similar to the Excitation Double Monochromator, the Emission Double Monochromator is used to select any wavelength of the fluorescence signal. It acts like an adjustable filter to discriminate scatter of excitation light and nonspecific fluorescence. The wavelength range is selectable from 330 – 600 nm in the standard instrument and from 280 – 850 nm in the spectrally enhanced instrument. The bandwidth is 20 nm.

Filter Wheel PMT

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

PMT Detector

A photo-multiplier tube (PMT) is used for the detection of such low light levels associated with fluorescence. The **infinite® M200** is available in two versions: The PMT of the standard version is sensitive up to 600 nm. The PMT of the spectrally enhanced version of the **infinite® M200** is sensitive up to the near infrared (NIR) while still having low dark current. Electronic circuitry uses analog to digital conversion of PMT output current. Adjusting the PMT gain enables measurement of a wide range of concentrations in lower or higher concentration domains. For details see Section 6.3.1 Instrument Parameters.

5.2 Fluorescence Intensity System — infinite® F200

The following parts constitute the fluorescence intensity system of the **infinite® F200** instrument:

- Light Source (1)
- Fluorescence Optics (2)
- Fluorescence Detection System (3).

The fluorescence top system is shown in *Figure 5-4*, the bottom system in *Figure* 5-5. The solid arrows indicate the excitation light path; the dashed arrows determine the emission light path.

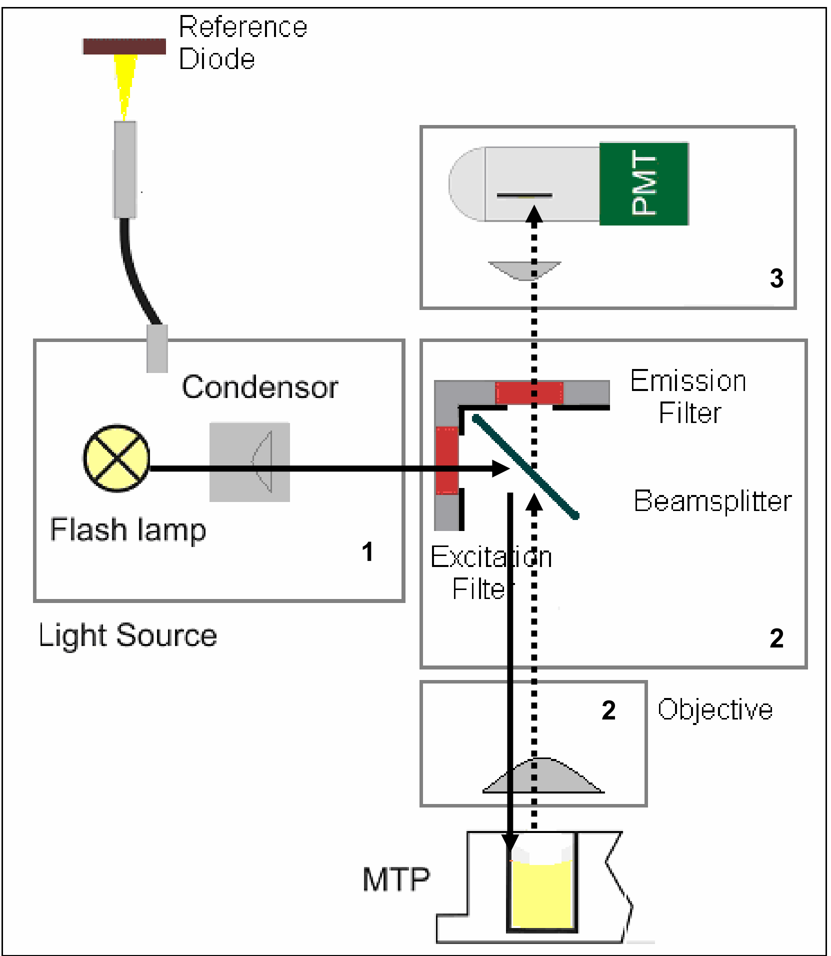


Figure 5-4: Fluorescence intensity top system of the **infinite® F200**

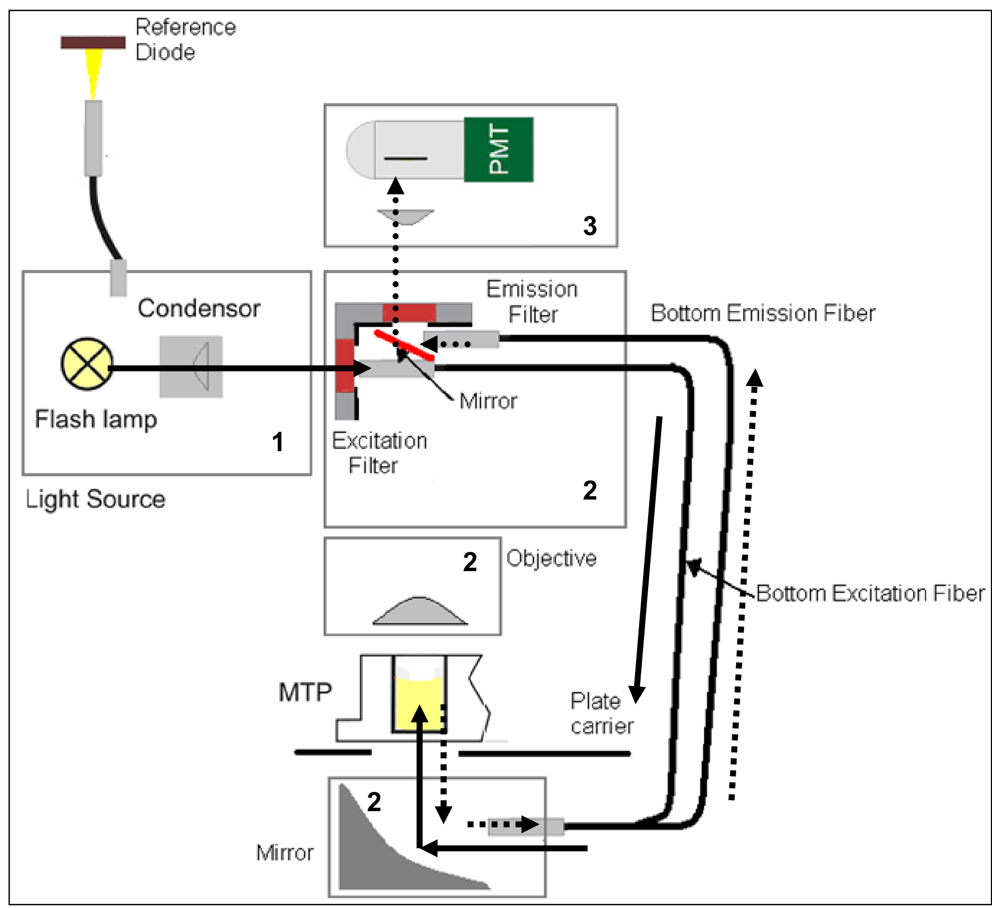


Figure 5-5: Fluorescence intensity bottom system of the **infinite® F200**

5.2.1 Light Source System

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

The **infinite® F200** light source system is built from the following components:

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

Flash lamp

The **infinite® F200** utilizes a high energy Xenon arc discharge lamp (Flash lamp). The flash sparks across a small gap between two electrodes. The lamp bulb contains a high pressure Xenon atmosphere. The flash decays within some microseconds.
The flash frequency is 40 Hz.

The **infinite® F200** 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:

1. High intensity from the deep UV to the near IR
2. Very long lifetime
3. Many applications - only one kind of lamp
4. No warm up time required

Condenser

Condenser type optics focuses the light through the entrance orifice to the fluorescence optical system.

Band Pass Filter

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

Excitation Filter

In many cases, fluorescence emission spectra do not depend on the exact excitation wavelength. Therefore, for a maximum total fluorescence signal, relatively broad excitation band pass filters (10 – 40 nm) may be used.

The spot diameter for the **infinite® F200** is about 2 mm for the top optics and 2 mm for the bottom optics.

Flash Monitor

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

5.2.2 Fluorescence Optics Top

Flash light enters the optical system by being focused through an orifice. This opening acts as a color specific light source. A semi-transparent mirror (beam splitter) reflects 50% of the light towards the microplate. The objective lens system focuses the light into the sample.

Fluorescence Emission is measured from above the well. Fluorescence light is collected by the objective, directed through the 50% mirror, and focused through the exit orifice for detection.

Objective Lens System

The objective is designed to collect as much of the fluorescent light from a well and focus it through the exit orifice to the detection system.

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

5.2.3 Fluorescence Optics Bottom

Flash light enters the optical system by being focused through an orifice. This opening acts as a color specific light source. The excitation bottom fiber guides the light to the bottom measurement head, which consists of an elliptical mirror focusing the light through the bottom of the microplate into the well. The emitted light is focused onto the excitation bottom fiber, which guides the light over a mirror through the emission filter into the fluorescence detection system.

5.2.4 Fluorescence Detection

Emission Filter

The emission filter discriminates scatter of excitation light and unspecific fluorescence. The emission filter is part of a filter set containing excitation filter, emission filter and a 50 % mirror.

PMT Detector

A photomultiplier tube (PMT) is used for the detection of such low light levels as involved with fluorescence. For details see 5.1.3 Fluorescence Intensity Detection.

5.3 Fluorescence Polarization System — infinite® F200

For technical details please refer to chapter 5.2 Fluorescence Intensity System — **infinite® F200**.

An **infinite® F200** configured for Fluorescence Polarization (FP) measurements is delivered with a standard FP filter slide. The filter slide is equipped with filters and polarizers for excitation and emission, at 485 and 535 nm respectively, and can be applied for measuring, for example, fluorescein-based FP applications.

For details on how to mount polarizers and FP filters please refer to chapter 4 Defining Filter Slides (**infinite® F200** only).

5.4 Absorbance System — infinite® M200

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 5.1.1 Light Source System Fluorescence Intensity. A fiber bundle guides the light from the excitation monochromator to the absorbance microplate (MTP) optics (3), which focuses the light into the wells. The absorbance MTP measurement module (4) is located underneath the plate carrier. These modules measure the light being transmitted through the sample. Before measurement of the microplate, a reference measurement is performed with the plate carrier moved out of the light beam.

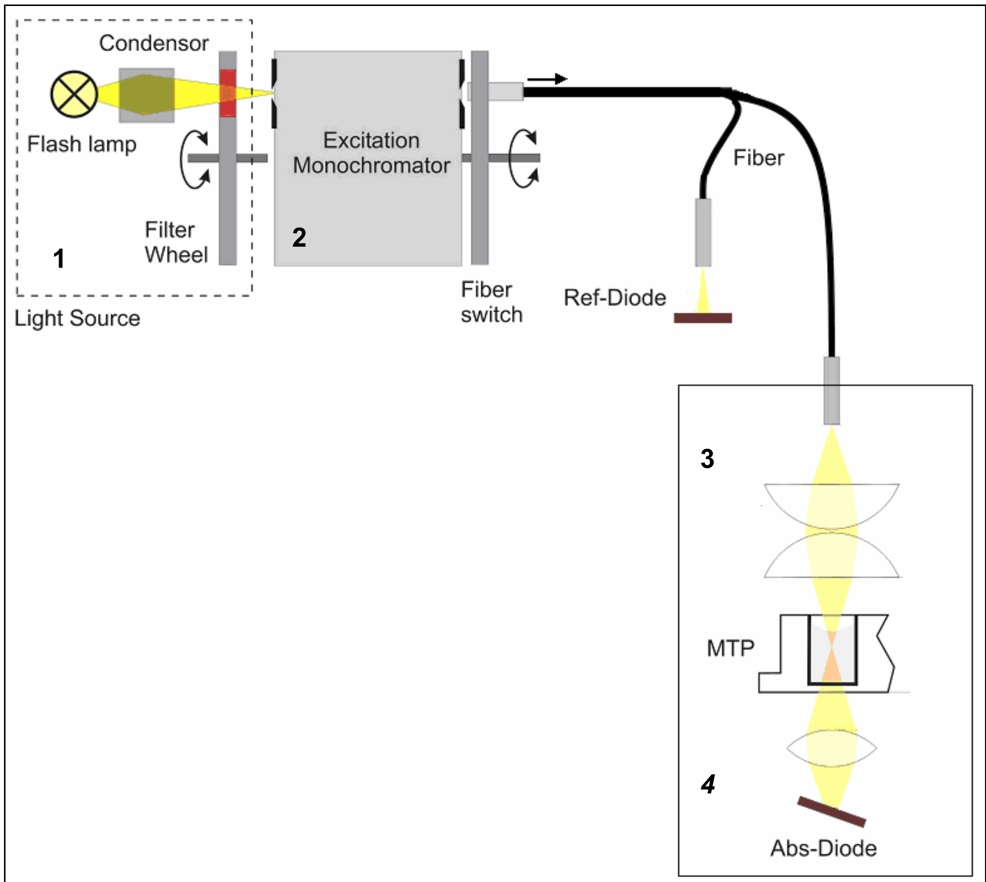


Figure 5-6 Optical System Absorbance **infinite® M200**

5.4.1 Absorbance Optics MTP

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

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

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

5.4.2 Absorbance Detection MTP

A silicon photodiode is used for the measurement of the transmitted light. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels being encountered with absorbance measurements up to 3 OD.

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

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

For further information please contact your local Tecan distributor or visit:

www.tecan.com

5.5 Cuvette Port (infinite® M200)

The **infinite® M200** may be optionally equipped with a cuvette port for absorbance measurements.

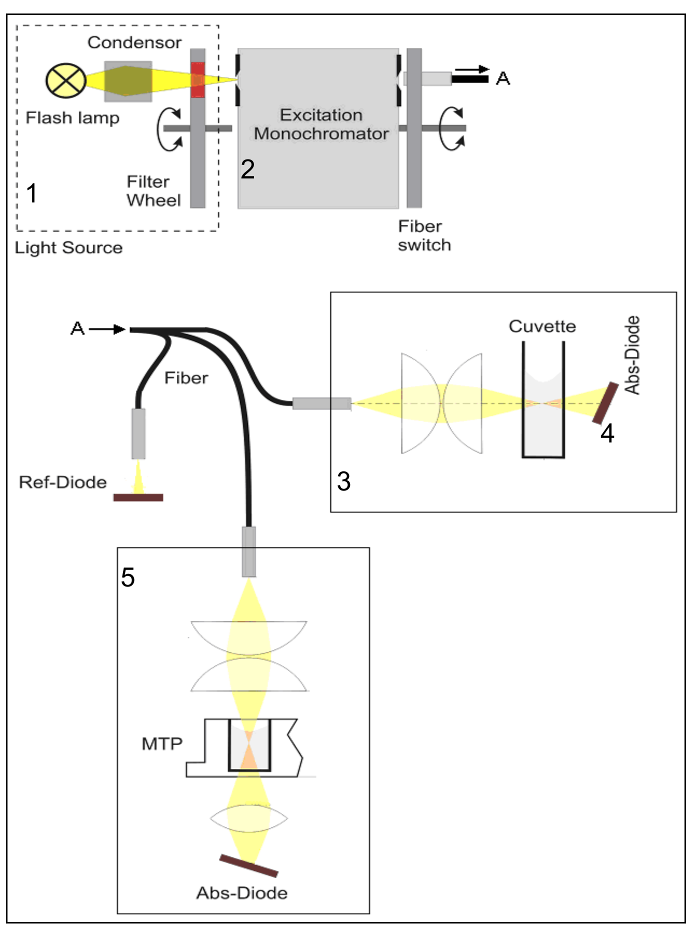


Figure 5-7: Optical System of the absorbance module of **infinite® M200** including the cuvette port

For absorbance measurements with the cuvette port of **infinite® M200** 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 2.2.1 Fluorescence/A) Fluorescence Intensity (FI). A fiber bundle guides the light from the excitation monochromator to the absorbance cuvette optics (3), which focuses the light through the cuvette. The absorbance cuvette measurement module (4) is located right after the cuvette port. A silicon photo diode measures the light being transmitted through the sample. Before measurement of the cuvette, a reference measurement against air is performed with the cuvette port moved out of the light beam. The figure also shows the light path of the absorbance microplate module (5).

Note
The cuvette port is an option of the infinite® M200 only. This option is not available for infinite® F200. With the infinite® F200, cuvettes may be measured using a Tecan Cuvette Adapter placed on the plate transport.

5.5.1 Absorbance Optics Cuvette

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

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

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

5.5.2 Absorbance Detection Cuvette

A silicon photodiode is used for the measurement of the transmitted light. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels being encountered with absorbance measurements below 3 O.D. Measurement values above 3 OD are marked as ‘OVER’ in the result sheet.

5.5.3 Cuvette types

The cuvette port is compatible with the following cuvettes:

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

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

Caution

Always use a valid filling volume. Make sure that the liquid level in the cuvette exceeds 20 mm (height). Otherwise the light path in the cuvette might not be filled completely with liquid which can lead to wrong measurement results.

Caution

The cuvette port of the infinite® M200 cannot be used for cuvettes with a measurement window < 2 mm (diameter) and a center height below 15 mm.

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

5.5.4 Inserting the Cuvette

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

Figure 5-8: Cuvette Port **infinite® M200**

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

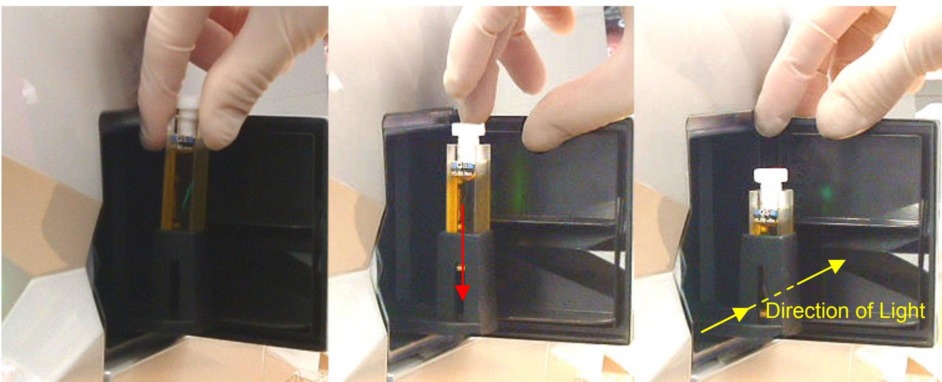


Figure 5-9: How to insert the cuvette into the cuvette holder

5.5.5 *i-Control and the Cuvette Port*

Cuvette Strip

For performing cuvette measurements, a 'Cuvette' strip is necessary (see Figure 5-10).

Figure 5-10: Cuvette strip

For a few applications it might be necessary to combine a microplate measurement with a cuvette measurement. The i-Control software therefore allows the usage of one cuvette strip and one plate strip within one measurement script. The cuvette measurement has to be positioned before the microplate measurement. To perform an accurate microplate measurement, the cuvette door must not be open. The software therefore does not allow the user to use a 'Move cuvette OUT' strip before the microplate measurement (see also chapter 5.5.6)

Cuvette Movements

The cuvette can be moved in and out with the 'cuvette in' and 'cuvette out' buttons (see Figure 5-11) or by selecting **Cuvette in/Cuvette out** in the **Instrument/Movements** dialog box.

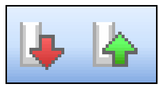


Figure 5-11: Cuvette 'out' and 'in' button

Blanking

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

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

Caution

Blanking data will be overwritten without a warning message when starting another blanking measurement. Blanking data will be deleted without a warning message when closing the i-Control software.

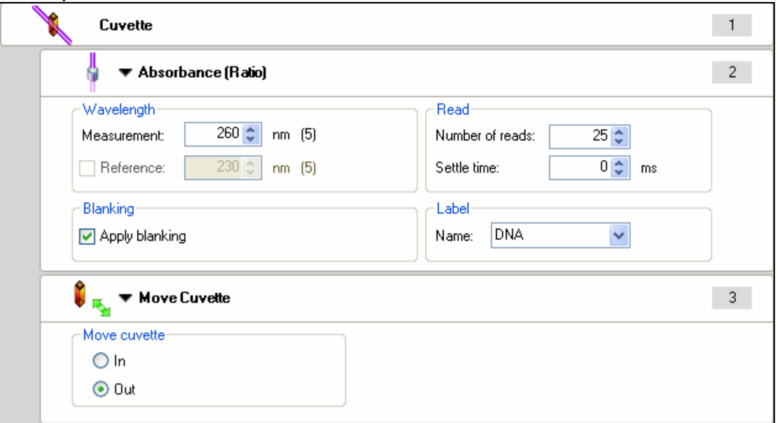
5.5.6 *i-Control Cuvette Examples*

Example 1:

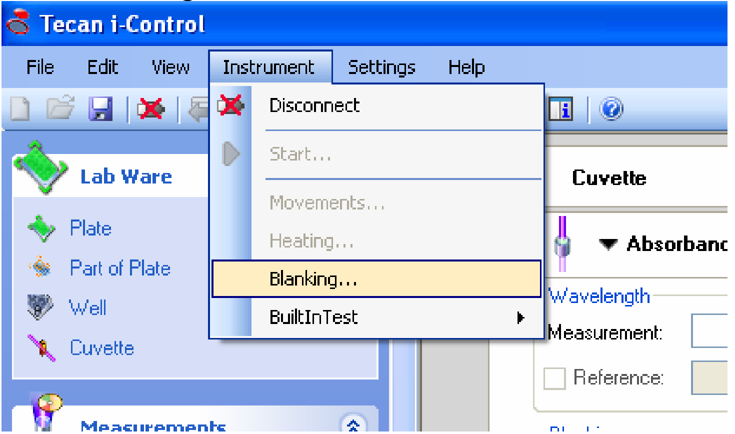
Example of how to use the 'Blanking' measurement when measuring a DNA sample:

1) Prepare cuvette with sample buffer

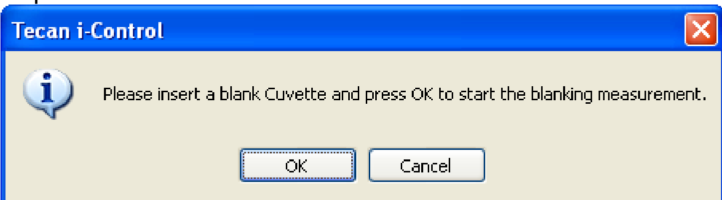
2) Set up the DNA measurement in the i-Control software:



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

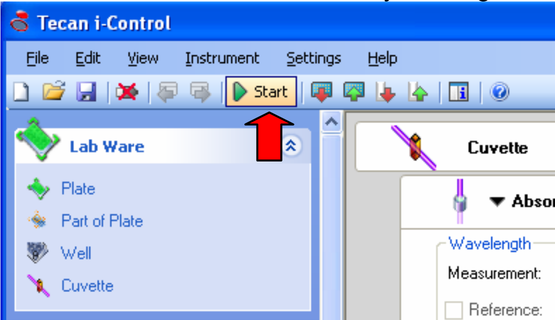


4) The instrument is initialized and the cuvette holder moves out. The user is requested to insert the blank cuvette:



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

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

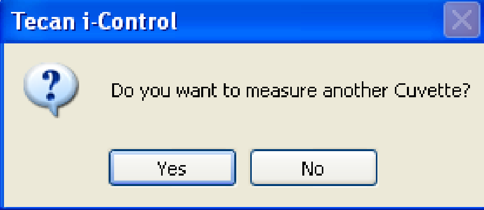


6) The cuvette holder is moved in and the measurement is performed. The measured data (Value) as well as the blank data (Blank) and the blanked data (Diff) are displayed in an Excel® spreadsheet:

Example for data display when measuring two cuvettes:

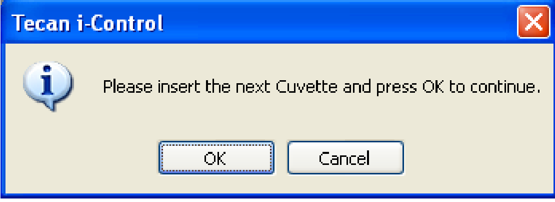
Info:	Blank data from: 20.03.2006 15:34:31				
	Value	Blank	Diff		
Cuv: 1	1.032	0.054	0.978		
Cuv: 2	1.409	0.054	1.355		

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



Click **No** to finish the measurement.

Click **Yes** to continue:

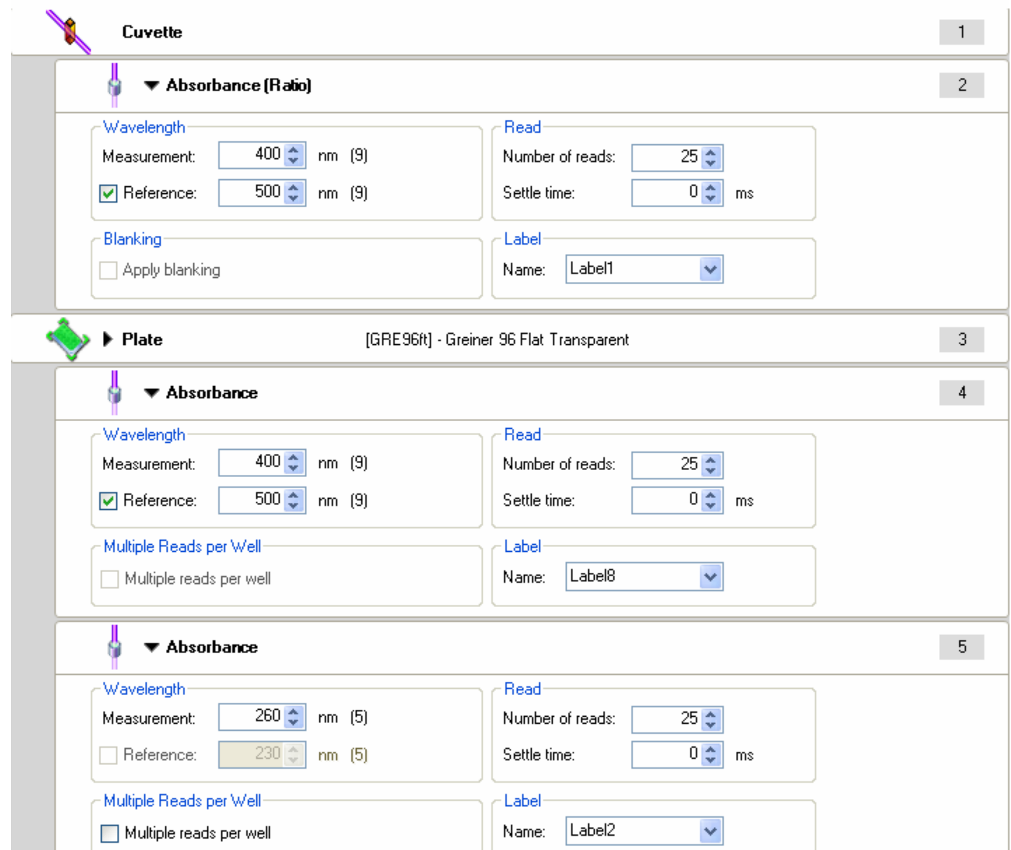


8) Insert the next sample cuvette and click **OK** to continue the measurement.

Example 2:

Combination of microplate and cuvette measurement:

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



Cuvette
Necessary for cuvette measurements.

Absorbance strip (cuvette)
Up to 4 absorbance fixed wavelength strips are allowed. Reference wavelength is only selectable when using one absorbance fixed wavelength strip. ‘Apply blanking’ is disabled when a reference wavelength is selected.

Select the appropriate measurement parameters (wavelength, number of flashes and settle time)

Plate
Necessary for microplate measurements. Select an appropriate plate type for the measurement.

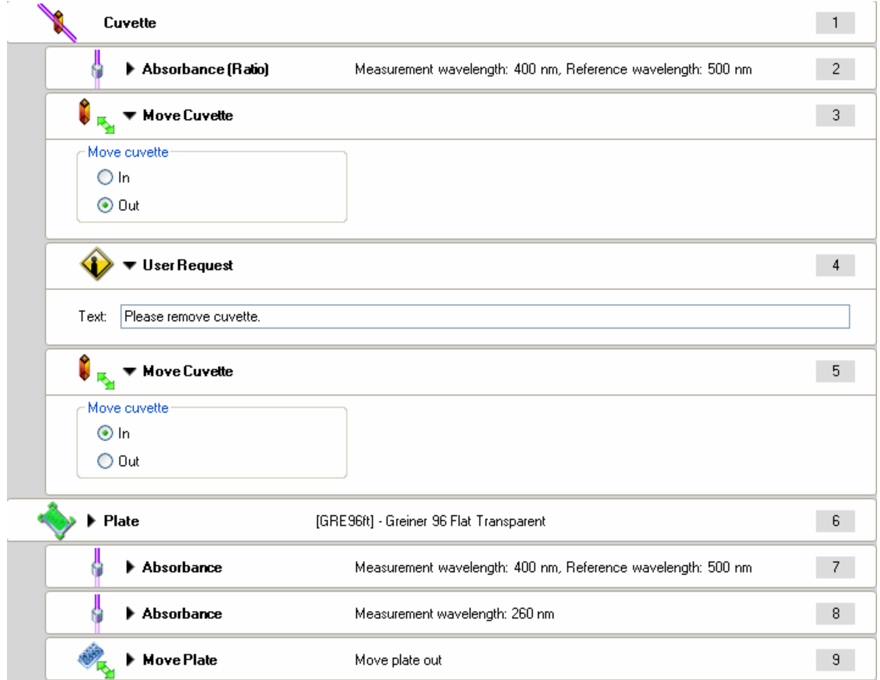
Part of Plate (not shown)
Optional. Use the ‘part of plate’ strip if only a part of the plate shall be measured.

Absorbance strip (microplate)
Up to 10 absorbance fixed wavelength strips are allowed. Reference wavelength is only allowed on the first absorbance strip. Reference wavelength is disabled on absorbance strips 2 to 10.

Select the appropriate measurement parameters (wavelength, number of flashes and settle time) for your application.

Example 3:

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



Cuvette
Necessary for cuvette measurement

Absorbance strip (cuvette)
Up to 4 absorbance fixed wavelength strips are allowed. Reference wavelength is only selectable when using one absorbance fixed wavelength strip. 'Apply blanking' is disabled when reference wavelength is selected.

Select the appropriate measurement parameters (wavelength, number of flashes and settle time)

Move Cuvette (Out)
The cuvette holder is moved 'out'.

User Request
The user request interrupts the measurement and therefore allows removing the cuvette from the cuvette port. When confirming the request the measurement continues.

Move Cuvette (In)
The cuvette port is moved in.

Plate
Necessary for microplate measurements. Select an appropriate plate type for the measurement.

Part of Plate (not shown)
Optional. Use the 'part of plate' strip if only a part of the plate will be measured.

Absorbance strip (microplate)
Up to 10 absorbance fixed wavelength strips are allowed. Reference wavelength is only allowed on the first absorbance strip. Reference wavelength is disabled on absorbance strips 2 to 10.

Select the appropriate measurement parameters (wavelength, number of flashes and settle time) for your application.

Move Plate
Optional. To move the microplate automatically out of the instrument when finishing the measurement, select the 'Move plate OUT'.

5.6 Absorbance System — infinite® F200

For absorbance measurements a similar optical path is used as for fluorescence excitation. The absorbance measurement module is located underneath the plate carrier. It measures the light being transmitted through the sample. Before measurement of the microplate, a reference measurement is performed with the plate carrier moved out of the light beam (see also 2.2.2 Absorbance).

The absorbance system is shown in *Figure 5-12* and consists of the following components:

- Light Source (1)
- Absorbance Optics (2)
- Absorbance Detection Unit (3)

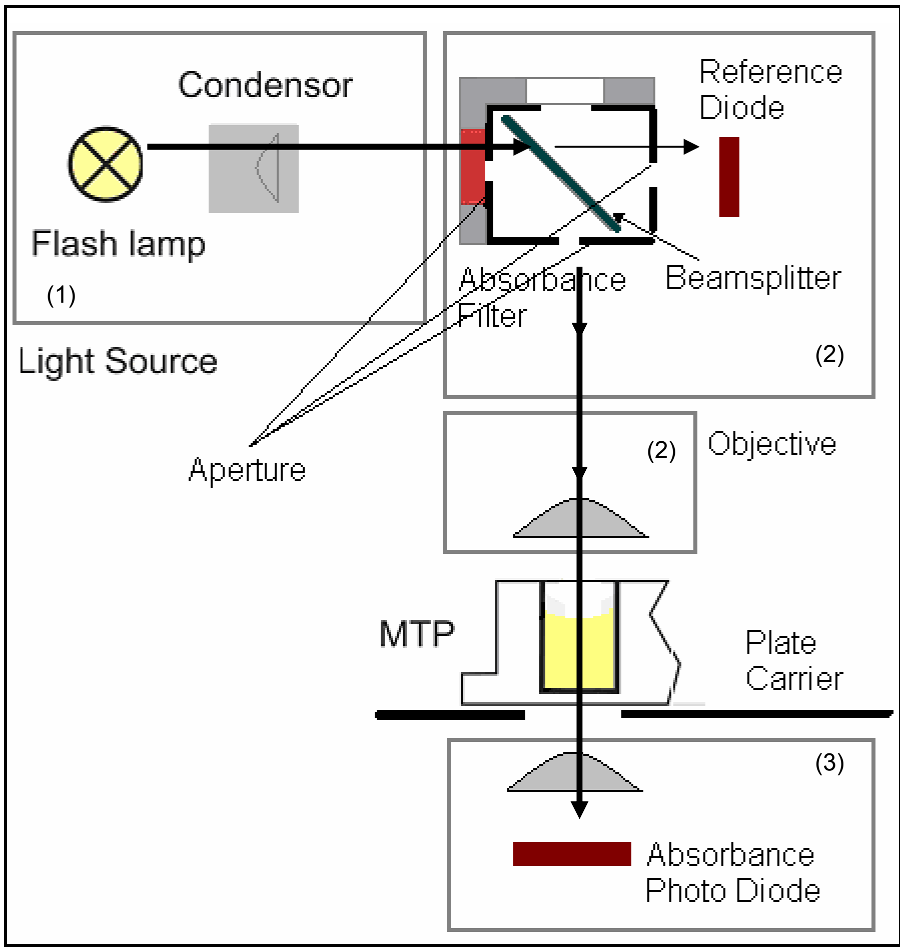


Figure 5-12: Absorbance System of the **infinite® F200**

5.6.1 Light Source System

The absorbance light source system is similar to the fluorescence top system. Please refer to 5.2.1 Light Source System.

Band Pass Filter

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

Absorbance Filter

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

5.6.2 Absorbance Optics

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

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

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

5.6.3 Absorbance Detection

A silicon photodiode is used for the measurement of the light beam. It is sensitive to a wide range of wavelengths. The photodiode is well suited for the light levels being encountered with absorbance measurements up to 3 OD.

Note
For absorbance measurement of nucleic acids of small volumes (2µl) use Tecans'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

5.7 Luminescence System

The **infinite**® **200** Luminescence System consists of the following parts:

- Luminescence Optics
- Detection Unit (Basic or Standard PMT)

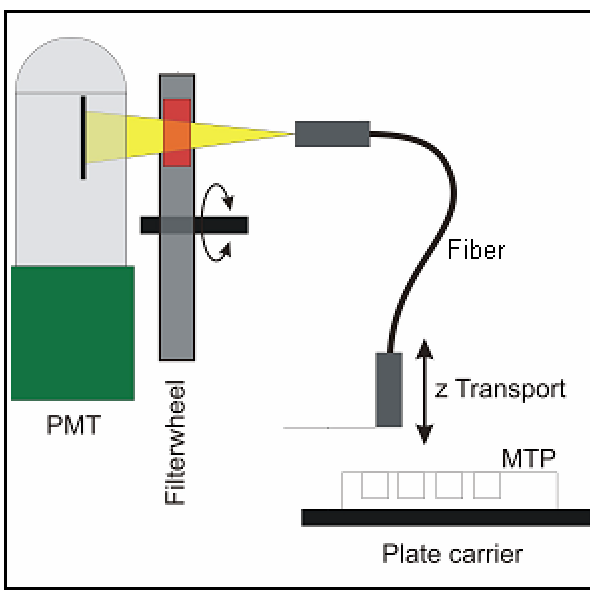


Figure 5-13: Optical System Luminescence

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

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

5.7.1 Luminescence Optics

In luminescence measurement mode, the **infinite**® **200** uses fixed microplate position and a moveable luminescence measurement head (see *Figure 5-13: Optical System Luminescence*). The plate thickness is defined by selecting the corresponding plate type in the software (see i-Control Instructions for Use).

Fiber

A glass fiber guides the light from the sample to the detection unit. The fiber is designed to measure 96-well plates as well as 384-well plates.

Filter Wheel

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

Filter Wheel Position	Filter
Position 1	Lumi Green*
Position 2	Lumi Magenta*
Position 3	1 OD neutral density filter
Position 4	No attenuation
Position 5	Blue 1**
Position 6	Green 1**

* recommended for BRET ™2 and ChromaGlo™-Luciferase Assay

** recommended for BRET™ 1

See *Figure 5-14* to *Figure 5-17* for transmission spectra of luminescence filters.

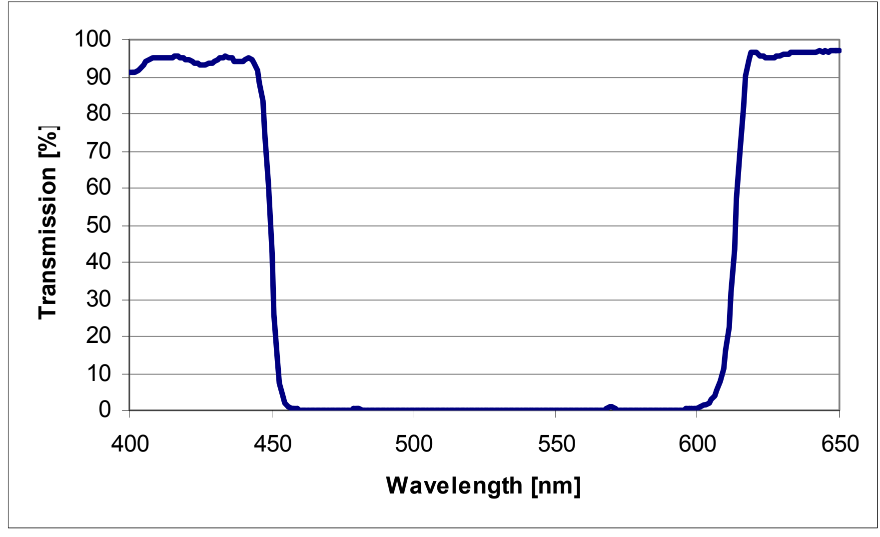


Figure 5-14: Transmission spectrum of filter 'Lumi Magenta'

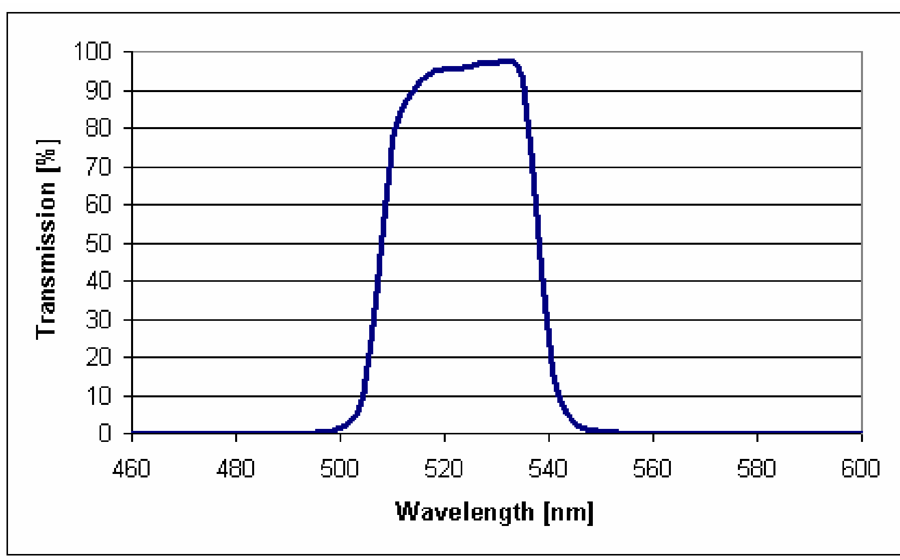


Figure 5-15: Transmission spectrum of filter 'Lumi Green'

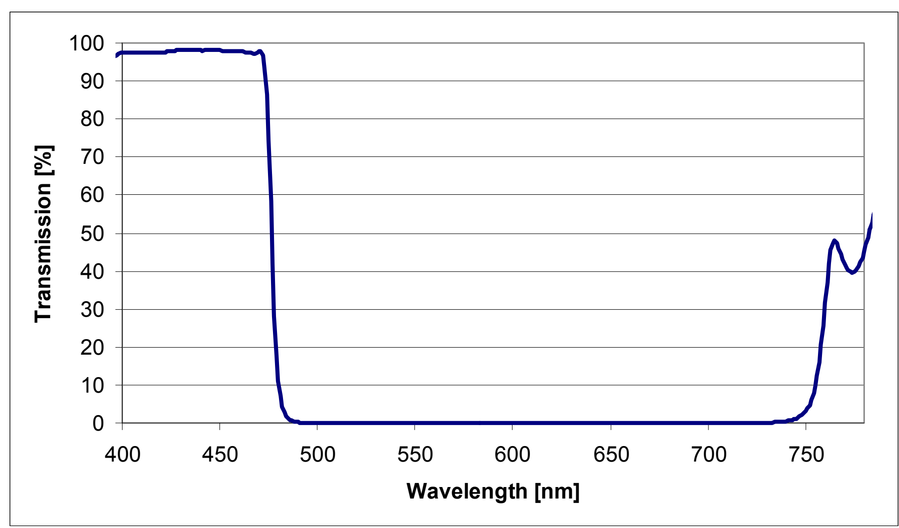


Figure 5-16: Transmission spectrum of filter 'Blue 1'

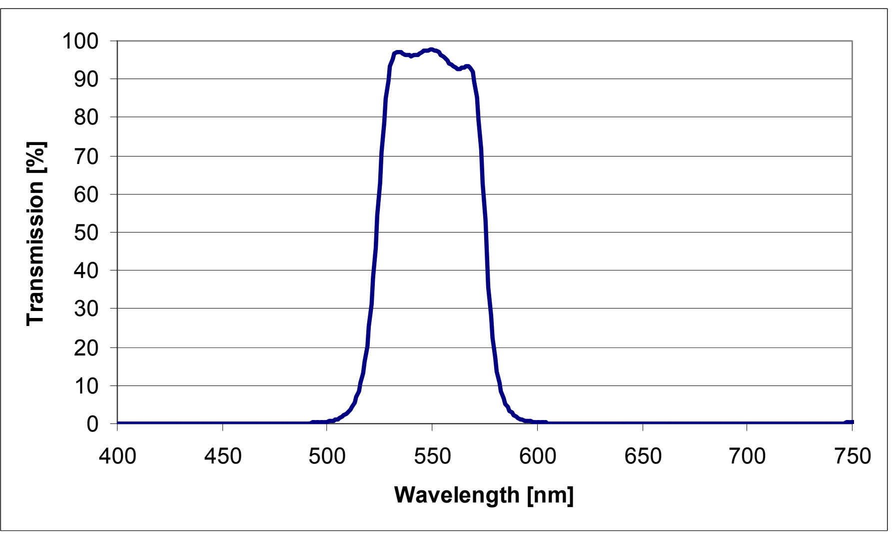


Figure 5-17: Transmission spectrum of filter 'Green 1'

5.7.2 Luminescence Detection

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

The **infinite**® **200** luminescence detection system utilizes the single photon counting measurement technique. This is based on a dedicated luminescence PMT with appropriate measurement circuitry. This technique is very robust against noise. It is preferred for measurement of very low light levels.

For best performance it is recommended to use white plates for luminescence measurements. For details see 6.7 Optimize Luminescence Measurements.

Note

Results of luminescence measurements are always displayed in RLU (Relative Luminescence Unit). 1 RLU corresponds to 1 count/s.

Caution

The instrument must be placed in a location away from direct sunlight. Illumination > 500 lux can negatively influence luminescence measurements.

6. Operating the infinite® 200

6.1 Introduction

The **infinite**® **200** is operated using a personal computer based software control. *i-Control* or *Magellan* software may be used as the user interface. For details see the corresponding software Instructions for Use. This short introduction is for a general understanding of instrument parameters and operation. Suggestions are made on how to optimize instrument parameters for your applications.

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

Caution

When placing a microplate into the plate carrier, always make sure that the correct plate definition file (plate height) has been selected in the software before you do anything else.

Maximum plate height is 23 mm (including lid).

Caution

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

Caution

In case of significant soiling of the plate transport, the spring mechanism might not work properly, and can lead to wrong positioning. Please contact your local service center.

Important

When operating the infinite® 200 always work according to GLP guidelines.

Caution

The infinite® 200 has a fan on the backside of the instrument that draws in air. The air filter has to be checked every 4 weeks and be replaced when dirty. The air filter must be replaced after 6 months.

6.2 General Operating Features

The **infinite**® **200** has some general behavior and options, which are independent from a particularly selected measurement technique.

6.2.1 Instrument Start Up

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

Instrument Power On

When switching ON the instrument no initialization steps are performed.

Connect to Instrument

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

The following steps are performed:

- Initialization of OS filter wheels (M200 only)
- Initialization of luminescence filter wheel
- Initialization of z-transport of luminescence optics
- Initialization of plate transport
- The plate transport is not moved out automatically.

The current versions of firmware and software are displayed.

The instrument is ready to be operated.

6.2.2 Finish a Measurement Session

Disconnect from Instrument

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

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

Instrument Shut Down

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

6.2.3 General Options

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

> ***Note***
> ***To keep temperature on a constant level and provide uniformity across the plate, the plate must be placed in "incubation position".***
>
> ***When the "heating" function is used during shaking, the temperature may vary slightly.***

Temperature Control

Some assays ask for an exact operating temperature. The **infinite® 200** can set up a specific temperature within a specific range, provide uniformity across the plate, and keep temperature constant above ambient. The main cooling fans stop ventilation.

Heating up the measurement chamber will take some time. Please check the temperature control display. If not incubated externally, the microplate should be left for equilibration before the measurement is started.

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

Kinetic Measurements

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

Microplate Shaking

infinite® 200 provides two shaking modes: linear and orbital. The shaking amplitude can be selected from 1 – 6 mm in steps of 0.5 mm. The frequency is a function of the amplitude. The shaking duration is selectable from 1 – 999 s.

Multi Labeling

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

6.3 Optimize Fluorescence Measurements

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

6.3.1 Instrument Parameters

Gain Settings

The **infinite® 200** fluorescence detection system uses an analog to digital (ADC: Analog Digital Converter) conversion of PMT signal. The gain setting controls the amplification of the PMT when converting fluorescence light into electrical current. The ADC needs a suitable input range of PMT current to provide a proper signal to noise ratio (S/N) on the one hand, and linearity on the other hand. Therefore, the gain should be tuned to make highest concentration microplate wells give highest possible readings. Then, readings of lower concentration microplate wells separate from background - as far as the background noise level allows for that.

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

PMT Properties

The **infinite® M200** and F200 may be equipped optionally with a 'standard' and a 'spectrally enhanced' PMT.

The gain for fluorescence intensity is selectable from 1 – 255. The performance of the PMT depends on the supply voltage. The **infinite® 200** PMTs are specified from 300 to 1250 V. The relationship between the gain settings of the **infinite® 200** and the voltage supply is described in Equation 6.3-1. The intended use of the **infinite® 200** PMT is therefore specified for gain settings from 60 to 255. Gain settings below 60 are possible and might be useful for special applications, but the performance of the PMT is not specified for voltage supply < 300 V. Tecan therefore does not take responsibility for measurement results of **infinite® 200** when using gain settings below 60.

Equation 6.3-1: $$U = \frac{Gain}{255} * 1250V$$

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

Example:

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

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

Flash Settings

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

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

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

Timing Parameters for Time Resolved Fluorescence

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

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

Settle Time

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

6.3.2 FI Ratio Mode

Ratio Mode

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

Filter Switch Time (**infinite® F200**)/Wavelength Switch Time (**infinite® M200**)

The **infinite® F200** can switch between two filters within 250 ms in case that the selected labels are measured with the same gain. Otherwise, the switching time is 400 ms. In this case the high voltage level at the PMT needs to be changed. The high voltage applied to the PMT needs some time to stabilize.

The **infinite® M200** can switch between two wavelengths within 150 ms in case that the selected labels are measured with the same gain and no order sorting (OS) switching point is involved (see Table 6.3-1:for switching points). Otherwise, the switching time is 400 ms. In this case the high voltage level at the PMT needs to be changed. The high voltage applied to the PMT needs some time to stabilize. The OS filter wheel needs to be moved.

Table 6.3-1: OSF (Order Sorting Filter) Switching Points (**infinite® M200**)

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

Example:

Fura-2: This application involves a filter/wavelength switch between 340 and 380 nm on the excitation side. The emission is measured at about 510 nm. The excitation filter/wavelength switch does not include an OS switch, therefore the switch is possible within 150 ms on an **infinite® M200** and 250 ms on a **infinite® F200**.

6.3.3 Measurement Accessories

Recommended Filters (infinite® F200 Only)

Please ask your local Tecan dealer for a recommended filter set. Filters designed for a different type of instrument will not necessarily perform well with the **infinite® F200**.

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

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

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.

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.

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

All standard microplates from 6 to 384 wells (maximum plate height 23 mm including lid) that conform to the following standards can be measured: ANSI/SBS 1-2004, ANSI/SBS 2-2004; ANSI/SBS 3-2004 and ANSI/SBS 4-2004.

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

Plate Definition File (*.pdfx)	Catalog Number	Manufacturer
GRE6ft	657 160 657 185	Greiner Bio-One, www.gbo.com/bioscience
GRE12ft	665 180 665 102	Greiner Bio-One, www.gbo.com/bioscience
GRE24ft	662 160 662 102	Greiner Bio-One, www.gbo.com/bioscience
GRE48ft	677 180 677 102	Greiner Bio-One, www.gbo.com/bioscience
GRE96ft	655 101 655 161	Greiner Bio-One, www.gbo.com/bioscience
GRE96fb_chimney	655 079 655 086 655 077 (Fluotrac 600) 655 076 (Fluotrac 200)	Greiner Bio-One, www.gbo.com/bioscience
GRE96fw_chimney	655 073 655 083 655 074 (Lumitrac 600) 655 075 (Lumitrac 200)	Greiner Bio-One, www.gbo.com/bioscience

Plate Definition File (*.pdfx)	Catalog Number	Manufacturer
GRE96ut	650 101 650 161 650 160 650 180 650 185	Greiner Bio-One, www.gbo.com/bioscience
GRE96vt	651 101 651 161 651 160 651 180	Greiner Bio-One, www.gbo.com/bioscience
GRE384fb	781 079 781 086 781 077 (Fluotrac 600) 781 076 (Fluotrac 200) 781 094 (µClear) 781 095 (µClear)	Greiner Bio-One, www.gbo.com/bioscience
GRE384ft	781 061 781 101 781 162 781 185 781 186 781 165 781 182	Greiner Bio-One, www.gbo.com/bioscience
GRE384fw	781 073 781 080 781 074 (Lumitrac 600) 781 075 (Lumitrac 200) 781 097 (µClear) 781 096 (µClear)	Greiner Bio-One, www.gbo.com/bioscience
GRE384sb	784 209	Greiner Bio-One, www.gbo.com/bioscience
GRE384st	784 201	Greiner Bio-One, www.gbo.com/biosciencer
GRE384sw	784 207	Greiner Bio-One, www.gbo.com/bioscience
COS6ft	3506 3516	Corning, www.corning.com/lifesciences/
COS12ft	3512 3513	Corning, www.corning.com/lifesciences/
COS24ft	3524 3526 3527	Corning, www.corning.com/lifesciences/
COS48ft	3548	Corning, www.corning.com/lifesciences/
COS96fb	3916 (TC-Treated) 3915 (Non-Treated) 3925 (Treatment: High)	Corning, www.corning.com/lifesciences/
COS96ft	3370 3628	Corning, www.corning.com/lifesciences/
COS96fw	3362 (TC-Treated) 3912 (Non-Treated) 3922 (Treatment: High)	Corning, www.corning.com/lifesciences/
COS96rt	3360 3367 3788 3795 3358	Corning, www.corning.com/lifesciences/
COS96ft_half area	3690 (High Binding) 3695 (Non-Treated) 3697 (TC-Treated)	Corning, www.corning.com/lifesciences/
COS384fb	3708 (Treatment: High) 3709 (TC-Treated) 3710 (Non-Treated)	Corning, www.corning.com/lifesciences/

Plate Definition File (*.pdfx)	Catalog Number	Manufacturer
COS384ft	3680 (Non-Treated) 3700 (Treatment: High) 3701 (TC-Treated) 3702 (Non-Treated)	Corning, www.corning.com/lifesciences/
COS384fw	3703 (Treatment: High) 3704 (TC-Treated) 3705 (non-Treated)	Corning, www.corning.com/lifesciences/
COS384fb_assay plate clear bottom	3711 (Non-Treated) 3712 (TC-Treated)	Corning, www.corning.com/lifesciences/
COS384fw_assay plate clear bottom	3706 (Non-Treated) 3707 (TC-Treated)	Corning, www.corning.com/lifesciences/
COS384fb_low volume	3676 (NBS) 3677 (Med. Binding)	Corning, www.corning.com/lifesciences/
COS384fw_low volume	3673 (NBS) 3674 (Med. Binding)	Corning, www.corning.com/lifesciences/
NUN96ft	269620 269787 439454 442404 475094	Nunc, www.nuncbrand.com
NUN96fb LumiNunc/FluoroNunc	137101 137103 237105 237107 237108 437111 437112	Nunc, www.nuncbrand.com
NUN96fw LumiNunc/FluoroNunc	136101 136102 236105 236107 236108 436110 436111	Nunc, www.nuncbrand.com
NUN384ft	164688 242757 242765 265196 464718	Nunc, www.nuncbrand.com
NUN384fb LumiNunc/FluoroNunc	164564 264556 460518	Nunc, www.nuncbrand.com
NUN384fw LumiNunc/FluoroNunc	164610 264572 460372	Nunc, www.nuncbrand.com

Table 6.3-2: Plate definition files and the corresponding catalog numbers

6.4 FP Measurements

6.4.1 Fluorescence Polarization

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

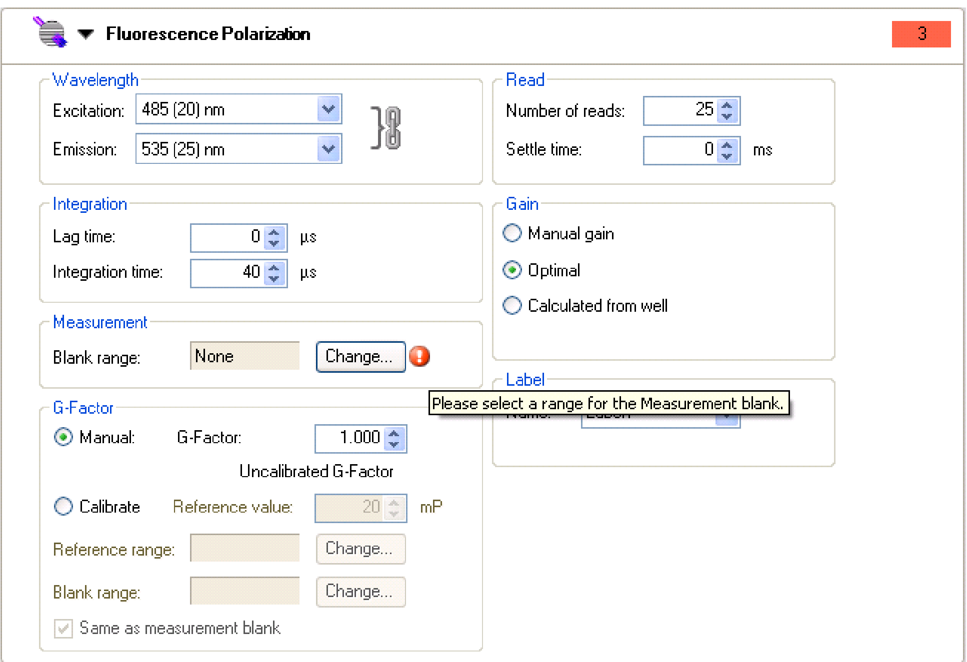
$$P = \frac{\left(I_{\parallel} - I_{\perp}\right)}{\left(I_{\parallel} + I_{\perp}\right)}$$

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

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

6.4.2 Measurement Blank Range

Measurement blank reduction is performed automatically at each fluorescence polarization measurement; the mean value of the respective blank wells will be subtracted from each sample value (see 6.4.8).



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

6.4.3 G-Factor Settings

The given equation for calculation of fluorescence polarization assumes that the sensitivity of the detection system is equivalent for parallel and perpendicular polarized light. This is generally not the case and either the parallel or perpendicular intensity must be corrected by so called 'G-Factor'. The G-factor compensates for differences in optical components between parallel and perpendicular measurement.

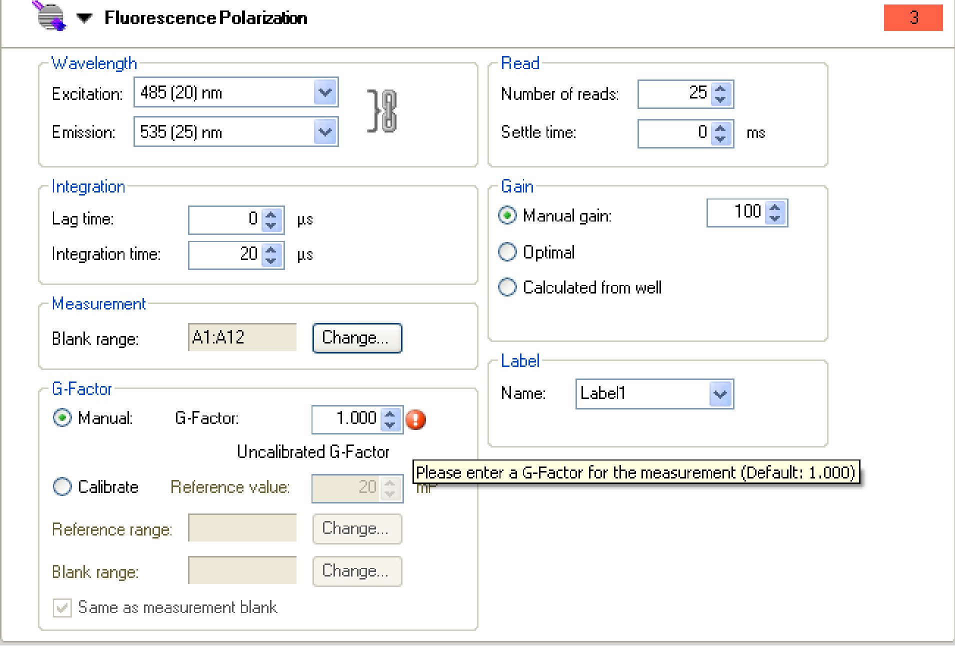
The G-Factor is the correction factor that can be determined for the wavelength of the fluorophore by measuring a sample with a known polarization value. A valid calibration of the instrument resulting in a G-factor is an important requirement for each fluorescence polarization measurement.

Caution
Make sure that the filter slide contains polarizers together with the filters defined for fluorescence polarization. Measurements without the polarizeres will result in a false G-Factor and false measurement data.

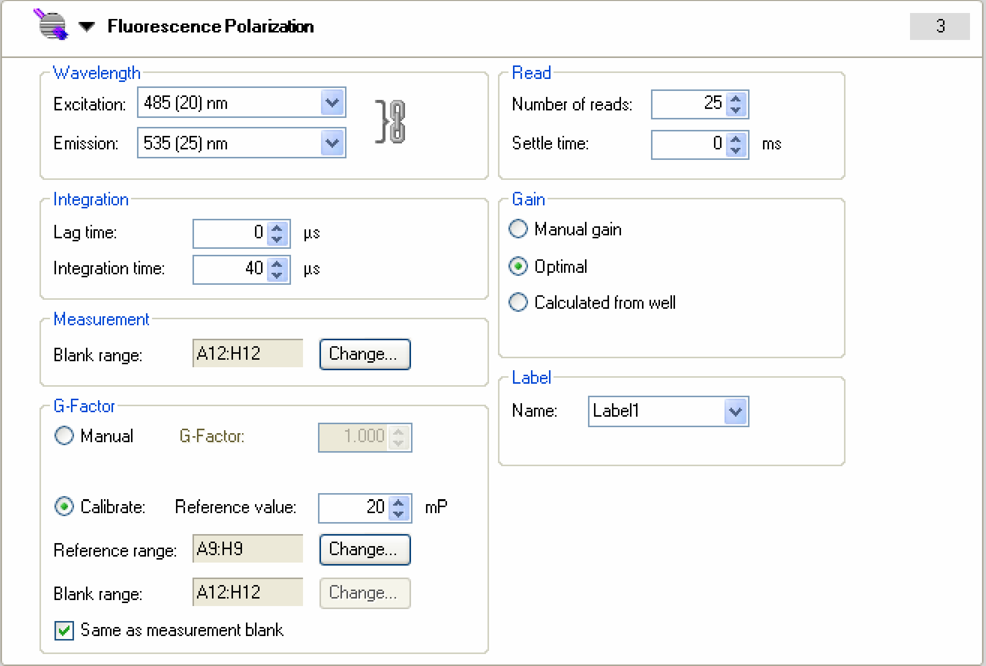
6.4.4 Measurement with an Uncalibrated G-Factor

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

For the G-Factor calibration see 6.4.5.



6.4.5 Measurement with a Simultaneous G-Factor Calibration



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

- **Reference value**: select a polarization value for the reference used, e.g. 20 mP for a 1 nm fluorescein solution in 0.01 M NaOH.
- **Reference range**: click **Change** and select the wells filled with the reference.
- **Blank range**: click **Change** and select the wells filled with the reference blank. Select **Same as measurement blank** if the reference blank is the same as the measurement blank.

Note

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

(Translation for German User)

Anmerkung:

Bei Verwendung von mehr als einem ‚Well' für die Positionen ‚Polarization Reference' und ‚Polarization Blank', werden für die Kalibration und für die weiteren Berechnungen die Mittelwerte herangezogen. Die Kalibration wird duch Mehrfachbestimmung genauer.

G-Factor Storage

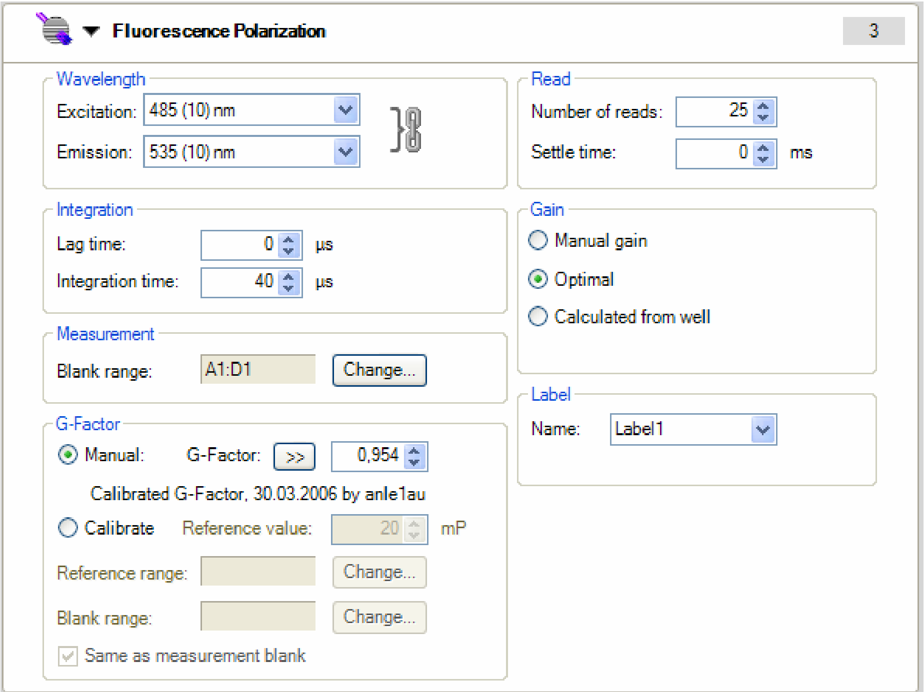
The calculated G-Factor is automatically stored on the computer's hard drive. Each G-Factor entry corresponds to the filter pair selection as well as the filter slide description. There is always only one G-Factor available for the respective filter pair combination and filter slide description, unless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

Caution
The filter slide description is part of the G-Factor key value. Avoid using the same filter slide description for different filter slides as this will affect the correct G-Factor recognition.

6.4.6 Measurement with a Calibrated G-Factor

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

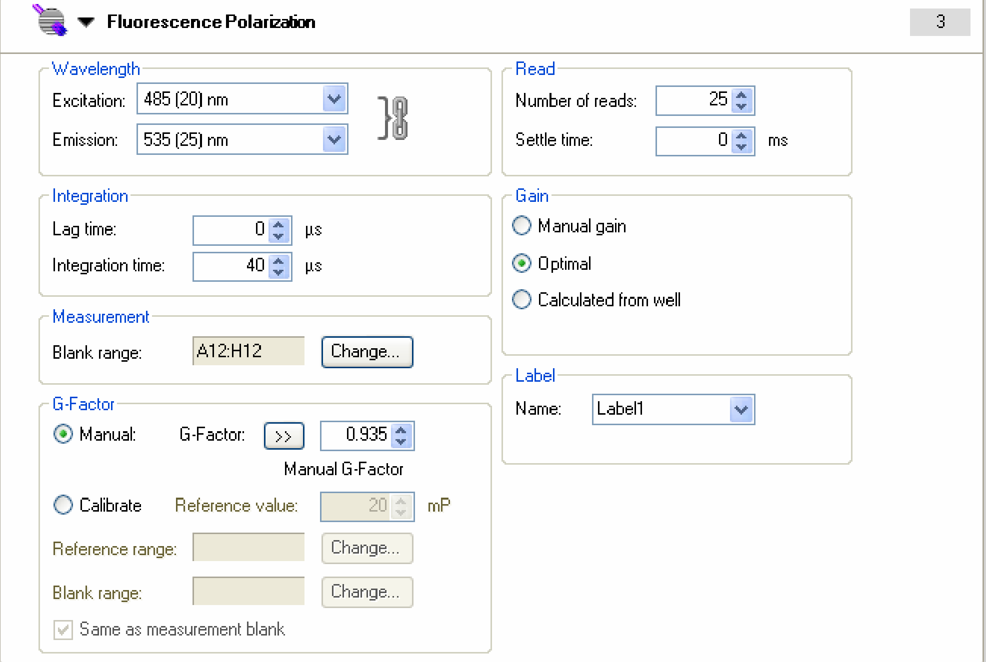
A calibrated G-factor will be displayed automatically or can be loaded by clicking the '>>' button only if it matches the selected fluorescence polarization filter pair and the filter slide description.



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

6.4.7 Measurement with a Manual G-Factor

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



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

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

6.4.8 Calculation of Fluorescence Polarization 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 [P]

$\overline{RFU}_{ref}$ ... Averaged relative fluorescence units of reference

$\overline{RFU}_{buf}$ ... Averaged relative fluorescence units of buffer

Blank Reduction:

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

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

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

Intensities:

Parallel and perpendicular intensities are calculated using the following formulas:

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

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

Polarization:

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

Anisotropy:

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

Total Intensity:

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

6.5 Optimize Absorbance Measurements

6.5.1 Measurement Parameters

Flash Settings

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

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

Settle Time

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

6.5.2 Absorbance Ratio Mode

Ratio Mode

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

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

Wavelength (infinite® M200)/Filter (infinite® F200) Switch Time

The **infinite® F200** can switch between two neighboring filters within 250 ms.

The **infinite® M200** can switch between two wavelengths in 150 ms. For conditions see 6.3.2 FI Ratio Mode.

6.6 Multiple Reads Per Well

The i-Control software allows multiple reads per well (MRW) to be performed in absorbance, fluorescence top and fluorescence bottom mode.

The multiple reads per well functions can be activated on an absorbance or fluorescence intensity program strip by selecting the 'Multiple reads per well' check box (see Figure 6-1 below).

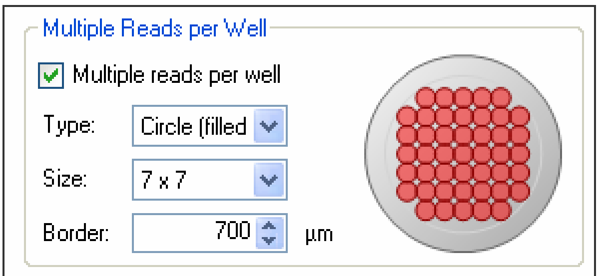


Figure 6-1: **Multiple reads per Well**

Note:
The function 'multiple reads per well' is only available for the fixed wavelength reading modes 'absorbance', 'fluorescence intensity top' and 'fluorescence intensity bottom'. The function is not available for scan measurements.

6.6.1 MRW Type

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

1) Square
2) Square (filled)
3) Circle
4) Circle (filled)
5) X-line
6) Y-line
7) XY-line

Pattern examples:

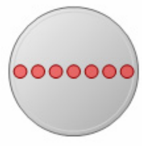
Square:

Square (filled):

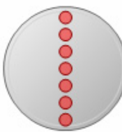
Circle:

Circle (filled):

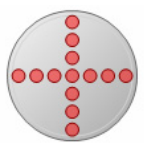
X-line:



Y-line:



XY-line:



6.6.2 MRW Size

The MRW size determines the number of points to be measured in a well. Depending on the microplate type and instrument (**infinite® F200** or **infinite® M200**) the 'size' is selectable from 1 x 1 to a maximum of 15 x 15 points. The diameter of the single measurement points corresponds to the theoretically calculated diameter of the light beam at the focal point (see Table 6.6-1).

Measurement Mode	M200	F200
Fluorescence Intensity Top	3 mm	2 mm
Fluorescence Intensity Bottom	2 mm	2 mm
Absorbance (microplate optics)	0.7 mm	0.5 mm

Table 6.6-1: Theoretically calculated beam diameter at the focal point.

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

6.6.3 MRW Border

In addition to 'Size' and 'Type', a 'Border' function allows the user to select a certain distance between light beam and the wall of the microplate well (distance in µm). As already stated in chapter 6.6.2, the software displays only a schematic overview of the measurement pattern. The border is calculated from the theoretical beam diameter of the instrument. However, when measuring liquid samples, the light beam diameter is influenced by the type and amount of liquid in a well.

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

Caution

All absorbance and fluorescence intensity specifications given in this document are only valid for single point measurements (one measurement point per well). When using the multiple reads per well option the specifications are not valid.

Caution

The software displays only a schematic view of the measurement pattern. Therefore optimize the multiple reads per well parameters for every new application. Make sure that the selected border is sufficient to avoid an overlap between the light beam and the well wall of the microplate.

Caution

A 'border' value that is too small may cause wrong measurement results due to overlap between the light beam and the well wall of the microplate.

6.6.4 Result Display in MS Excel®

The MS Excel® result sheet generated by the i-Control software displays a schematic graphical overview (‘Multiple Reads Per Well – Alignment’; see Figure 6-2) of the measurement points. A number is assigned to each measurement point. The results are presented in list form: number of measurement point versus result value (OD or RFU; see Figure 6-3 for result of a fluorescence measurement). In addition, the standard deviation (‘Stdev’) and the average value (‘Mean’) of the measurements points/well are also displayed:

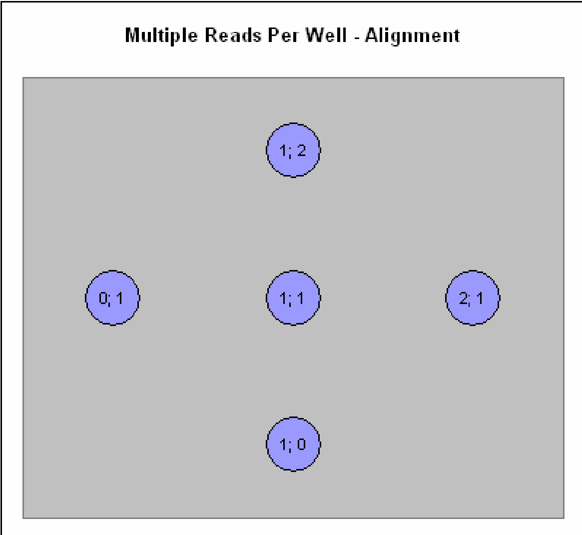
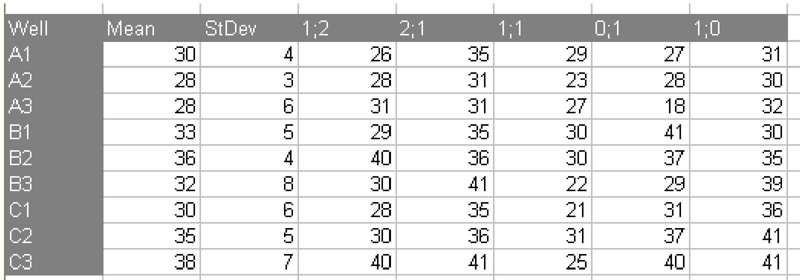


Figure 6-2: Alignment graphic (XY-Line, 3 x 3)



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

Figure 6-3: Example of MS Excel® result list generated by i-Control.

6.6.5 Miscellaneous Software Features of MRW

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

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

‘Reference Wavelength’ (located on the absorbance strip) is not available in combination with ‘Multiple Reads Per Well’.

6.7 Optimize Luminescence Measurements

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

6.7.1 Integration Time

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

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

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

6.7.2 Light Level Attenuation

When using photon counting detection, optical attenuation of higher luminescence light levels (> 10,000,000 RLU) is necessary. In such a case, too many photons entering the PMT 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 RLU (without attenuation) are marked as “INVALID” on the result sheet.

The **infinite® 200** hardware can attenuate light levels by a fixed factor of either 1 (none) or 10 (1 OD). Correspondingly, the usable measurement range will be shifted to higher light levels (< 100,000,000 RLU).

7. Instrument Features

7.1 Introduction

> ***Note***
> ***All specifications are subject to change without prior notification.***

The following types of measurement are provided with the fully equipped **infinite® 200** microplate reader:

Measurement Type	Description
Fluorescence Intensity Top/Bottom	See 7.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)
Fluorescence Time Resolved	See 7.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)
Fluorescence Polarization	See 7.4 Fluorescence Polarization (FP- **infinite® F200** only)
Absorbance	See 7.5 Absorbance
Glow Type Luminescence	See 7.6 Glow Type Luminescence
Dual Color Luminescence	See 7.8 Dual Color Luminescence (e.g. BRET ™)
Flash Type Luminescence	See 7.7 Flash Type Luminescence

- All standard microplates from 6 to 384-wells that conform to the following standards, can be measured in any of the above measurement types: ANSI/SBS 1-2004; ANSI/SBS 2-2004; ANSI/SBS 3-2004 and ANSI/SBS 4-2004.
- The instrument can perform kinetic measurements.
- Reading may be restricted to one part of the microplate.

7.2 Instrument Specifications

The table below lists the technical specifications of the instrument:

Parameters	Characteristics	
Measurement	Software controlled	
Interface	USB	
Filter Handling **infinite® F200**	*External filter exchange*	
Filter Handling **infinite® M200**	Monochromator-based wavelengths change – no filters necessary	
Microplates to be Measured	From 6-well to 384-well plates (SBS standard formats)	
Plate Definition	Via scanning software	
Temperature Control	From 5 °C above ambient up to 42 °C	
Plate Shaking	Linear and orbital shaking, amplitude selectable from 1 – 6 mm in 0.5 mm steps	
Parameters	**Characteristics**	
Light Source	High energy Xenon Flash lamp, life time: 10^8 flashes	
Optics	Fused Silica Lenses	
Fluorescence Detector	Standard: PMT Spectrally enhanced: red-sensitive PMT	
Luminescence Detector	Low dark count photomultiplier tube photon-counting electronics (Basic and Standard PMT)	
Absorbance Detector	Silicon photodiode	
Power Supply	Auto-sensing: 100 – 120 V/220 – 240 V, 50-60 Hz	
Power Consumption	150VA	
Physical		
Outer Dimensions Basic instrument	Width: 425 mm Height: 253 mm Depth: 457 mm	16.73 inches 9.96 inches 17.99 inches
Weight **infinite® F200**	14 kg	
Weight **infinite® M200**	15.8 kg	
Outer Dimensions Pump Module	Width: 250 mm Height: 155 mm Depth: 156 mm	9.84 inches 6.10 inches 6.14 inches
Weight Pump Module	3.4 kg	
Environmental		
Ambient Temperature		
Operation	+ 15 °C to + 30 °C	+ 59 °F to + 86°F
Non-operation	- 20 °C to + 60 °C	-4 °F to + 140 °F
Relative Humidity		
Operation	< 90 % non condensing	
Over-voltage Category	II	
Pollution Degree	2	
Usage	General Laboratory Instrument	
Noise Level	< 60 dBA	
Method of Disposal	Electronic waste (infectious waste)	

7.3 Fluorescence Intensity and Time Resolved Fluorescence (TRF)

Parameters	Characteristics
Wavelength Range - **infinite® M200**:	230 - 850 nm
	selectable in 1 nm steps
Wavelength Range - **infinite® F200**:	230 - 850 nm
Standard Filter:	Not applicable – instruments are equipped with user-defined filters

Parameters	Instrument type	Characteristics
Wavelength Range	standard	Excitation: 230 – 600 nm
	spectrally-enhanced	Excitation: 230 – 850 nm
	standard	Emission: 330 – 600 nm
	spectrally-enhanced	Emission: 280 – 850 nm

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

7.3.1 Definition of the Detection Limit

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

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

7.3.2 Fluorescein (Fluorescence Intensity) Top

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

7.3.3 Fluorescein (Fluorescence Intensity) Bottom

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

7.3.4 Europium (Time Resolved Fluorescence)

Plate Type (number of wells)	96
Dispensed Volume [µl]	200
Flashes (Reads) per Well	25
Europium Detection Limit (**infinite® F200**)	< 3 pM
Europium Detection Limit (**infinite® M200**)	< 5 pM (Typical value)

7.4 Fluorescence Polarization (FP- infinite® F200 only)

> ***Note***
> *The option 'Fluorescence Polarization' is only available for the infinite® F200. This module cannot be installed on a infinite® M200.*

Parameters	Instrument type	Characteristics
Wavelength Range	standard	Excitation: 300 – 600 nm
	spectrally-enhanced	Excitation: 300 – 850 nm
	standard	Emission: 330 – 600 nm
	spectrally-enhanced	Emission: 330 – 850 nm
Standard Filter	both	Configuration of Default filter slide: **Exc 485 (20) nm** Position on Exc filter slide: Exc1: 485 (20) - parallel Exc2: 485 (20) - perpendicular **Em 535 (25) nm** Position on Em filter slide: Em1: 535 (25) - parallel Em2: 535 (25) - parallel

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

FP Parameters	Characteristics
Integration Time	20 - 2000 µs
Lag Time	0 - 2000 µs
FP Precision (F200 only)	< 5 mP @ 1nM Fluorescein

7.5 Absorbance

Parameters	Instrument Type	Characteristics
Wavelength Range	**infinite® F200**	230 – 1000 nm
	infinite® M200	230 – 1000 nm no filter necessary, selection in 1 nm steps possible
Measurement Range	Both	0 – 3 OD

The following specifications are valid for the wavelength range from 300 – 700 nm for **infinite® 200** series.

Plate type (number of wells)	96
Accuracy 0 – 2 OD	< ± (1 % + 10 mOD)
Accuracy 2 – 3 OD	< ± 2.5 %
Baseline Flatness	± 10 mOD (1 sigma)
M200	
Wavelength Accuracy	≤ ± 1.5 nm λ > 315 nm; ≤ ± 0.8 nm λ ≤ 315 nm
F200	
Wavelength Accuracy	Dependent on filters used

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

7.6 Glow Type Luminescence

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	380 – 600 nm
Lin. Dynamic Range	6 orders of magnitude 7 orders of magnitude (extended dynamic range)
Integration Time/well	1 – 20000 ms
Cross Talk % (black plate)	< 0.01 %
Measurement range	> 6 orders of magnitude
Attenuation of Light	10, 1 (no attenuation)

7.6.1 ATP Glow Luminescence

Plate Type (number of wells)	96
Total Dispensed Volume[µl]	200
Integration Time/well [ms]	1000
ATP Detection Limit (Standard PMT)	3 fmol/well
ATP Detection Limit (Basic PMT)	20 fmol/well

Caution
Specifications are only valid when the instrument is placed in a location with illumination < 500 lux.

7.7 Flash Type Luminescence

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Luminescence Detection uses the photon counting technique.

Parameters	Characteristics
Wavelength Range	380 – 600 nm
Measurement Range	> 6 orders of magnitude
Integration Time/well	1 – 20000 ms
Cross talk % (black plate)	< 0.01 %
Attenuation of Light	10, 1 (no attenuation)
ATP Detection Limit (Standard PMT)	< 80 amol/well
ATP Detection Limit (Basic PMT)	< 500 amol/well

Caution

Specifications are only valid when the instrument is placed in a location with illumination < 500 lux.

7.8 Dual Color Luminescence (e.g. BRET ™)

Caution

Switch on the instrument at least 15 minutes before starting a luminescence measurement. Some components need to warm up to guarantee stable conditions for the measurement.

Parameters	Characteristics
Built-in Wavelength:	See 5.7 Luminescence System
Integration Time:	1 - 20000 ms. Different integration times are possible for each wavelength.
Plate Type:	96 and 384-well microplates
Dynamic Range	6 decades

7.9 "On the Fly" Measurements

"On the Fly" measurements are the fastest measurements possible using the **infinite® 200**. These measurements are performed using only one flash (number of flashes).

- 96-well plates (FI, TRF, Absorbance) Measurement time: 20 s
- 384-well plates (FI, TRF, Absorbance) Measurement time: 30 s

(Plate-in/out movement not included).

7.10 Cuvette Measurements (infinite® M200 only)

Note
The option 'Cuvette' is only available for the infinite® M200. This module cannot be installed on a infinite® F200.

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

Parameters	Characteristics
Wavelength Range	230 – 1000 nm no filter necessary, selection in 1 nm steps possible
Measurement Range	0 – 3 OD

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

Cuvette types	12.5 x 12.5 mm, maximum height 55 mm (including lid); micro cuvettes: center height 15 mm, measurement window > 2 mm (diameter).
Accuracy 0 – 2 OD	< ± (1 % + 10 mOD)
Accuracy 2 – 3 OD	< ± 2.5 %
Uniformity at 1 OD	< 3 % CV
Reproducibility 0 – 2 OD	< ± (1 % + 10 mOD)
Reproducibility 2 – 3 OD	< ± 2.5 %
Linearity 0 – 2 OD	R^2 > 0.998
Baseline Flatness	± 10 mOD (1 sigma)

Caution

The cuvette port of infinite® M200 cannot be used for cuvettes with a measurement window < 2 mm (diameter) and a center height below 15 mm.

7.11 Injector

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

8. Quality Control

8.1 Periodic Quality Control Tests

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

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

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

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

Caution

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

Caution

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

8.2 Specifications - Passed/Failed Criteria

Note
All specifications are subject to change without prior notification.

The following table gives an overview of the passed/failed criteria for the specification test of the **infinite® 200**.

Specification Microplate	Passed/Failed Criteria
Fluorescence Top Sensitivity	< 20 pM Fluorescein
Fluorescence Top Uniformity	< 3 % CV
Fluorescence Top Precision	< 2 % CV
Fluorescence Bottom Sensitivity	< 100 pM Fluorescein
Fluorescence Bottom Uniformity	< 3 % CV
Fluorescence Bottom Precision	< 2 % CV
Time Resolved Fluorescence Sensitivity (F200)	< 3 pM
Time Resolved Fluorescence Precision (F200)	< 2 % CV
FP Precision (F200 only)	< 5 mP
Luminescence Sensitivity Glow Type	< 3 fmol/well (Standard PMT)
Luminescence Sensitivity Glow Type	< 20 fmol/well (Basic PMT)
Luminescence Sensitivity Flash Type	< 80 amol/well (Standard PMT)
Luminescence Sensitivity Flash Type	< 500 amol/well (Basic PMT)
Absorbance Accuracy	0 – 2 OD: ≤ ± 1 % + 10 mOD 2 – 3 OD: ≤ ± 2.5 %
Absorbance Baseline Flatness (1 sigma)	< ± 10 mOD
Absorbance Wavelength Accuracy	≤ ± 1.5 nm λ > 315 nm; ≤ ± 0.8 nm λ ≤ 315 nm

Specification Cuvette (M200 only)	Passed/Failed Criteria
Absorbance Accuracy	0 – 2 OD: ≤ ± 1 % + 10 mOD 2 – 3 OD: ≤ ± 2.5 %
Absorbance Baseline Flatness (1 sigma)	< ± 10 mOD

8.3 Specifications - Test Instructions

8.3.1 Fluorescence Top

For the **infinite® 200** with the option 'Fluorescence Top', the following tests can be performed to prove the specifications:

- Sensitivity
- Uniformity
- Precision

These test instructions are valid for the **infinite® 200**:

- **infinite® F200**
- **infinite® M200**
- Standard version
- Spectrally enhanced version

Sensitivity

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

Measurement Parameters:

Parameter	**Setting**
Reading Mode	Fluorescence Top
Exc Wavelength	**infinite® F200**: 485 (20 nm) **infinite® M200**: 485 nm
Em Wavelength	**infinite® F200**: 535 (25 nm) **infinite® M200**: 535 nm
Number of flashes	25
Integration Time	40
Settle Time	0
Gain	Optimal
Plate Type	GRE96fb

Plate Layout:

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

<table>
<tr><th><></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td></tr>
<tr><td>D</td></tr>
<tr><td>E</td></tr>
<tr><td>F</td></tr>
<tr><td>G</td></tr>
<tr><td>H</td></tr>
</table>

Material/Reagents:

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

Calculation of Detection Limit (Sensitivity):

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

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

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

Uniformity

Perform the following measurement to determine the Uniformity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Exc Wavelength	**infinite® F200**: 485 (20 nm) **infinite® M200**: 485 nm
Em Wavelength	**infinite® F200**: 535 (25 nm) **infinite® M200**: 535 nm
Number of flashes	25
Integration Time	40
Settle Time	0
Gain	Optimal
Plate Type	GRE96fb

Plate Layout:

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

<table>
<tr><th><></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td></tr>
<tr><td>D</td></tr>
<tr><td>E</td></tr>
<tr><td>F</td></tr>
<tr><td>G</td></tr>
<tr><td>H</td></tr>
</table>

Material/Reagents:

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

Calculation of Uniformity:

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

mean_F Average RFU value of wells filled with fluorophore
stdev_F Standard deviation of RFU values of wells filled with fluorophore
The result of the formula determines the uniformity in % CV.

Precision

Perform the following measurement to determine the precision/reproducibility:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Exc Wavelength	**infinite**® **F200**: 485 (20 nm) **infinite**® **M200**: 485 nm
Em Wavelength	**infinite**® **F200**: 535 (25 nm) **infinite**® **M200**: 535 nm
Number of flashes	25
Integration Time	40
Settle Time	0
Gain	Optimal
Plate Type	GRE96fb
Part of the Plate	A1
Kinetic	20 Cycles
Interval Time	Minimal

Plate Layout:

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

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

Material/Reagents:

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

Calculation of Precision:

$$\mathrm{Precision(CV\%)} = \frac{\mathrm{stdev}_{\mathrm{wellA1}} * 100}{\mathrm{mean}_{\mathrm{wellA1}}}$$

$\mathrm{mean}_{\mathrm{wellA1}}$ Average RFU value of well A1 over the 20 kinetic
$\mathrm{stdev}_{\mathrm{wellA1}}$ Standard deviation of RFU values of Well A1 over the 20 cycles
The result of the formula determines the Precision in % CV.

8.3.2 Fluorescence Bottom

For the **infinite® 200** with the option 'Fluorescence Bottom' the following tests may be performed to prove the specifications:

- Sensitivity
- Uniformity
- Precision/Repeatability

These test instructions are valid for the **infinite® 200**:

- **infinite® F200**
- **infinite® M200**
- Standard version
- Spectrally enhanced version

Sensitivity

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

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Exc Wavelength	**infinite® F200**: 485 (20 nm) **infinite® M200**: 485 nm
Em Wavelength	**infinite® F200**: 535 (25 nm) **infinite® M200**: 535 nm
Number of flashes	25
Integration Time	40
Settle Time	0
Gain	Optimal
Plate Type	GRE96fb

Plate Layout:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A–H	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank

Material/Reagents:

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

Calculation of Detection Limit (Sensitivity):

See 8.3.1 Fluorescence Top: Sensitivity.

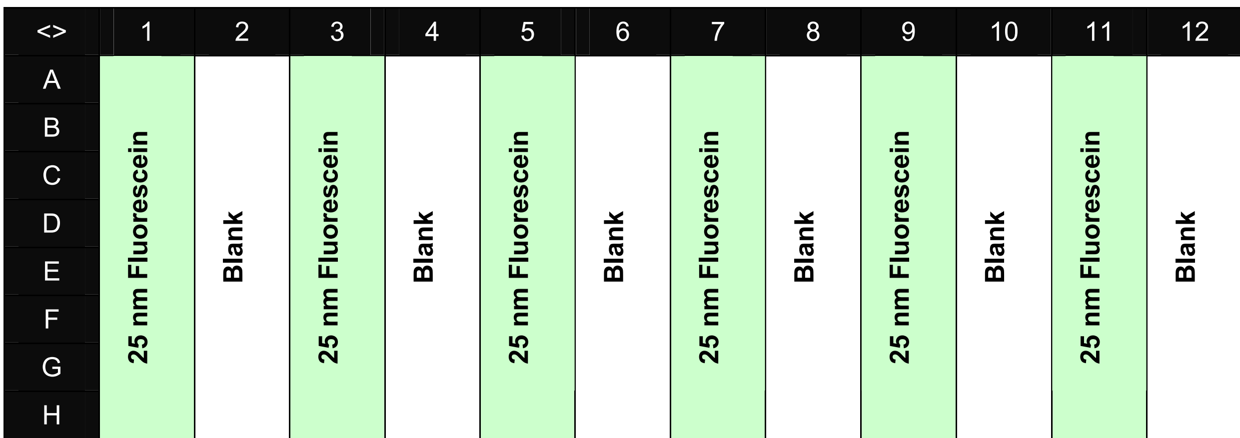
Uniformity

Perform the following measurement to determine the Uniformity:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Exc Wavelength	**infinite® F200**: 485 (20 nm) **infinite® M200**: 485 nm
Em Wavelength	**infinite® F200**: 535 (25 nm) **infinite® M200**: 535 nm
Number of flashes	25
Integration Time	40
Settle Time	0
Gain	Optimal
Plate Type	GRE96fb

Plate Layout:



Filling volume: 200 µl

Material/Reagents:

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

Calculation of Uniformity:

See 8.3.1 Fluorescence Top: Uniformity

Precision

Perform the following measurement to determine the precision/reproducibility:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Bottom
Exc Wavelength	**infinite® F200**: 485 (20 nm) **infinite® M200**: 485 nm
Em Wavelength	**infinite® F200**: 535 (25 nm) **infinite® M200**: 535 nm
Number of flashes	25
Integration Time	40
Settle Time	0
Gain	Optimal
Plate Type	GRE96fb
Part of the Plate	A1
Kinetic	20 Cycles
Interval Time	Minimal

Plate Layout:

<>	1	2	3	4	5	6	7	8	9	10	11	12
A–H	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank	25 nm Fluorescein	Blank

Filling volume: 200 µl

Material/Reagents:

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

Calculation of Precision:

See 8.3.1 Fluorescence Top: Precision.

8.3.3 *Time Resolved Fluorescence*

For the **infinite**® **200** with the option 'Fluorescence Top' the following tests may be performed to prove the specifications:

- Sensitivity
- Precision/Repeatability

These test instructions are valid for the **infinite**® **200**:

- **infinite**® **F200**
- Spectrally enhanced version

Sensitivity

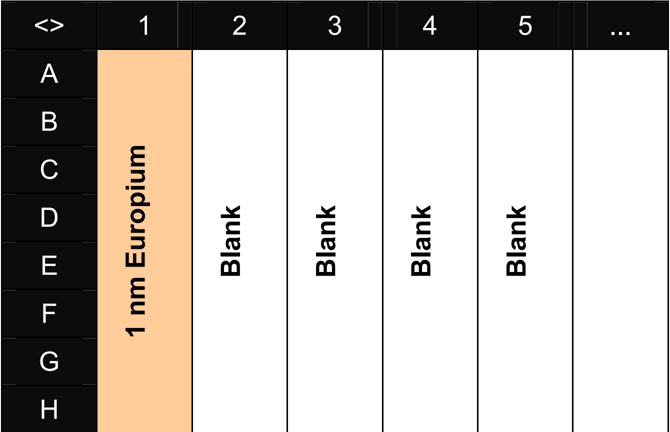
Perform the following measurement to determine the sensitivity:

Measurement Parameters:

Parameter	**Setting**
Reading Mode	Fluorescence Top
Exc Wavelength	**infinite**® **F200**: 340 nm (35) **infinite**® **M200**: 340 nm
Em Wavelength	**infinite**® **F200**: 612 nm (10 nm) **infinite**® **M200**: 617 nm
Number of flashes	25
Integration Time	400
Lag Time	100
Settle Time	0
Gain	Optimal
Plate Type	GRE96fw

Plate Layout:

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



Material/Reagents:

1 nm Europium (B119-100, HVD Live Sciences)
Enhancement Solution (=Blank) (1244-105, HVD Live Sciences)
1 Greiner 96-well plate white
200 µl Pipette + tips

Calculation of Detection Limit (Sensitivity):

See 8.3.1 Fluorescence Top: Sensitivity.

Precision

Perform the following measurement to determine the precision/reproducibility:

Measurement Parameters:

Parameter	Setting
Reading Mode	Fluorescence Top
Exc Wavelength	**infinite® F200**: 340 nm (35) **infinite® M200**: 340 nm
Em Wavelength	**infinite® F200**: 612 nm (10 nm) **infinite® M200**: 617 nm
Number of flashes	25
Integration Time	400
Lag Time	100
Time between Move and Flash	0
Gain	Optimal
Plate Type	GRE96fw
Part of the plate	A1
Kinetic	20 Cycles
Interval Time	Minimal

Plate Layout:

See 8.3.3 Time Resolved Fluorescence Precision.

Calculation of Precision:

See 8.3.1 Fluorescence Top: Precision.

8.3.4 Fluorescence Polarization (infinite® F200 only)

For the **infinite® F200** with the option 'Fluorescence Polarization' the following tests may be performed to prove the specifications:

- Precision/Repeatability

These test instructions are valid for the **infinite® F200**:

- Standard version
- Spectrally enhanced version

Precision

Perform the following measurement to determine the precision:

Measurement Parameters:

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

Plate Layout:

<table>
<tr><th><></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td><td rowspan="8">1 nm Fluorescein</td><td rowspan="8">Blank</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td></tr>
<tr><td>D</td></tr>
<tr><td>E</td></tr>
<tr><td>F</td></tr>
<tr><td>G</td></tr>
<tr><td>H</td></tr>
</table>

Filling volume: 200 µl/well

Material/Reagents:

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

Calculation of Precision:

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

8.3.5 Glow Luminescence

For the **infinite**® **200** with the option 'Luminescence the following tests may be performed to prove the specifications:

- Sensitivity

Sensitivity

Perform the following measurement to determine the Sensitivity:

Measurement Parameters:

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

Plate Layout:

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

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

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

Material/Reagents:

BioThema ATP Kit (ATP-Kit SL 144-041, BioThema AB)
1 Greiner 96-well plate white
200 µl Pipette + tips

Calculation of the Sensitivity (Detection Limit):

$$\text{DetectionLimit}(\text{fmol}/\text{well}) = \frac{2 \cdot 10^{-8} * 3 * \text{Stdev}_B}{\text{mean}_{ATP} - \text{mean}_B} * 0.0002 * \frac{1}{1e^{-15}}$$

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

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

8.3.6 Absorbance Accuracy

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

8.3.7 Absorbance Wavelength Accuracy

The wavelength accuracy defines the deviation of the set measurement wavelengths from the nominal wavelength. This test is only valid for the **infinite® M200**.

Measurement Parameters:

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

Material/Reagents:

MultiCheck plate

Calculation of Wavelength Accuracy:

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

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

Max_t theoretical Maximum
Max_m measured Maximum

8.3.8 Absorbance Baseline Flatness (infinite® M200)

Perform the following measurement to determine the baseline-flatness:

Measurement Parameters:

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

Plate Layout:

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

Material/Reagents:

No material or reagents necessary for this test.

Calculation of Baseline Flatness:

Calculate the standard deviation.

8.3.9 Absorbance Baseline Flatness (infinite® F200)

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

Measurement Parameters:

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

Plate Layout:

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

Material/Reagents:

No material or reagents necessary for this test.

Calculation of Baseline Flatness:

Calculate the standard deviation over 20 cycles for each wavelength.

8.3.10 Absorbance Cuvette (*infinite*® M200 only)

For the **infinite**® **M200** with the option 'Cuvette' the following test may be performed to prove the accuracy specification:

Accuracy

Perform the following measurement to determine the absorbance accuracy:

Measurement Parameters:

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

Material:

Starna® reference material RM-N1N35N + D3 cuvette
(for more information please refer to www.starna.co.uk)

Calculation of Accuracy:

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

9. Cleaning and Maintenance

9.1 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.

This section contains the following procedures:

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

WARNING

ALL PARTS OF THE INSTRUMENT THAT COME INTO CONTACT WITH POTENTIALLY INFECTIOUS MATERIAL MUST BE TREATED AS POTENTIALLY INFECTIOUS AREAS.

IT IS ADVISABLE TO ADHERE TO APPLICABLE SAFETY PRECAUTIONS, (INCLUDING THE WEARING OF POWDER-FREE GLOVES, SAFETY GLASSES AND PROTECTIVE CLOTHING) TO AVOID POTENTIAL INFECTIOUS DISEASE CONTAMINATION WHEN PERFORMING CLEANING PROCEDURES AND ALSO WHEN MAKING ADJUSTMENTS TO THE INSTRUMENT.

9.2 Liquid Spills

1. Switch OFF the instrument.
2. Wipe up the spill immediately with absorbent material.
3. Dispose of contaminated material appropriately.
4. Clean the instrument surfaces with a mild detergent.
5. For biohazardous spills clean with B30 (Orochemie, Max-Planck-Str. 27, D-70806 Kornwestheim).
6. Wipe cleaned areas dry.

WARNING

ALWAYS SWITCH-OFF THE INSTRUMENT BEFORE REMOVING ANY KIND OF SPILLS ON THE INSTRUMENT. ALL SPILLS MUST BE TREATED AS POTENTIALLY INFECTIOUS. THEREFORE, ALWAYS ADHERE TO APPLICABLE SAFETY PRECAUTIONS, (INCLUDING THE WEARING OF POWDER-FREE GLOVES, SAFETY GLASSES AND PROTECTIVE CLOTHING) TO AVOID POTENTIAL INFECTIOUS DISEASE CONTAMINATION.

ADDITIONALLY, ALL RESULTING WASTE FROM THE CLEAN-UP MUST BE TREATED AS POTENTIALLY INFECTIOUS AND THE DISPOSAL MUST BE PERFORMED ACCORDING TO THE INFORMATION GIVEN IN CHAPTER 9.5 DISPOSAL.

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

WARNING

ENSURE THAT THE MICROPLATE IS REMOVED FROM THE INSTRUMENT BEFORE IT IS PREPARED FOR SHIPMENT.IF A MICROPLATE IS LEFT IN THE INSTRUMENT, FLUORESCENT SOLUTIONS MAY SPILL ONTO THE OPTICAL PARTS AND DAMAGE THE INSTRUMENT.

9.3 Instrument Disinfection

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

WARNING

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

WARNING

ALL PARTS OF THE INSTRUMENT THAT COME INTO CONTACT WITH POTENTIALLY INFECTIOUS MATERIAL MUST BE TREATED AS POTENTIALLY INFECTIOUS AREAS.

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

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

9.3.1 Disinfection Solutions

The instrument should be disinfected using the following solution:

- B30 (Orochemie, Max-Planck-Str. 27; D-70806 Kornwestheim)

9.3.2 Disinfection Procedure

WARNING

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

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

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

See 9.4 Disinfection Certificate for an example of the disinfection certificate, which must be completed before the instrument is returned to the service center for service or repair.

9.4 Disinfection Certificate

A disinfection certificate label **MUST** be completed and attached to the top of the package **(visible from the outside of the shipping container!)** in which the instrument is returned, before shipping it to the service center for service or repair.

The instrument **MUST** be disinfected at the operating authority's site.

The disinfection procedure must be performed in a well-ventilated room by authorized and trained personnel wearing disposable powder-free gloves, protective glasses and protective clothing.

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

I declare that the instrument in this package has been decontaminated or disinfected to remove or inactivate any biological material, which could be dangerous to service personnel, or that it has never been exposed to any hazardous biological material.
Contact person ....
Company: ....
Function: ....
Phone/Fax: ....
E-mail: ....
Date of decontamination: ....
Method of decontamination applied: ....
....
Date: ....
Signature: ....

9.5 Disposal

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

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

Caution

Observe all federal, state and local environmental regulations.

ATTENTION

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

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

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

9.5.1 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.

9.5.2 Disposal of Operating Material

WARNING

BIOLOGICAL HAZARDS CAN BE ASSOCIATED WITH THE WASTE MATERIAL (MICROPLATE) OF PROCESSES RUN ON THE infinite® 200.

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.

9.5.3 Disposal of the Instrument

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

Caution

Always disinfect the instrument before disposal.

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

WARNING

DEPENDING ON THE APPLICATIONS, PARTS OF THE HYDROFLEX PLATFORM MAY HAVE BEEN IN CONTACT WITH BIOHAZARDOUS MATERIAL.

- **MAKE SURE TO TREAT THIS MATERIAL ACCORDING TO THE APPLICABLE SAFETY STANDARDS AND REGULATIONS.**
- **ALWAYS DECONTAMINATE ALL PARTS BEFORE DISPOSAL (I.E. CLEAN AND DISINFECT).**

10. Troubleshooting

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

Error #	Error Text	Description
17	z drive init error	Hardware Failure z-drive Module. Please report this error to your local Tecan customer support office.
18	Injector A not available	Hardware Failure Injector A. Please report this error to your local Tecan customer support office.
19	Injector B not available	Hardware Failure Injector A. Please report this error to your local Tecan customer support office.
20	Injector Init Error:	Hardware failure Injector Module. Please report this error to your local Tecan customer support office.
21	Invalid Command: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
22	Invalid Operand: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
23	Invalid Command Sequence: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
24	N/A	N/A
25	Injector not init.: [cmd]	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
26	Plunger Overload:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
27	Valve Overload:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
28	Plunger Move not allowed:	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
29	Command Overflow	Unspecific internal communication error. Please report this error to your local Tecan customer support office.
30	Prepare: [s]: Gain:[g], Counts: [cts]	Unspecific Hardware failure. Please report this error to your local Tecan customer support office.
31	[ERR] at module [mod] (cmd:[cmd])	Unspecific Hardware failure. Please report this error to your local Tecan customer support office.
32	"MTP is in Out-Position",	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
33	[val] ... not set at (Ratiolabel [n])	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
34	Injectors are not enabled	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
35	Invalid Parameter Length (max: [n] char allowed)	Unspecific error in the Instrument - Computer communication protocol. Please report this error to your local Tecan customer support office.
36	Checksum Error	Communication Error on USB interface. Please report this error to your local customer support office.
37	Init Error at module [mod#]	Unspecific Hardware Failure. Please report this error to your local Tecan customer support office.

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

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