

## LanthaScreen® TR-FRET Assay

### Implementation on Tecan's Infinite® F200 Multimode Reader

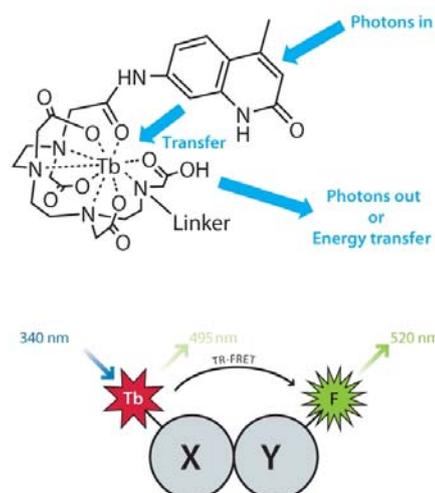


#### Introduction

This Technical Note describes the successful implementation of Invitrogen's LanthaScreen TR-FRET assay system on Tecan's Infinite F200 filter-based multi modular detection system. The Infinite F200 has performed according to Invitrogen's LanthaScreen certification program criteria and was successfully validated by Invitrogen as "LanthaScreen® Certified". Attached are recommended instrument settings to maximize assay performance.

#### Principle of LanthaScreen assay technology

The LanthaScreen assay technology is a powerful TR-FRET based technique developed by Invitrogen aiming to study a number of protein kinases, nuclear receptors, proteases and other cellular targets [1]. LanthaScreen TR-FRET assays are based on the energy transfer between two fluorescent labels, a long-lifetime lanthanide chelate as donor fluorophore, namely Terbium (Tb) and fluorescein or GFP as acceptor species (figure 1). The long lasting fluorescence of terbium enables to measure the FRET signal after all short-living background fluorescence has disappeared, resulting in high signal to blank (S/B) ratios. Furthermore TR-FRET technology offers a possibility to correct for differences in assay volume and concentration between wells as well as quenching effects caused by colored compounds.



**Figure 1:** LanthaScreen TR-FRET assay principle (1)

### **Infinite F200 – New horizon in microplate detection**

The Infinite 200 series is an easy to use and affordable instrument, available with a monochromator or a filter system. It offers the choice of different detection modes to suit individual needs and is designed with a modular concept, which allows later upgrades and integration with other systems.

Beside its high sensitivity in fluorescence-based technologies, which includes fluorescence intensity top (FI top) and fluorescence intensity bottom (FI bottom), fluorescence polarisation (FP), time resolved fluorescence (TRF), fluorescence resonance energy transfer (FRET) and time-resolve fluorescence resonance energy transfer (TR-FRET), the Infinite F200 offers a series of other capabilities, like the possibility to measure all kind of absorbance-based applications, the compatibility with Tecan's low volume DNA-quantitation tool, the NanoQuant Plate™, a 2-syringe-based injector module and a high-performance photo counting luminescence module, offering the possibility to measure glow and flash luminescence, as well as dual colour luminescence (figure 2).

Infinite F200 – Filter	
	Fluorescence intensity top reading incl Time Resolved Fluorescence
	Fluorescence intensity bottom reading incl Time Resolved Fluorescence
	Spectrally enhanced photo multiplier tube
	Absorbance
	Photon counting luminescence incl dual color luminescence
	Fluorescence polarization
	Temperature control
	Injectors
	NanoQuant Plate™

**Figure 2:** Infinite F200 – major instrument features.

Together with Tecan's unique system-modularity the Infinite F200 represents an inexpensive excess also to sophisticated assay systems, which are usually used in drug discovery applications.

In contrast to other detection systems, the Infinite F200 offers an affordable alternative to high-end instrumentation and provides a satisfying and straightforward solution for measuring LanthaScreen TR-FRET technology in various plate formats and sample volumes. The ultra-flexible and intuitive Tecan i-control™ software, which features an easy-to-use software interface that allows the user to define a specific workflow for each application, emphasizes the comprehensive and outstanding user friendliness.

## Material and methods

### **Instrument**

- Infinite F200 filter-based multimode reader

### **Microplates**

- Costar® 384 well low volume, white, flat bottom (Corning, USA)

### **Reagents**

- LanthaScreen Tb Instrument Control Kit

### **Sample Preparation**

Sample preparation was performed according to LanthaScreen Tb Instrument Control Kit Protocol (Catalog No. PV5591).

**Measurement settings**

Measurement Parameter	Donor Emission	Acceptor Emission
Mode	Fluorescence Top Reading	Fluorescence Top Reading
Excitation Wavelength	340 nm	340 nm
Excitation Bandwidth	35 nm	35 nm
Emission Wavelength	495 nm	520 nm
Emission Bandwidth	10 nm	10 nm
Gain	optimal	optimal
Number of Flashes	25	25
Integration Time	200 µs	200 µs
Lag Time	100 µs	100 µs
Settle Time	0 ms	0 ms

**Table 1:** Optimized instrument settings for LanthaScreen TR-FRET Technology on Infinite F200.

**Data Analysis**

Please refer questions regarding the LanthaScreen technology and data analysis directly to Invitrogen [3].

## Results and Discussion

To validate the performance of the Infinite F200 for LanthaScreen Technology the LanthaScreen TR-FRET instrument control kit was used. This kit was designed for the calibration of LanthaScreen certified readers as well as for the validation / certification of their compatibility with LanthaScreen technology [2]. For details concerning assay procedure and data evaluation please refer to the corresponding manual: LanthaScreen Tb Instrument Control Kit Protocol (Catalog No. PV5591).

A series of experiments in low-volume, white Costar 384 well plates have been performed in order to calibrate and fine-tune the measurement settings for the Infinite F200. The corresponding results are presented in table 1.

It has to be noted, that the LanthaScreen Certified status implicates the use of *white* microplates for all LanthaScreen assays. Therefore, Tecan recommends the use of white microplates, but in terms of assay volume and plate format, no compromise must be made, which means that all sample volumes between 20 µl and 200 µl in a number of different plate formats can be used.

Please refer question regarding instrument performance concerning to LanthaScreen technology directly to Tecan Austria [4].

## Conclusion

This Technical Note describes the instrument settings for successful performance of Invitrogen’s LanthaScreen technology on Tecan’s Infinite F200 filter-based multimode detection system. All validation/certification experiments have been performed at Invitrogen (Madison /USA) with the result, that the Infinite F200 has performed according to the assay criteria and was successfully validated and certified by Invitrogen as “LanthaScreen® Certified” [2].



## Literature

[1] [www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/DD-Misc/instrument-compatibility-portal/tecan.html](http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/DD-Misc/instrument-compatibility-portal/tecan.html)

[2] [www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/DD-Misc/instrument-compatibility-portal/tecan.html](http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/DD-Misc/instrument-compatibility-portal/tecan.html)

[3] [expertline-at@tecan.com](mailto:expertline-at@tecan.com)

[4] [expertline-at@tecan.com](mailto:expertline-at@tecan.com)

## List of Abbreviations

FI	fluorescence intensity
FP	fluorescence polarization
FRET	fluorescence resonance energy transfer
Tb	Terbium
TRF	time resolved fluorescence
TR-FRET	time resolved fluorescence resonance energy transfer

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