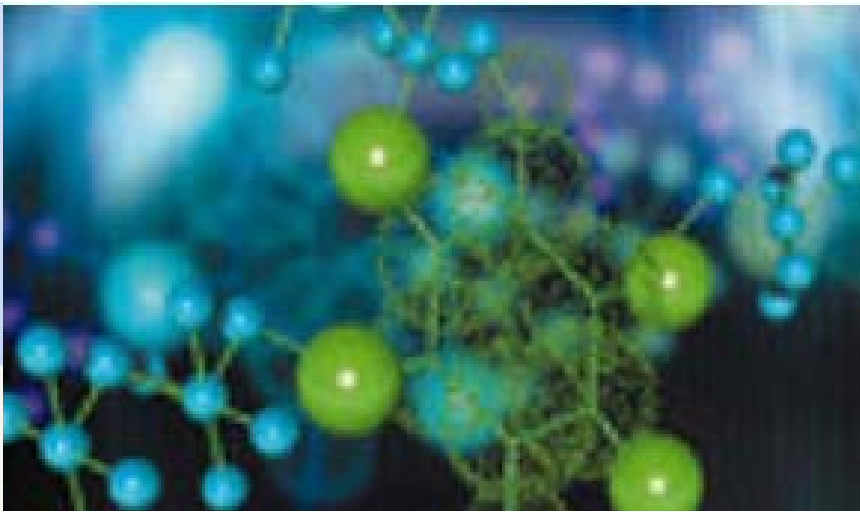
Technical Note

LanthaScreen™ TR-FRET Assay

Implementation on Tecan's Infinite® F500 Multimode Reader



Introduction

The Infinite® F500 is Tecan´s most sensitive filter-based multimode microplate reader and has been granted **LanthaScreen™ Certified Plus** status by Invitrogen.

In this technical note we describe the instrument settings on the basis of experiments with the LanthaScreen™ TR-FRET Control Kit on Tecan´s Infinite F500 multifunctional detection system.

Assay principle

Time resolved fluorescence (TRF) measurement techniques, using long-lifetime fluorophores, e.g. lanthanides as labelling species, have become very popular for many research and pharmacological applications. TRF permits an efficient reduction of background signals e.g. from autofluorescent compounds or scattered light by time gated signal detection (1). *Fluorescence resonance energy transfer* (FRET) is a technique based on the following principles: when a suitable pair of fluorophores (the so called FRET pair) is brought into close proximity (1-10 Å) of one another, excitation of the donor-fluorophore results in a transfer of energy to the acceptor-fluorophore, resulting in an increase of the acceptor emission signal and a decrease in donor-emission. The LanthaScreen™ TR-FRET (*time resolved fluorescence resonance energy transfer*) assay is based on the energy transfer between two fluorescent labels, the long-lifetime terbium lanthanide chelate as donor fluorophore and a common short-lifetime fluorophore (i.e. Fluorescein) as acceptor dye.

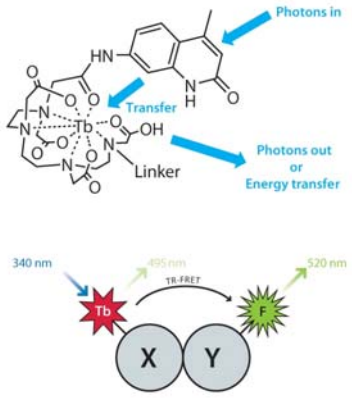


Figure 1: LanthaScreen™ TR-FRET assay principle (3)

Material and Methods

Instrument

Tecan Infinite F500 filter-based multimode microplate reader equipped with a fluorescence intensity top module and the relevant filters (Tecan Austria)

Microplates

384 well micro plate, flat bottom, black, polystyrol (Corning®, NY, USA)

Reagents

LanthaScreen™ TR-FRET Control Kit (Invitrogen, Carlsbad, USA), please refer inquiries related to reagents directly to Invitrogen

Assay procedure

The assay was performed as described in the LanthaScreen™ TR-FRET Control Kit manual (2)

Instrument Settings

Measurement parameter	Donor settings	Acceptor settings
Plate	[COS384fb.pdfx]	[COS384fb.pdfx]
Part of Plate	A1-C24	A1-C24
Mode	Fluorescence Top	Fluorescence Top
Excitation Wavelength	340 [35] nm	340 [35] nm
Emission Wavelength	495 [10] nm	520 [25] nm
Gain	optimal	optimal
Number of Flashes	10	10
Integration Time	200 µs	200 µs
Lag Time	100 µs	100 µs
Settle Time	0 ms	0 ms
Z-Pos.	cal. from well	cal. from well

Table 1: Measurement parameters for Infinite® F500

Further experiments with a variety of different filter sets at Tecan revealed that due to the high sensitivity of the Infinite F500 the following filters, i.e. excitation 340 [35], emission 495 [10] and emission **520 [10]**, can be recommended from Tecan´s filter product list to be used for LanthaScreen™ measurements on the Infinite F500.

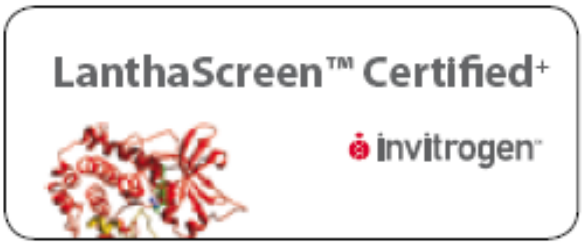
Data Analysis

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

Results and Conclusion

This technical note describes the instrument settings for successful performance of Invitrogen´s LanthaScreen™ TR-FRET Control Kit on the Tecan´s Infinite F500 filter-based multimode microplate reader. The validation/certification experiments have been performed at Invitrogen (Madison, USA).

With regard to the obtained measurement data, the Infinite F500 has performed according to the high level assay criteria and was successfully validated and certified by Invitrogen as “LanthaScreen™ Certified Plus”.



Acknowledgement

We express our acknowledgements to Mark Koeppel, PhD, Gary Prescott, PhD and Kevin Lowitz from Invitrogen (Madison, USA), who provided the validation and certification support for the Infinite F500.

Literature

[1] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, chapter 1, page 1-25, Springer Science & Business Media, 3rd edition, 2006

[2] LanthaScreen™ TR-FRET Control Kit manual

[3] Invitrogen homepage, section LanthaScreen™: www.invitrogen.com/LanthaScreen

List of Abbreviations

TRF	Time Resolved Fluorescence
F	Fluorescein
FRET	Fluorescence Resonance Energy Transfer
TR-FRET	Time Resolved Fluorescence Resonance Energy Transfer
Tb	Terbium

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