Technical Note

LanthaScreen™ TR-FRET Assay

Implementation on Tecan's Infinite® M1000 Multimode Reader

Introduction

The Infinite® M1000 multimode microplate reader is the first monochromator-based instrument to date that meets the high level **LanthaScreen™ Certified Plus** status.
In this technical note we describe the instrument settings on the basis of experiments with the LanthaScreen™ TR-FRET Control Kit on Tecan´s new Infinite M1000 multifunctional detection system.

The Principle of LanthaScreen™

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 assay (*time resolved fluorescence resonance energy transfer*) 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 as acceptor dye.

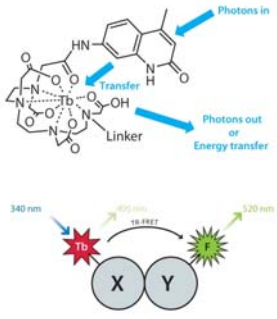


Figure 1: LanthaScreen™ TR-FRET assay principle (3)

Material and methods

Instrument

Tecan Infinite M1000 premium Quad4 Monochromators™ multimode microplate reader (Tecan Austria)

Microplates

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

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)

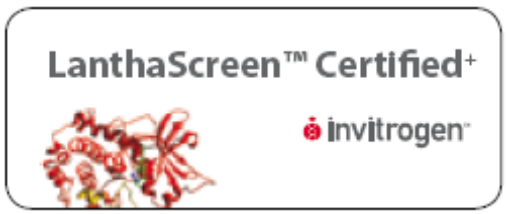
Instrument Settings

Measurement	Label 1	Label 2
Plate	[COS384fb.pdfx]	[COS384fb.pdfx]
Part of Plate	A1-C24	A1-C24
Mode	Fluorescence Top	Fluorescence Top
Excitation Wavelength	332 [20] nm	332 [20] nm
Emission Wavelength	485 [20] nm	515 [20] nm
Gain	200 (manual)	200 (manual)
Number of Flashes	20 - 50	20 - 50
Flash Frequency	100 Hz	100 Hz
Integration Time	200 µs	200 µs
Lag Time	100 µs	100 µs
Settle Time	0 ms	0 ms
Z-Pos. (cal. from B1)	24969 µm	24969 µm

Table 1: Measurement parameters for Infinite® M1000

Data Analysis

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



Acknowledgement

We express our acknowledgements to Mark Koeppel, PhD, Gary Prescott, PhD and Kevin Lowitz from Invitrogen (Madison), who provided the validation and certification support for the Infinite® M1000.

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
FRET	Fluorescence Resonance Energy Transfer
TR-FRET	Time Resolved Fluorescence Resonance Energy Transfer
Eu	Europium
Tb	Terbium
APC	Allophycocyanin

Results and Conclusion

This technical note describes the instrument settings for successful performance of Invitrogen´s LanthaScreenTM TR-FRET Control Kit on the Tecan´s new Infinite M1000 premium Quad4 Monochromators-based multimode detection system.

The validation/certification experiments have been performed at Invitrogen (Madison, USA).

With regard to the obtained measurement data, the Infinite M1000 has performed according to the high level assay criteria and was successfully validated and certified by Invitrogen as “LanthaScreen™ Certified Plus”.

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395 538 V1.0, 07-2008