

HTRF[®] Homogenous TR-FRET Assay

Implementation on Tecan's Infinite[®] M1000 Multimode Reader



Introduction

Tecan's Infinite[®] M1000 premium Quad4 Monochromators[™] multimode microplate reader has been validated and certified by Cisbio Bioassays, France for compatibility with the HTRF[®] technology.

In this technical note we describe the implementation and instrument settings on the basis of validation experiments conducted with the Infinite M1000 and the HTRF[®] Reader Control Kit, HTRF[®] cAMP assay and HTRF[®] cytokine (TNF α) assay from Cisbio.

Assay principle

HTRF[®] combines *time resolved fluorescence* (TRF) with *fluorescence resonance energy transfer* (FRET) [1,3]. It is based on the energy transfer between two fluorescent labels, a long-lifetime europium (Eu³⁺) cryptate donor and either XL 665 (chemically modified allophycocyanin) or the d2 acceptor.

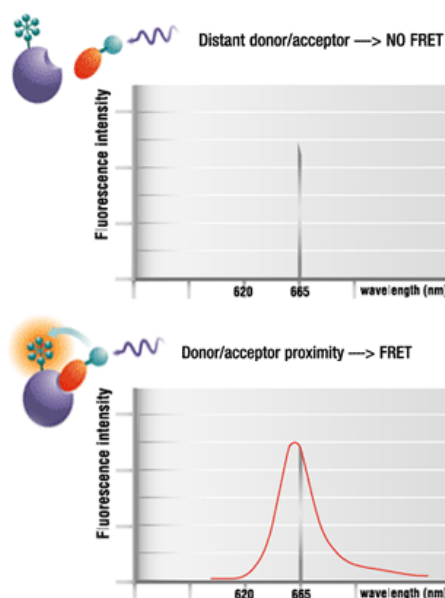


Figure 1: FRET-principles for HTRF[®]: Detection of acceptor-specific fluorescence (665 nm) indicates that FRET has occurred, caused by the proximity of the two interacting partners. FRET is often expressed as ratio of acceptor- and donor-emission intensities, offering a possibility to correct for pipeting imprecision and coloured compound filter effect [3].

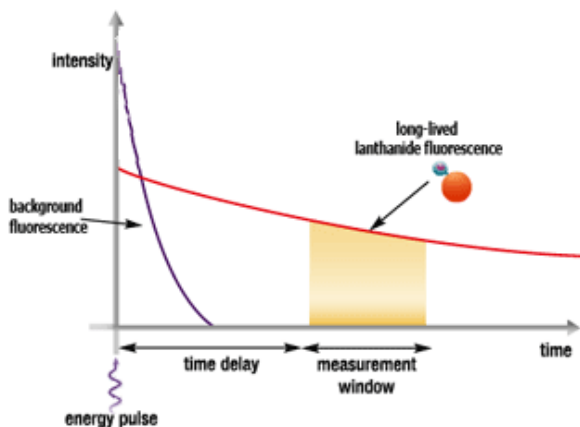


Figure 2: *Europium³⁺ cryptate* as donor fluorophore: A delay between excitation and fluorescence measurement allows elimination of non-specific short-lived emissions. Depending on reader type, the optimal delay may vary from 50 to 150 µseconds [3].

Material and Methods

Instrument

Tecan Infinite M1000 premium Quad4 Monochromators microplate reader including a fluorescence intensity top detection module

Microplates

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

Reagents

HTRF® Reader Control Kit (Cisbio, France)
 HTRF® TNFα Assay (Cisbio, France)
 HTRF® cAMP HiRange Assay (Cisbio, France)

Assay procedure

The assays and data analysis were performed as described in the HTRF® assay manuals [2].

Instrument Settings

Measurement Parameter	Donor Settings	Acceptor Settings
Plate	[COS384fw.pdfx]	[COS384fw.pdfx]
Mode	Fluorescence Top	Fluorescence Top
Excitation Wavelength	317 nm	317 nm
Excitation Bandwidth	20 nm	20 nm
Emission Wavelength	620 nm	665
Emission Bandwidth	10 nm	10 nm
Gain	optimal	optimal
Number of Flashes	50 - 165	50 - 165
Flash Frequency	100 Hz	100 Hz
Integration Time	500 µs	500 µs
Lag Time	60 µs	60 µs
Settle Time	0 ms	0 ms
Z-Pos.	calculated from well	same as donor

Table 1: Measurement parameter and settings for Infinite M1000

Results

A series of HTRF® measurements have been performed on the Infinite M1000 by using the HTRF Reader Control Kit in white flat bottom 384 well plates in order to calibrate and fine-tune the measurement settings. The influence of the number of flashes on the assay performance is outlined in Table 2.

	50 flashes	125 flashes	165 flashes
S/B	250	275	275
deltaF _{low}	20	25	25
deltaF _{high}	850	875	950
CV [%]	7	3.7	3.2
Z'	0.82	0.87	0.89
Meas. time	~ 2 min 30 sec	~ 4 min 30 sec	~ 5 min 15 sec

Table 2: Number of flashes and assay performance. The exemplified data represent typical performance values using the HTRF® Reader Control Kit in white microplates (values represent mean values of four different serial instruments, the assay was run in triplicate under each excitation setting. The measurement times correspond to the plate layout described in the HTRF® control kit protocol (A1-H8).

For further validation of the instrument, experiments have been performed with the HTRF[®] cAMP HiRange detection kit and the HTRF[®] TNF α detection kit.

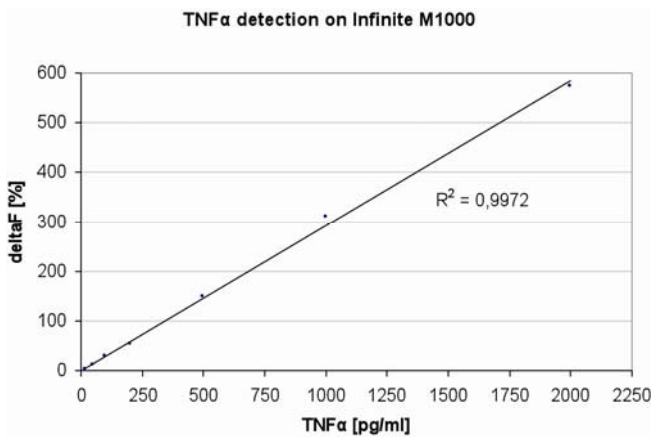


Figure 3: TNF α detection using the Infinite M1000 instrument and the HTRF[®] TNF α detection kit. Shown are DeltaF values [%] versus increasing TNF α concentrations (20, 50, 100, 200, 500, 1000, and 2000 pg/ml).

Figure 3 shows the high linear correlation ($R^2 > 0.99$) between the calculated DeltaF values and increasing TNF α concentrations especially at low TNF α concentrations.

Conclusion

Tecan's premium Quad4 Monochromators multimode microplate reader Infinite M1000 has been successfully validated and certified for HTRF[®] in white plates by Cisbio. The validation was based on experiments conducted with the HTRF[®] Reader Control Kit, the HTRF[®] TNF α detection kit and the HTRF[®] cAMP detection kit.

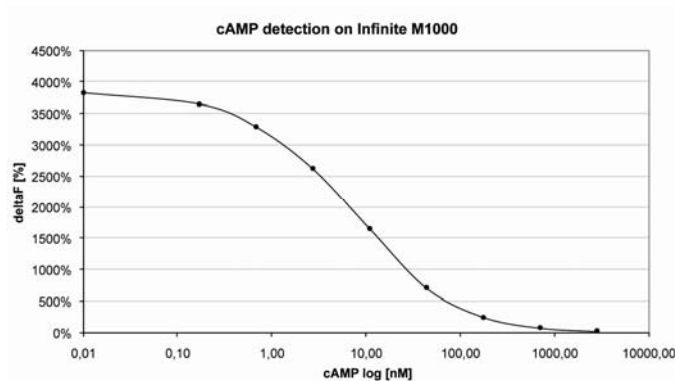


Figure 4: Detection of cAMP with the Infinite M1000 instrument using the cAMP detection kit. DeltaF values [%] are shown in dependence of increasing cAMP concentrations (0.17, 0.68, 2.73, 10.94, 43.75, 175, 700, and 2800 nM)

Figure 4 shows the obtained DeltaF values versus increasing concentrations of cAMP with an IC₅₀ value determined of approximately 22 nM. According to Cisbio, France, the results presented are typical for the cAMP HiRange kit.

Acknowledgement

We express our acknowledgements to Alexandre Jean, Laurence Jacquemart and Marie-Laure Lebreton from Cisbio Bioassays, France, who provided excellent support for the validation of 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] HTRF Reader Control Kit manual (Cisbio International, France); HTRF[®] TNF α detection kit manual (Cisbio International, France); HTRF[®] cAMP detection kit manual (Cisbio International, France)
- [3] <http://www.htf.com/technology>

List of Abbreviations

APC	Allophycocyanine
cAMP	cyclic Adenosine Monophosphate
Eu	Europium
FRET	Fluorescence Resonance Energy Transfer
HTRF	Homogenous Time Resolved Fluorescence
S/B	Signal to Blank
TNF α	Tumor Necrosis Factor α
TRF	Time Resolved Fluorescence
TR-FRET	Time Resolved Fluorescence Resonance Energy Transfer

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