Application Note:
AP-MIB-VARIO13-0708

LanthaScreen™ TR-FRET Assay Performance with Thermo Scientific Varioskan Flash Spectral Scanning Multimode Reader

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Summary

LanthaScreen TR-FRET assay technology from Invitrogen is a widely used application in biotech and pharmaceutical research. This paper describes the performance of the Varioskan® Flash multimode reader using LanthaScreen assays. The results show that Varioskan Flash is compatible with LanthaScreen assays, and Varioskan Flash is an excellent spectral scanning multimode reader for TR-FRET based applications.

Introduction

LanthaScreen technology uses time-resolved fluorescence resonance energy transfer (TR-FRET) to study multiple target classes including protein kinases, nuclear hormone receptors, as well as proteases and ubiquitinated proteins. LanthaScreen TR-FRET works on the principles that when suitable pairs of fluorophores are in close proximity of one another, excitation of the terbium chelate donor fluorophore results in energy transfer to the fluorescein or GFP acceptor fluorophore (Figure 1). The benefit of this assay is the long fluorescence lifetime of the terbium lanthanide donor fluorochrome excited at 340 nm. This allows ratiometric measurement of acceptor fluorescence emission (FRET signal) at 520 nm over the 495 nm donor emission long after the background fluorescence has dissipated (Figure 2). The time-resolved measurement reduces assay interference (e.g. fluorescent compounds) and increases data quality.

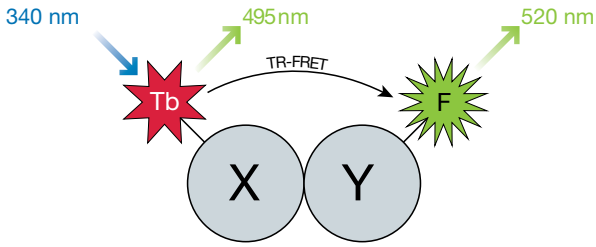

Figure 1. Principle of LanthaScreen TR-FRET detection

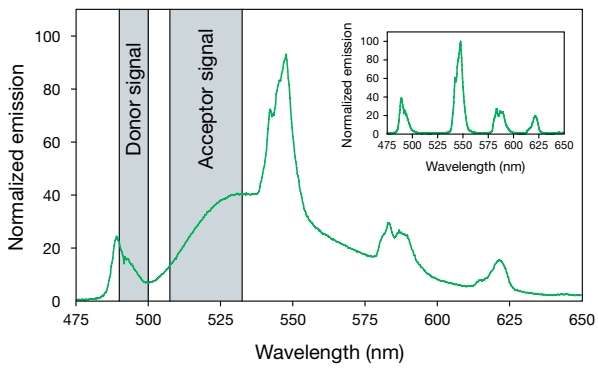

Figure 2. Fluorescence Spectra of Terbium and Fluorescein (FRET) emission. The time-resolved spectra above illustrate energy transfer occurring when terbium (donor) and fluorescein (acceptor) are brought into proximity via biomolecular interactions. The inset shows the time-resolved spectra in the absence of energy transfer.

Materials and methods

The performance of Varioskan Flash was tested using a LanthaScreen Tb-anti-GST Antibody Kit (catalogue number PV4216) that was obtained from Invitrogen (Carlsbad, CA). Solid white 384-well microplates were purchased from Corning (Corning, NY). The assay was performed according to the kit instructions.

Briefly, the fluorescein labelled GST-MBP positive control provided was serially diluted from 200nM stock solution and the Tb-anti-GST antibody was diluted to 4 nM concentration using the LanthaScreen TR-FRET dilution buffer provided in the kit. For reactions in standard white 384-well plates 20 µL of Tb-anti-GST antibody (2nM final concentration) and 20 µL of the fluorescein-GST-MBP positive control dilution were aliquoted in the plate with four replicates. When low volume white 384-well plates were used, aliquots of 10 µL of Tb-anti-GST antibody and 10 µL of the fluorescein-GST-MBP positive control dilution were added into the plate using same number of replicates. The reactions were allowed to incubate for 60 minutes at room temperature and then measured with Varioskan Flash controlled with the Thermo Scientific SkanIt Software (Thermo Fisher Scientific, Finland). The measurement parameters programmed into the SkanIt® Software to perform time-resolved fluorescence dual emission measurement are shown in Table I. Required dual emission measurement was performed using multiwavelength measurement option of the SkanIt Software that makes it possible to measure both donor and acceptor emissions easily from the sample without any additional time differences. The structure of the measurement protocol and multiwavelength measurement step used is shown in Figure 3.

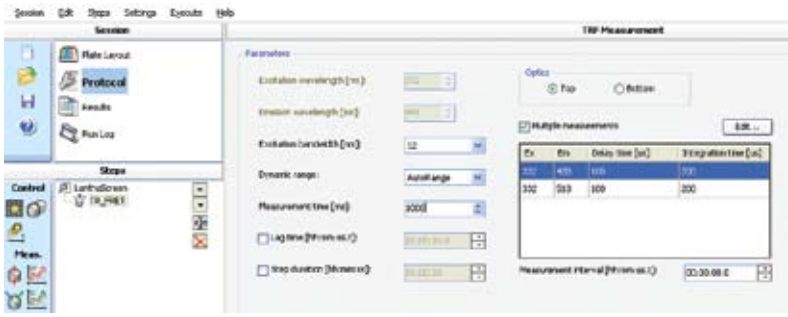

Figure 3. SkanIt Software measurement protocol and parameters for LanthaScreen TR-FRET assays.

	Measurement 1 (Tb-donor)	Measurement 2 (Fluorescein acceptor)
Excitation wavelength (nm)	332	332
Emission wavelength (nm)	488	518
Excitation bandwidth (nm)	12	12
Emission bandwidth (nm)	12	12
TRF dealy time (us)	100	100
TRF integration time (us)	200	200
Measurement time (ms)	1000 (100 flashes)	1000 (100 flashes)

Table 1. Varioskan Flash measurement parameters for LanthaScreen assays

After the measurement, the data from both standard and low volume 384-well plate assays was transported to Microsoft Excel for further calculations. TR-FRET ratio between acceptor and donor emission signals were calculated for each positive control sample and calibration curves were drawn. Then, theoretical assay sensitivities for both plate formats using standard IUPAC 3*SD principle and Z-prime values for each positive control concentration were calculated.

Results

Calibration curves of the assays with 384- and low volume 384-well plates are shown in Figure 4. and Figure 5. shows Z-prime values against positive control concentrations.

According to these results, both standard and low volume white 384-well plates perform well in LanthaScreen assays with Varioskan Flash. Measured calibration curves show almost identical behavior with both plate types and very similar theoretical assay sensitivities are obtained with both plate formats.

When assay robustness is estimated based on Z-prime values, similar behavior is also noticed between standard and low volume 384-well plates. Z-prime values are well over required limit of 0.5 with all sample concentrations over 1 nM.

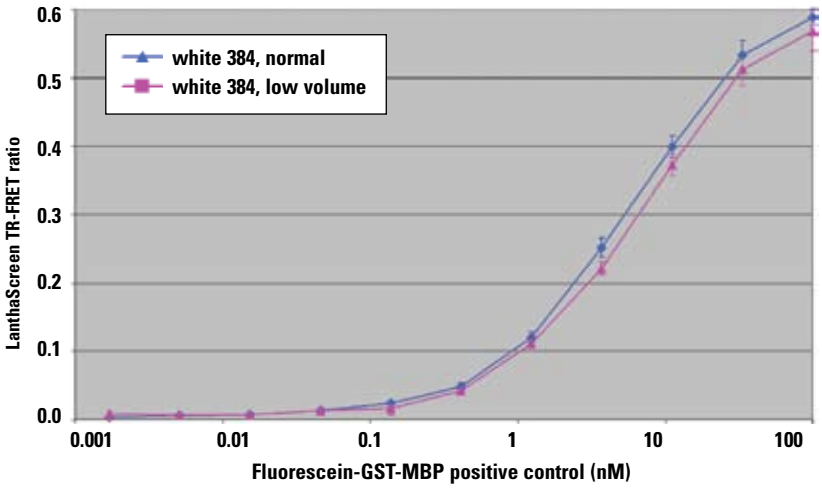

Figure 4. Calibration curves of LanthaScreen assays with standard and low volume white 384-well plates

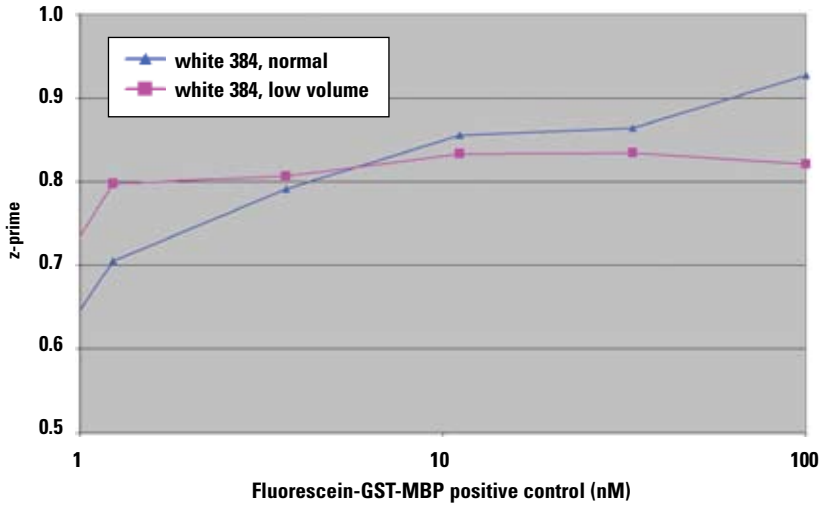

Figure 5. Z-prime values of LanthaScreen assays with Varioskan Flash

Conclusions

These results show that Varioskan Flash fulfills all requirements for LanthaScreen assays and can be well used to measure TR-FRET based assays with good sensitivity. Furthermore, measuring TR-FRET assays that require dual emission measurement is easy to program and can be done with only one measurement step using multiwavelength feature of the software. LanthaScreen assays can also be performed with Varioskan Flash using low total assay volume and low volume 384-well plates that makes this Varioskan Flash/LanthaScreen combination specially useful for screening purposes where low sample consumption is exceptionally important.

Further information

For further information about the Varioskan Flash spectral scanning multimode reader or Invitrogen LanthaScreen technology, please refer to the following web pages:

www.thermo.com/readingroom

www.thermo.com/varioskan

www.invitrogen.com/lanthascreen

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