Application Note

LanthaScreen™ TR-FRET Assay from Invitrogen™ on Synergy™ 4

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Introduction

Here we describe the use of the Synergy™ 4 Hybrid Microplate Reader to measure the output from Invitrogen's LanthaScreen™ TR-FRET assay technology.

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 fluor excited at 340 nm. This allows measurement of the signal long after the background fluorescence has dissipated (Figure 2). The time-resolved measurement reduces assay interference (e.g. fluorescent compounds) and increases data quality.

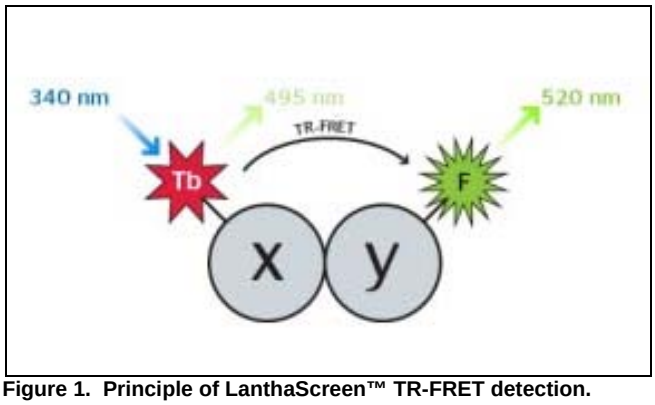

Figure 1. Principle of LanthaScreen™ TR-FRET detection.

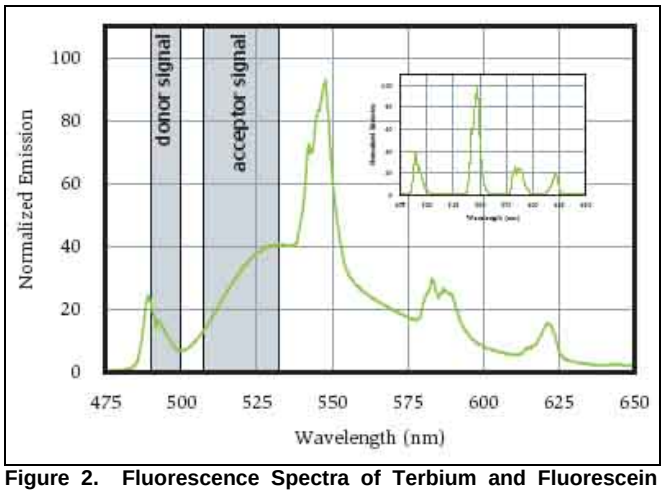

Figure 2. Fluorescence Spectra of Terbium and Fluorescein (FRET) emission. The time-resolved spectra above illustrate energy transfer occurring when terbium (donor) and fluorescein (acceptor) are brought into proximity via biomolecular interactions. The inset shows the time-resolved spectra in the absence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

Materials and Methods

A LanthaScreen™ Tb-anti-GST Antibody Kit (catalog number PV4216) was obtained from Invitrogen (Carlsbad, CA). Solid white 384-well (catalog number 3912) and 1536-well (catalog number 3725) microplates were purchased from Corning (Corning, NY). The assay was performed according to the kit instructions. Briefly, the fluorescein labeled GST-MBP positive control provided was serially diluted from 200nM, and the Tb-anti-GST antibody was diluted to 4 nM using the LanthaScreen™ TR-FRET dilution buffer provided. For reactions in 384-well plates 20 µL of Tb-anti-GST antibody (2nM final) and 20 µL of the fluorescein-GST-MBP positive control dilution were aliquoted in replicates of 8. Reactions in 1536-well plates contained 5 µL of antibody and 5 µL of positive control. The reactions were allowed to incubate for 60 minutes in the dark at room temperature. The plates were then read on a Synergy 4 Multi-Mode Microplate Reader, using the reader's filter system. The reader was controlled and the data collected and analyzed using Gen5™ Data Analysis Software.

Results

The emission spectrum data presented in Figure 2 indicates that the fluorescein acceptor emission is a narrow band centered around 520 nm when excited at 340 nm, and typical fluorescein emission filters at 530 or 535 nm are not suitable for detection. Samples with only terbium-labeled antibody exhibit a narrow band emission centered around 495 nm. Using a suitable emission filter set for wavelength selection as on the Synergy 4 Hybrid Reader, the fluorescein emission can be distinguished from that of terbium.

The data presented in Figures 3 and 4 demonstrate the ability of the Synergy 4 Hybrid Reader to quantitatively measure TR-FRET signal using the Invitrogen LanthaScreen™ assay in either 384- or 1536-well plates. When the signal-to-background ratio is plotted against fluorescein-GST-MBP concentration a sigmoidal shaped curve is observed. This sigmoidal relationship can be described using a 4-parameter logistic fit of the data.

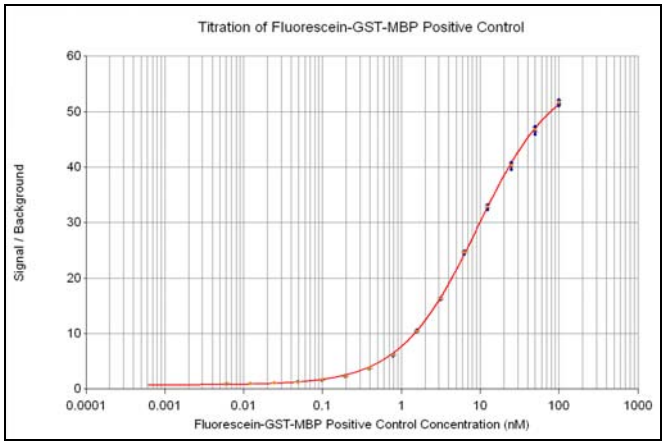

Figure 3. Titration of fluorescein-GST-MBP with Tb-Anti-GST antibody in 384-well microplates.

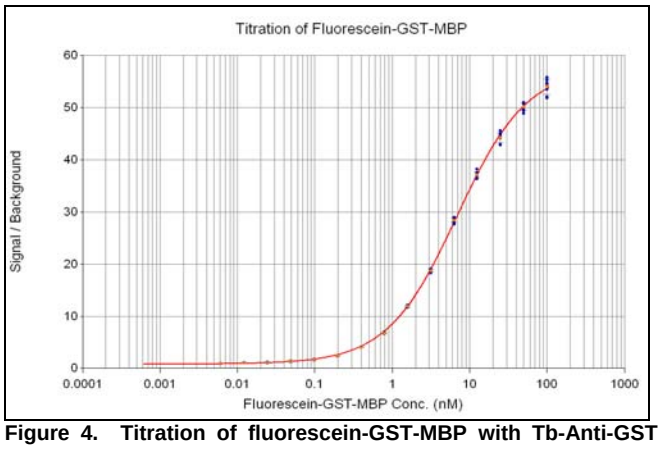

Figure 4. Titration of fluorescein-GST-MBP with Tb-Anti-GST antibody in 1536-well microplates.

LanthaScreen™ is a trademark of Invitrogen.

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