LanthaScreen™ Terbium-Labeled TR-FRET Anti-Epitope Tag Reagents - User Guide

Cat. nos. PV3568, PV3569, PV4216, PV4217 **Shipping Condition: Frozen** **Storage: -20°C**

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Reagents Available

Reagents	Size	Cat. no.
LanthaScreen™ Tb-anti-His-Tag Antibody Kit	25 µg	PV3568
	1 mg	PV3569
LanthaScreen™ Tb-anti-GST Antibody Kit	25 µg	PV4216
	1 mg	PV4217

For a list of components in each kit, please refer to the certificate of analysis provided with each kit. The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

Introduction

Principle of FRET and TR-FRET

For screening libraries of compounds, time-resolved fluorescence resonance energy transfer (TR-FRET) is a recognized method for overcoming interference from compound autofluorescence or light scatter from precipitated compounds. The premise of a TR-FRET assay is the same as that of a standard FRET assay: when a suitable pair of fluorophores are brought within close proximity of one another, excitation of the first fluorophore (the donor) results in energy transfer to the second fluorophore (the acceptor). An increase in the fluorescence emission of the acceptor coupled with a decrease in the fluorescence emission of the donor demonstrates energy transfer between the donor and acceptor. In high throughput screening (HTS) assays, using a ratio of the intensities of the acceptor and donor fluorophores corrects for differences in assay volumes between wells, and corrects for quenching effects because of colored compounds.

Difference Between FRET and TR-FRET

TR-FRET assays use a long-lifetime lanthanide chelate as the donor species. Lanthanide chelates are unique in that their excited state lifetime (the average time that the molecule spends in the excited state after accepting a photon) is a millisecond or longer. This is in sharp contrast to the lifetime of common fluorophores used in standard FRET assays, which are typically in the nanosecond range. Because interference from autofluorescent compounds or scattered light is also on the nanosecond timescale, these factors negatively impact standard FRET assays. To overcome these interferences, perform TR-FRET assays by measuring FRET after a suitable delay, typically 50 to 100 microseconds, after excitation by a flashlamp excitation source in a microtiter plate reader. This delay not only overcomes interference from background fluorescence or light scatter, but also avoids interference from direct excitation because of the non-instantaneous nature of the flashlamp excitation source.

Use of Terbium as Donor Species

The most common lanthanides used in TR-FRET assays for HTS are terbium and europium. Terbium offers unique advantages over europium when used as the donor species in a TR-FRET assay. In contrast to europium-based systems that use allophycocyanin (APC) as the acceptor, terbium-based TR-FRET assays use common fluorophores such as fluorescein as the acceptor. Because it is straightforward (and inexpensive) to label a molecule such as a peptide with fluorescein, directly labeled biomolecules may be used in terbium-based TR-FRET assays, rather than biotinylated molecules that must then be indirectly labeled via streptavidin-mediated recruitment of APC. The use of directly labeled molecules in a terbium-based TR-FRET assay reduces costs, improves kinetics, avoids problems because of steric interactions involving large APC conjugates, and simplifies assay development since there are fewer independent variables requiring optimization in a directly labeled system.

Instrument Settings

Excitation and Emission Spectra

The excitation and emission spectra of terbium and fluorescein are shown below in Figure 1. Four sharp emission peaks characterize the terbium emission spectrum, with silent regions between each peak. The first terbium emission peak (centered between approximately 485 and 505 nm) overlaps with the maximum excitation peak of fluorescein. Energy transfer to fluorescein is then measured in the silent region between the first two terbium emission peaks.

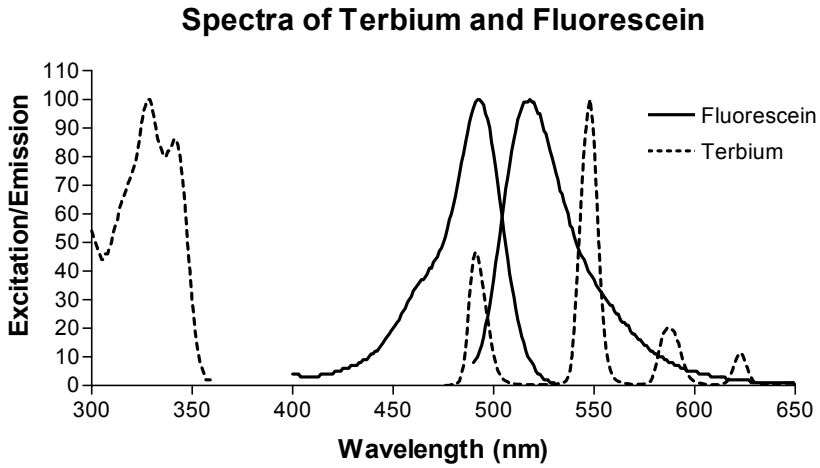

Figure 1: Excitation and emission spectra of fluorescein and terbium.

Excitation Filter

The terbium donor is excited using a 340 nm excitation filter with a 30 nm bandpass. However, the exact specifications of the excitation filter are not critical, and filters with similar specifications will work well. In general, excitation filters that work with europium-based TR-FRET systems will perform well with the LanthaScreen™ terbium chelates.

Fluorescein Emission Filter

The specifications of this emission filter are more critical than those of the excitation filter. To measure energy transfer to fluorescein without interference from terbium, use a filter centered at 520 nm with a 25 nm bandpass. We do not recommend standard "fluorescein" filters because such filters also pass light associated with the terbium spectrum.

Terbium Emission Filter

In addition, the emission of fluorescein because of FRET is referenced (or "ratioed") to the emission of the first terbium peak using a filter that isolates this peak. Use a filter centered at 490 or 495 nm, with a 10 nm bandpass. In general, a 490 nm filter will reduce the amount of fluorescein emission that "bleeds through" into this measurement, although instrument dichroic mirror choices (such as those on the Tecan Ultra Evolution™ instrument) may necessitate the use of a 495 nm filter. The effect on the quality of the resulting measurements is minimal in either case. Filters suitable for LanthaScreen™ assays are available from Chroma (www.chroma.com) as filter set PV001, or from other vendors. A LanthaScreen™ filter module for the BMG LABTECH PHERAStar is available direct from BMG.

Other Instrument Settings

Other instrument settings are typical to the settings used with europium-based technologies. Use the guidelines provided by the instrument manufacturer as a starting point for optimization. For a LanthaScreen™ assay, a delay time of 100 µs, followed by a 200 µs integration time, are typical. The number of flashes or measurements per well is highly instrument-dependent and is set as advised by your instrument manufacturer. Most filter-based instruments capable of time-resolved FRET, such as the Tecan Ultra Evolution™, BMG PHERAStar, Molecular Devices Analyst®, or PerkinElmer EnVision™ are suitable for LanthaScreen™ assays. In addition, monochromator-based instruments such as the Tecan Safire^{2TM} are also suitable for LanthaScreen™ assays. Contact Technical Support for instrument-specific setup guidelines.

Applications of Anti-Epitope Tag Antibodies to Nuclear Receptor Assays

Using and Detecting Epitope Tags

Epitope tags facilitate the purification of proteins for use in many different applications. In addition, such tags indirectly label proteins through the use of LanthaScreen™ terbium-labeled anti-epitope tag antibodies. Indirect labeling of a protein through the use of an anti-epitope tag antibody is advantageous when working with partially purified protein, or in cases in which direct labeling of the protein using amine-reactive terbium (Tb) chelates negatively impacts protein activity.

An example of an application using such a labeling strategy is shown schematically in Figure 2. Tb-labeled anti-GST antibody indirectly labels the GST-tagged RXR-beta ligand-binding domain. Titrating the receptor with the agonist 9-cis retinoic acid recruits a fluorescein labeled peptide, PGC1a, which binds to the receptor upon 9-cis retinoic acid-induced conformational changes.

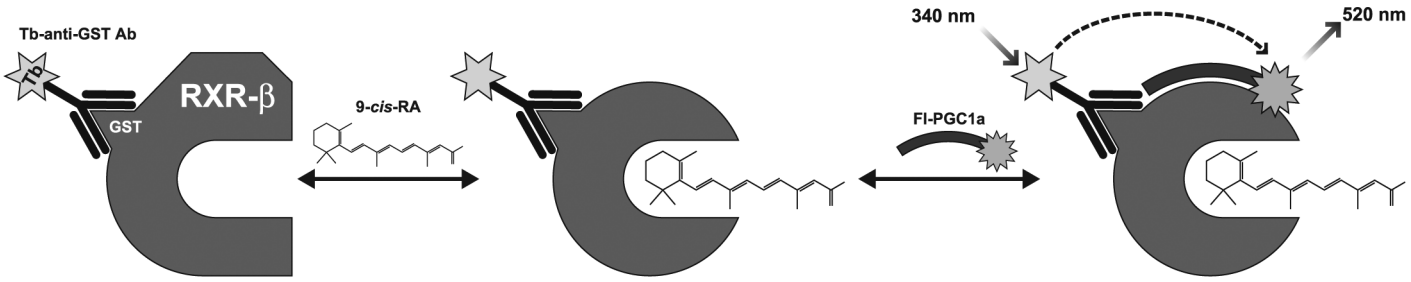

Figure 2: Schematic illustration of the use of a terbium-labeled anti-GST antibody in a nuclear hormone receptor assay.

The binding of the fluorescein labeled peptide causes an increase in the TR-FRET signal, as is illustrated in the data shown in Figure 3. Such a system characterizes agonist affinity for the receptor or could be configured to identify additional agonists or antagonists.

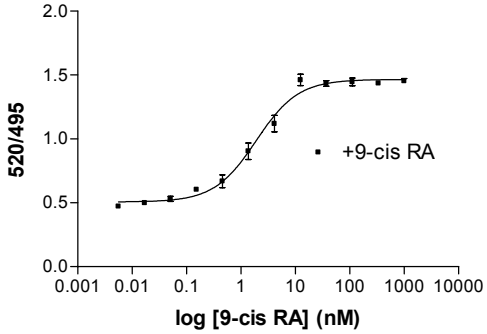

Figure 3: Titration of the agonist 9-cis retinoic acid into 10 nM RXR-β, 5 nM Tb-anti-GST Antibody and 350 nM Fluorescein-PGC1a peptide with a 1 hour incubation. Read on a Tecan Ultra Evolution™.

There is a range of variables associated with developing a TR-FRET assay utilizing Tb-labeled anti-epitope antibodies. Determine the reagent concentrations, order of reagent addition, incubation times, etc., for each application.

Determining Antibody Concentration

The optimal amount of antibody used will depend on the amount of tagged protein in the assay. Too little terbium-labeled antibody causes lack of signal while too much antibody increases background. Either too much or too little antibody may decrease the "read window". We recommend determining the optimal concentration of antibody by using 0.5, 1, 2, and 4 X concentrations of the epitope-tagged protein.

Assay Stability and Read Window

For a given assay system, assess signal stability and read window. In general, many assays will reach equilibrium within 2 hours, and will show a stable signal for at least 8 hours. However, depending on assay configuration and the specific demands of the assay, these times may vary and must be determined experimentally for the given assay system.

First Time User

Included in each terbium-labeled anti-epitope tag antibody kit is a corresponding fluorescein-labeled positive control. To verify proper instrument settings to run the LanthaScreen™ assay, we recommend that a dilution series of the fluorescein-labeled positive control be titrated against a fixed concentration of terbium-labeled antibody to generate a binding curve. The certificate of analysis provided with each antibody kit shows a binding curve that is representative of the data obtained in conducting such an experiment. For example, Figure 4 shows the result of a titration of Fluorescein-GST-Maltose Binding Protein (MBP) against 2 nM Tb-labeled Anti-GST Antibody. The plate was read on a Tecan Ultra Evolution™ plate reader after a 1 hour room temperature incubation.

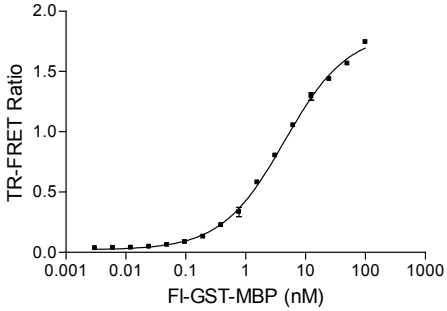

Figure 4: Titration of Fluorescein-GST MBP into Tb-Anti-GST antibody on a Tecan Ultra Evolution™.

Assessing Data Quality in Ratiometric Measurements

The TR-FRET value is a unitless ratio derived from the underlying donor and acceptor signals, which are dependent on instrument settings (such as instrument gain). The TR-FRET ratio and the resulting "top" and "bottom" of an assay window will depend on these settings as well, and will vary from instrument to instrument. Figure 5 below demonstrates the pitfalls of simply relying on the assay window as a measure of data quality. Normalizing the ratiometric data on the left and re-plotting the data on the right shows that the data is identical in quality (despite vastly different assay windows). What is important in determining the quality of an assay is not the size of the window as much as the size of the errors in the data relative to the difference in the maximum and minimum values. For this reason, the "Z prime" (Z') value proposed by Zhang and colleagues (*J Biomol Screen* 1999: 4(2) pp 67-73), which takes these factors into account, is the correct way to assess data quality in a TR-FRET assay. Typically, our assays have Z' values of greater than 0.70.

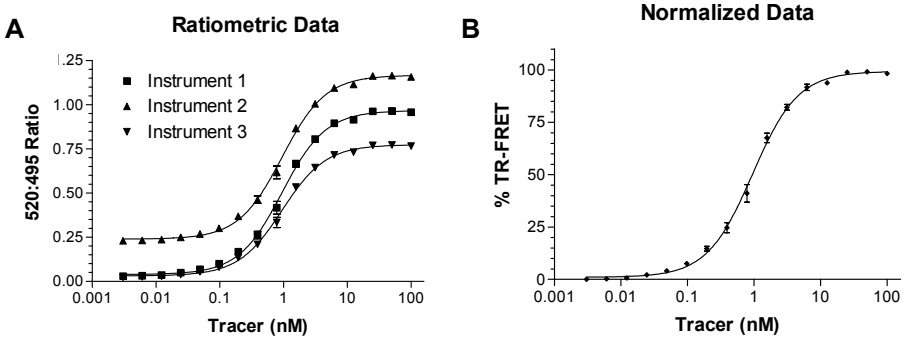

Figure 5: Assay window variability because of instrument type (A) does not affect the resulting data. Upon normalization, the above three curves are identical (B).

Related Products

Reagents	Amount	Cat. No.
LanthaScreen™ Tb-anti-Mouse Antibody	25 µg	PV3765
	1 mg	PV3767
LanthaScreen™ Tb-anti-Goat Antibody	25 µg	PV3769
	1 mg	PV3771
LanthaScreen™ Tb-anti-Rabbit Antibody	25 µg	PV3773
	1 mg	PV3775
LanthaScreen™ Tb-anti-Human Antibody	25 µg	PV3777
	1 mg	PV3779
LanthaScreen™ Tb-PY20 Antibody	25 µg	PV3552
	1 mg	PV3553
LanthaScreen™ Tb-PY72 Antibody	25 µg	PV3554
	1 mg	PV3555
LanthaScreen™ Tb-PY100 Antibody	25 µg	PV3556
	1 mg	PV3557
LanthaScreen™ Tb-PT66 Antibody	25 µg	PV3558
	1 mg	PV3559
LanthaScreen™ Tb-pSer (PKC) Antibody	25 µg	PV3560
	1 mg	PV3561
LanthaScreen™ Tb-IκB pSer32 Antibody	25 µg	PV3562
	1 mg	PV3563
LanthaScreen™ Tb-pCrosstide Antibody	25 µg	PV3564
	1 mg	PV3565
LanthaScreen™ Tb-CREB pSer133 Antibody	25 µg	PV3566
	1 mg	PV3567
LanthaScreen™ Tb-Streptavidin, 1 mg/mL	50 µg	PV3576
	1 mg	PV3577
Fluorescein-PKC Substrate, 1 mg/mL	1 mg	PV3506
Fluorescein-IKK Substrate, 1 mg/mL	1 mg	PV3507
Fluorescein-CREBtide Substrate, 1 mg/mL	1 mg	PV3508
Fluorescein-Crosstide Substrate, 1 mg/mL	1 mg	PV3509
Fluorescein-PTK Substrate 1, 1 mg/mL	1 mg	PV3513
Fluorescein-PTK Substrate 2, 1 mg/mL	1 mg	PV3511
Fluorescein-Poly GT, 30 µM	1 mL	PV3610
Fluorescein-Poly GAT, 30 µM	1 mL	PV3611
LanthaScreen™ Amine Reactive Tb Chelate	10 µg	PV3583
	100 µg	PV3582
	1 mg	PV3581
LanthaScreen™ Thiol Reactive Tb Chelate	10 µg	PV3580
	100 µg	PV3579
	1 mg	PV3578

Notice to Purchaser

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