

LanthaScreen® Tb-anti-GFP Antibody

Table of Contents

1. Potential Applications of Terbium labeled anti-GFP Antibody
2. Material Included
3. Guidelines for Cellular Detection of GFP or GFP-fusion proteins
 - a. Materials Required But Not Provided
 - b. LanthaScreen® Cellular Assay Protocol Using Tb-anti-GFP
 - c. LanthaScreen® Detection
 - d. Representative Cellular Detection Data
 - i. Quantitative Detection of Cells expressing GFP-fusions
 - ii. Detection of DNA Damage Induced GFP-p53 Accumulation
 - iii. Detection of IRAK1 Degradation Induced by IL-1 β
4. Guidelines for Biochemical Detection of Recombinant GFP or GFP-fusions
 - a. Materials Required But Not Provided
 - b. LanthaScreen® Biochemical Assay Protocol Using Tb-anti-GFP
 - c. Representative Biochemical Detection Data
5. Appendix
 - a. LanthaScreen® Technology Overview
 - b. LanthaScreen® Cellular Assay
 - c. Instrument Guidelines for First-time LanthaScreen® Users

Potential applications of Terbium labeled anti-GFP antibody

The LanthaScreen® Tb anti-GFP antibody has been developed to allow users to configure highly sensitive assays for the detection of GFP or GFP fusions. The anti-GFP antibody was raised against full length GFP from jellyfish *Aequorea Victoria*. The antibody is suitable for detection of native GFP and GFP variants including yellow fluorescent protein and emerald GFP, which is used throughout Life Technologies LanthaScreen® platform, and is available in a number of vectors from Life Technologies for user to configure their own assay systems. Because the antibody has been raised against GFP from jellyfish *Aequorea victoria*, GFP variants from other sources may not perform adequately in this system.

- Quantitative detection of GFP or GFP-fusion protein either as a recombinant protein or expressed in cells with high sensitivity
- Developing cellular assays for the detection of regulated GFP-fusion protein stabilization (accumulation/degradation)
- Unlike reading direct fluorescence intensity of GFP, LanthaScreen® Tb-anti-GFP allows more sensitive and robust detection of GFP as a transcriptional reporter gene

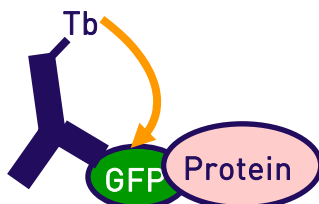


Figure 1 Schematic illustration of TR-FRET using LanthaScreen® Tb-anti-GFP. Tb-anti-GFP antibody binds to GFP or GFP-fusion (as shown). Excitation of Tb at around 337 nm results in time resolved-fluorescence resonance energy transfer (TR-FRET) from Tb to GFP. The TR-FRET value is determined as a ratio of the FRET-specific signal measured with a 520 nm filter (GFP) to that of the signal measured with a 490nm or 495 nm filter, which is specific to terbium.

Materials Included

Material	Amount	Storage	Handling
LanthaScreen® Tb-anti-GFP Antibody	25 µg (A13391) or 1 mg (A13392)	-20°C	<ul style="list-style-type: none"> Minimize freeze-thaw cycles Protect from light

Guidelines for Cellular Detection of GFP or GFP-fusions

Materials required but not provided

Materials	Recommended Source	Part #
6X LanthaScreen® Cellular Assay Lysis Buffer	Invitrogen	A12891
Cell line of interest	various	various
Protease Inhibitor Cocktail	Sigma	P8340
Fluorescence plate reader with top-read and TR-FRET capability	Visit www.invitrogen.com/instrumentsetup for details	
White tissue culture-treated, 384-well assay plates	Corning	3570

LanthaScreen® Cellular Assay Protocol using Tb-anti-GFP

- Begin with cells grown on an assay plate (e.g. 384-well white plate Corning #3570).
- Prepare **Complete 6X Lysis Buffer** by adding protease inhibitor cocktail to the 6X Lysis Buffer, at a 1:33 dilution of 100X stock (e.g., 30 µL of 100X stock inhibitors per 1 mL of 6X Lysis Buffer) followed by Tb-anti-GFP Antibody to a final concentration of 12 nM (the stock antibody is supplied at approximately 0.5 mg/mL, which is 3.3 µM based on an antibody molecular weight of 150,000). Mix gently by inversion. Store on ice until use.
Note: Other antibody concentrations may be tested to optimize the assay for your particular system.
- Add complete 6X Lysis Buffer to each well of the assay plate so that final lysis buffer concentration is 1X. Cover the plate.
- Incubate the covered plate at room temperature in the dark for 2 hours or other desired equilibration time. This incubation time may be optimized to shorter or longer periods, depending on your assay system.
Note: Assay plates may be stored at 4 °C overnight prior to reading. Let the plate warm to room temperature prior to reading.
- Proceed to reading the plate, as described in the next section.

LanthaScreen® Detection

Instruments and Filters

Detection can be performed on a variety of plate readers, including the PE Envision. The data presented on the next pages were generated using a BMG PHERAstar plate reader using the LanthaScreen® filter block available from BMG. Visit www.invitrogen.com/instrumentsetup or contact Invitrogen Discovery Sciences technical support (drugdiscoverytech@invitrogen.com or 760-603-7200 (enter 3 for “know your party’s extension”, then enter 40266)) for more information on performing LanthaScreen® Cellular Assays on your particular instrument.

Note: We do not recommend using monochromator-based instruments without adjustable bandwidth such as Tecan Safire2 and Molecular Devices M5, as the sensitivity of these instruments is not sufficient to adequately detect the TR-FRET signal.

Reading the Assay Plate and Data Analysis

All measurements should be taken at room temperature from the top of the wells.

1. Let the assay plate warm to room temperature before reading, if necessary.
2. Set the fluorescence plate reader to top/time-resolved read mode.
3. Allow the lamp in the plate reader to warm up for at least 10 minutes before making measurements.
4. Filter bandwidths and dichroic mirrors are critical and cannot be approximated. Please refer to instrument specific set up guides at www.invitrogen.com/instrumentsetup.
5. Calculate the acceptor (520nm)/donor (490nm or 495nm) Emission for each well, by dividing the acceptor emission values by the donor emission values. Do **not** average the 520 and 490 or 495 reading and then take the ratio.
6. **Optional:** Convert the data to a response ratio by dividing each emission ratio value by the value from cell-free wells (or cells not expressing any GFP).

Representative Cellular Detection Data

Quantitative detection of cells expressing GFP-fusion proteins

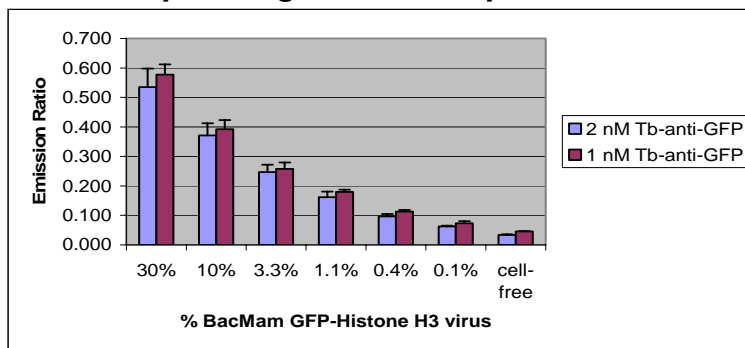


Figure 2 TR-FRET analysis of GFP-Histone H3 expression in BacMam-transduced cells. U-2 OS cells were transduced with the indicated amount of BacMam GFP-Histone H3 virus (Invitrogen, A12897) in a 384-well assay plate for ~24 h. Cells were lysed with 6X LanthaScreen® Cellular Assay Lysis Buffer (Invitrogen Catalog #A12891) containing LanthaScreen® Tb-anti-GFP and incubated for 8 minutes at room temperature prior to reading the plate on a BMG PHERAstar Plus. The emission ratio (520nm/490nm) of GFP vs. Tb is plotted against the concentrations of BacMam virus used (n=4).

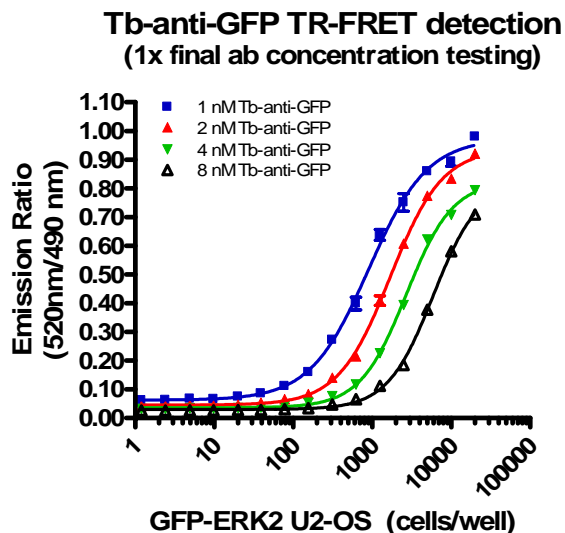


Figure 3 Tb-anti-GFP antibody concentration optimization testing using stably-expressing GFP-ERK2 cells. LanthaScreen® ERK2 U2-OS cells stably expressing GFP-ERK2 fusion proteins were plated at various seeding densities in a white 384-well assay plate (Corning 3570). Immediately after plating, cells were lysed with 6X LanthaScreen® Cellular Assay Lysis Buffer (Invitrogen Catalog #A12891) containing LanthaScreen® Tb-anti-GFP at various concentrations and incubated for 3 hours at room temperature prior to reading the plate on a BMG PHERAstar Plus. For each 1x final antibody concentration tested, the emission ratio (520nm/490nm) of GFP vs. Tb is plotted against the number of cells per well (n=4). In this case, 1 nM final Tb-anti-GFP antibody concentration generated the most sensitivity detection.

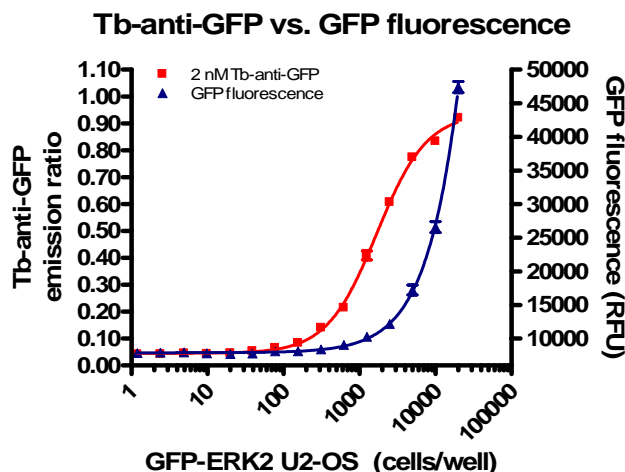


Figure 4 Tb-anti-GFP TR-FRET offers greater sensitivity than direct GFP fluorescence detection. LanthaScreen® ERK2 U2-OS cells stably expressing GFP-ERK2 fusion protein were plated at various seeding densities in a white 384-well plate (Corning 3570) or a black, clear-bottom 384-well plate (Corning 3712). Immediately after plating, cells in the white plate were lysed with 6X LanthaScreen® Cellular Assay Lysis Buffer (Invitrogen Catalog #A12891) containing 12 nM LanthaScreen® Tb-anti-GFP (1x final = 2 nM) and incubated for 3 hours at room temperature prior to reading the plate on a BMG PHERAstar Plus. The emission ratio (520nm/490nm) of GFP vs. Tb is plotted against the cells per well (n=4). For comparison, GFP fluorescence intensity of the cells plated into the black plate was measured using a Tecan Safire² plate reader and also plotted against the number of cells per well (n=4).

Detection of DNA damage induced GFP-p53 accumulation

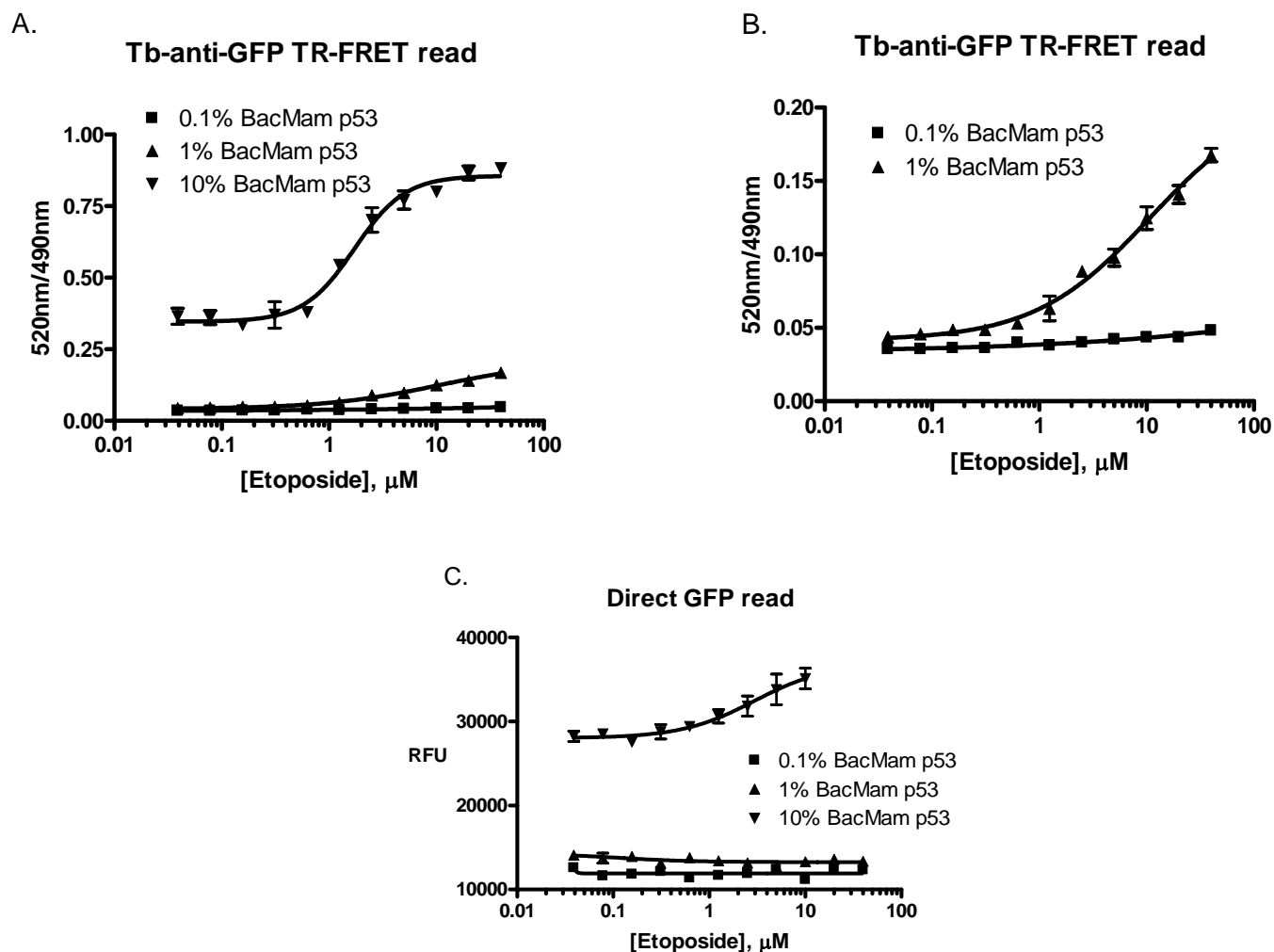
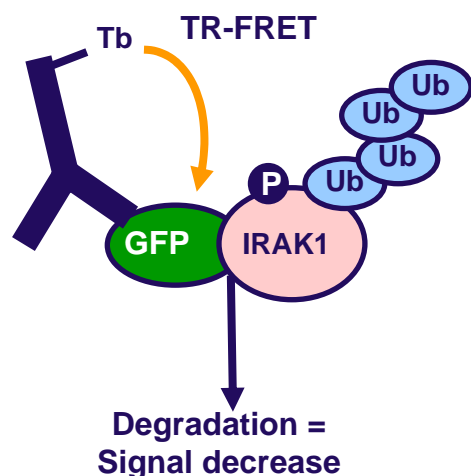


Figure 5 Using Tb-anti-GFP TR-FRET method to detect DNA damage induced GFP-p53 accumulation. U-2 OS cells plated on either white 384-well assay plate (Corning 3570) (A. and B.) or black wall transparent bottom 384-well plate (Corning 3712) (C.) were transduced with indicated amount of BacMam GFP-p53 and treated with indicated amounts of etoposide for 21 hours. A. and B. Cells were then 6X LanthaScreen® Cellular Assay Lysis Buffer (Invitrogen Catalog #A12891) containing 12 nM LanthaScreen® Tb-anti-GFP and incubated for 2 hrs at room temperature prior to reading the plate on a BMG PHERAstar Plus. The emission ratio (520nm/490nm) of GFP vs. Tb is plotted against the concentrations of etoposide used (n=4). C. The plate was read on Tecan Safire2 for GFP fluorescence intensity. After cell-free background subtraction, the GFP fluorescence intensity is plotted against the concentrations of etoposide (n=4). Tb-anti-GFP can detect dose-dependent GFP-p53 accumulation induced by etoposide when both 1% and 10% BacMam GFP-p53 were used (about 4 and 3 fold window, respectively).

Detection of IRAK1 degradation induced by IL-1 β



IRAK1 degradation in U-2OS cells: 0.001% BacMam & Tb-anti-GFP readout

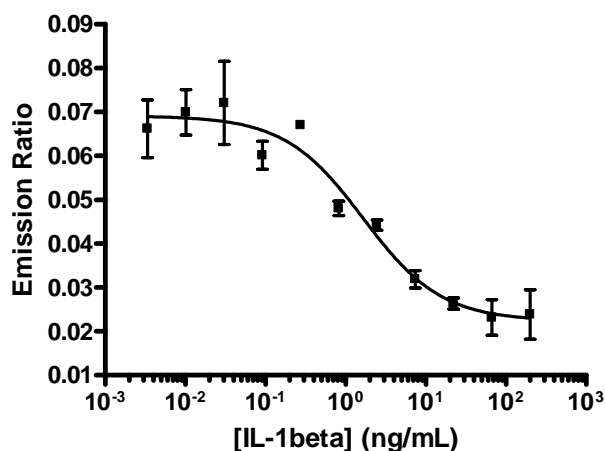


Figure 6 Using Tb-anti-GFP TR-FRET method to detect GFP-IRAK1 degradation induced by IL-1 β . U-2 OS cells plated on white 384-well assay plate (Corning 3570) were transduced with indicated amount of BacMam GFP-IRAK1 and treated with indicated amounts of IL-1 for one hour. Cells were then lysed with 6X LanthaScreen® Cellular Assay Lysis Buffer (Invitrogen Catalog #A12891) containing 12 nM LanthaScreen® Tb-anti-GFP and incubated for 2 hrs at room temperature prior to reading the plate on a BMG PHERAstar Plus. The emission ratio (520nm/490nm) of GFP vs. Tb is plotted against the concentrations of IL-1 β used (n=4).

Guidelines for Biochemical Detection of Recombinant GFP or GFP-fusions

Materials required but not provided

Materials	Recommended Source	Part #
TR-FRET Dilution Buffer	Invitrogen	PV3574
GFP protein or GFP-fusion protein of interest (The LanthaScreen Tb-anti GFP antibody has been raised against GFP from jellyfish <i>Aequorea Victoria</i> . Other GFP variants may not be suitable).	various	various
Fluorescence plate reader with top-read and TR-FRET capability	Visit www.invitrogen.com/instrumentsetup for details	
White tissue culture-treated, 384-well assay plates	Corning	3570

LanthaScreen® Biochemical Assay Protocol using LanthaScreen® Tb-anti-GFP

1. Prepare serial dilutions of GFP or GFP-fusion protein of interest with TR-FRET Dilution Buffer.
2. Prepare 4 nM Tb-anti-GFP solution with TR-FRET Dilution Buffer (For first users, we recommend preparing several concentrations of the antibody to determine the optimal concentration for your experiment).
3. Plate 10 µL/well of the GFP or GFP fusion protein serial dilution onto an assay plate (e.g. 384-well white plate Corning #3570).
4. Add 10 µL/well of the 4 nM Tb-anti-GFP solution onto the assay plate

Note: The final Tb-anti-GFP Antibody concentration is 2 nM. Other concentrations could be tested to find the optimal concentration for your experiment.

5. Incubate the covered plate at room temperature in the dark for 15 to 60 min. The equilibration time can be optimized for your experiment.

Note: Securely sealed assay plates may be stored at 4 °C overnight prior to reading. Let the plate warm to room temperature prior to reading.

6. Proceed to reading the plate as described in the above LanthaScreen® Detection section.

Representative Biochemical Detection Data

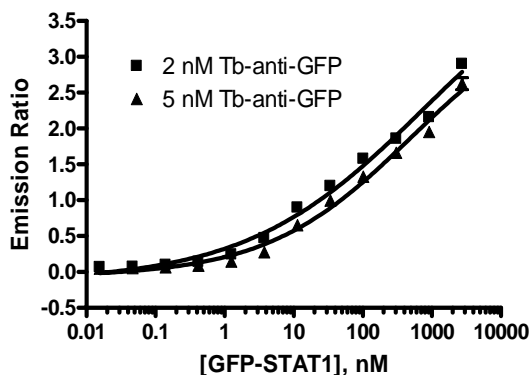


Figure 7 TR-FRET analysis of GFP-STAT1 protein level using Tb-anti-GFP. Dilution series of GFP-STAT1 protein (Invitrogen, PV5211) was prepared with TR-FRET Dilution Buffer (Invitrogen, PV3574) and plated in a 384-well assay plate. TR-FRET Dilution Buffer containing Tb-anti-GFP was added to reach the indicated final concentrations of the antibody. The plate was incubated for 15 min at room temperature prior to plate reading on BMG PHERAstar Plus. The emission ratio (520nm/490nm) of GFP vs. Tb is plotted against the concentrations of GFP-STAT1 (n=4).

Appendix

LanthaScreen® Technology Overview

Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET) is a preferred fluorescent assay format in drug discovery laboratories. TR-FRET assays are less susceptible to compound interference than other assay formats and may be applied to multiple target classes. To support this technology focus, Invitrogen uses two sets of FRET-pairs in developing our LanthaScreen® Assays: Terbium/Fluorescein (or GFP) and Europium/AlexaFluor® 647.

LanthaScreen® Cellular Assay

LanthaScreen® Cellular Assays are HTS-compatible immunoassays used to interrogate target-specific post-translational modifications in a cell-based format. Target proteins are expressed as fusions with green fluorescent protein (GFP) in living cells, and modification-specific antibodies labeled with Terbium (Tb) are used to detect stimulus-induced post-translational modifications in a time-resolved fluorescence resonance energy transfer (TR-FRET) format.

The use of GFP as a FRET acceptor circumvents the need to use complex antigen-capturing reagents, thereby providing a high-throughput alternative to commonly used analytical methods such as Western blot and ELISA.

For more information, visit www.invitrogen.com/lanthascreen.

Instrument Guidelines for First-time LanthaScreen® users

LanthaScreen® assays require the detection of terbium TR-FRET. For more information about your specific instrument and to purchase filters, visit www.invitrogen.com/instrumentsetup. For a protocol describing how to test whether your instrument is able to detect a terbium/GFP TR-FRET signal, please contact Drug Discovery Technical Support at drugdiscoverytech@invitrogen.com or 760-603-7200 (enter 3 for “know your party’s extension”, then enter 40266).