LanthaScreen® IkB alpha GripTite™ Cell-based Assay Protocol

Catalog no. K1489 **Shipping: Dry Ice** **Storage: Liquid Nitrogen**

Protocol part no. K1489.pps Rev. date: 6 May 2010

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1. Overview of Lanthascreen® Cellular Assay Technology

Time-resolved FRET (TR-FRET) has been recognized as a method to overcome interfering signals from compounds in high-throughput screening applications. Similar to standard FRET-based assays, TR-FRET relies on the proximity dependent energy transfer between an excited donor fluorophore and a suitable acceptor fluorophore, which can be detected by an increased emission from the acceptor molecule. LanthaScreen® TR-FRET technology uses a long lifetime terbium chelate (Tb) fluorophore as a donor species. The majority of fluorophores possess excited-state lifetimes on the order of nanoseconds. In contrast, terbium fluorophores display extended excited state lifetimes on the range of milliseconds. This unique feature allows the measurement of FRET between interacting donor and acceptor molecules after an extended time delay, typically 50–100 microseconds after excitation by a flash lamp excitation source. This delay overcomes interference caused by such things as autofluorescent compounds and precipitate induced light scatter. A complete guide to commonly asked questions and answers regarding LanthaScreen® technology can be found at www.invitrogen.com/lanthascreen.

The LanthaScreen® cellular assays represent a unique and sensitive way to utilize TR-FRET technology for the interrogation of target-specific phosphorylation events within endogenous signal transduction pathways. By expressing specific target proteins as fusions with green fluorescent protein (GFP, a suitable TR-FRET acceptor for the excited-state Tb fluorophore) in living cells, modification-specific antibodies labeled with Tb can be used to detect stimulus-induced post-translational modifications in a lysed-cell assay format.

2. Materials Supplied

Cell Line Name:	LanthaScreen® IkB alpha GripTite™
Description:	LanthaScreen® IkB alpha GripTite™ cells contain a stably integrated expression vector encoding for a GFP-IkB fusion protein under control of the CMV promoter. Treatment of LanthaScreen® IkB alpha GripTite™ cells with an agonist such as TNFalpha will lead to the transient phosphorylation of the GFP-IkB alpha fusion protein. The phosphorylated GFP-IkB alpha fusion protein can be quantitated in cell lysates using a terbium-labeled anti-IkB alpha [pSer32] antibody.
Catalog Number:	K1489
Shipping Condition:	Dry Ice
Storage Condition:	Liquid nitrogen. **Immediately upon receipt, cells must be stored in liquid nitrogen or thawed for immediate use. Cells stored at –80°C can quickly lose viability.**
Quantity:	~2,000,000 (2×10^6 cells/mL)
Application:	Detection of phosphorylation of GFP-IkB alpha
Growth Properties:	Adherent
Cell Phenotype:	Epithelial
Selection Marker:	Blasticidin (5 µg/mL)
Vector Used:	pcDNA™6.2/EmGFP-IkB
Mycoplasma Testing:	Negative
BioSafety Level:	2

3. Materials Required, but Not Supplied

Media/Reagents	Recommended Source	Part #
Recovery™ Cell Culture Freezing Medium	Invitrogen	12648-010
DMEM w/ GlutaMAX™	Invitrogen	10569-010
DMSO	Fluka	41647
Fetal Bovine Serum (FBS), dialyzed, tissue-culture grade **(DO NOT SUBSTITUTE!)**	Invitrogen	26400-044
Opti-MEM® (w/ HEPES/L-Gln, w/o Phenol Red)	Invitrogen	11058-021
Fetal Bovine Serum (Charcoal/Dextran Stripped)	Invitrogen	12676-029
Non-essential amino acids (NEAA)	Invitrogen	11140-050
HEPES Buffer Solution (1M)	Invitrogen	15630-080
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140-122
Phosphate-buffered saline (PBS) w/o Ca^{2+} and Mg^{2+}	Invitrogen	14190-136
Trypsin/EDTA	Invitrogen	25300-062
Blasticidin (antibiotic)	Invitrogen	R210-01
LanthaScreen® Tb-anti-IkBalpha [pSer32] Antibody	Invitrogen	PV3652
LanthaScreen® Cellular Assay Lysis Buffer	Invitrogen	PV5598
Protease Inhibitor Cocktail	Sigma Aldrich	P8340
Phosphatase Inhibitor Cocktail	Sigma Aldrich	P2850
Tumor necrosis factor alpha (TNFα), human recombinant	Invitrogen	PHC3015

Consumables	Recommended Source	Part #
White tissue culture treated, 384-well assay plates	Corning Life Sciences	3570

Equipment	Recommended Source
Fluorescence plate reader w/ top-read and TR-FRET capability	Various
Filters, if required for plate reader (see **Section 5.3**)	Chroma Technology Corp.

4. Cell Culture Conditions

4.1 Media Required

Note: We prepare our media by adding the listed components directly to the 500 mL medium bottle.

Note: Blasticidin can be added directly to the cell culture flask to reach 5 µg/mL. Similar methods are suitable.

Note: Unless otherwise stated, all media and solutions should be at room temperature or slightly higher (we recommend 37°C for optimal performance) before adding them to the cells.

Component	Growth Medium	Assay Medium	Freezing Medium	Thaw Medium
DMEM w/ GlutaMAX™	500 mL (90%)	—	—	500 mL (90%)
Opti-MEM®	—	500 mL (99%)	—	—
Dialyzed FBS	50 mL (10%)	—	—	50 mL (10%)
Charcoal / Dextran-stripped FBS	—	5 mL (1%)	—	—
NEAA	5 mL (0.1 mM)	5 mL (0.1 mM)	—	5 mL (0.1 mM)
HEPES	5 mL (0.01 M)	—	—	5 mL (0.01 M)
Penicillin (antibiotic)	5 mL (100 U/mL)	5 mL (100 U/mL)	—	5 mL (100 U/mL)
Streptomycin (antibiotic)	5 mL (100 µg/mL)	5 mL (100 µg/mL)	—	5 mL (100 µg/mL)
Sodium Pyruvate	5 mL (1 mM)	5 mL (1 mM)	—	5 mL (1 mM)
Blasticidin (antibiotic)	5 µg/mL	—	—	—
Recovery™ Cell Culture Freezing Medium	—	—	100%	—

4.2 Detailed Cell Handling Procedures

4.2.1 Thawing Method

1. Place 14 mL of Thaw Medium (without Blasticidin) into a T75 flask.
2. Place the flask in a humidified 37°C/5% CO_2 incubator for 15 minutes to allow medium to equilibrate to the proper pH and temperature.
3. Remove the vial of cells to be thawed from liquid nitrogen and thaw rapidly by placing at 37°C in a water bath with gentle agitation for 1–2 minutes. Do **not** submerge vial in water.
4. Decontaminate the vial by wiping with 70% ethanol before opening in a Class II biological safety cabinet.
5. Transfer the vial contents to a sterile 15-mL conical tube.
6. Add 10 mL of Thaw Medium (without Blasticidin) drop-wise into the cell suspension.
7. Centrifuge cells at 200 × g for 5 minutes.
8. Aspirate supernatant and resuspend the cell pellet in 1 mL of fresh Thaw Medium (without Blasticidin).

   Note: This step is important to fully remove the DMSO present from the Recovery™ Cell Culture Freezing Medium.
9. Transfer the contents to the T75 tissue culture flask containing pre-equilibrated Thaw Medium (without Blasticidin) and place flask in a humidified 37°C/5% CO_2 incubator.
10. Switch to passaging cells in Growth Medium with Blasticidin, once cells appear to be growing at consistent rates for the given cellular background.

4.2.2 Propagation Method

1. Cells should be passaged or fed at least two times a week. Cells should be maintained between 10% and 90% confluency. Do not allow cells to reach confluence. Cells which have grown to confluence many not show expected agonist response in the assay.
2. To passage cells, aspirate medium, rinse once with PBS, add Trypsin/EDTA (3 mL for a T75 flask, 5 mL for a T175 flask and 8 mL for T225 flask) and swirl to coat the cells evenly. Cells usually detach after ~2 minutes exposure to Trypsin/EDTA. Add Growth Medium (7 mL for a T75 flask, 10 mL for T175 and T225 flasks) to inactivate Trypsin and mix. Verify under a microscope that cells have detached and clumps have completely dispersed.
3. Transfer required amount to a new flask containing prewarmed Growth Medium.

4.2.3 Freezing Method

1. Harvest and count the cells, then spin cells down and resuspend in Recovery™ Cell Culture Freezing Medium at a density of $\geq 2 \times 10^6$ cells/mL.
2. Dispense 1-mL aliquots into cryogenic vials.
3. Place in an insulated container for slow cooling and store overnight at –80°C.
4. Transfer to liquid nitrogen the next day for storage.

4.2.4 Special Considerations for working with this Cell Line

1. This cell line is a clonal population isolated by Fluorescence Activated Cell Sorting (FACS). Assay performance can be expected to depend upon use of the specified media as responsive cells have been chosen based on these formulations.
2. For additional information about the GripTite™ 293 MSR cellular background, please contact Technical Support.
3. This cell line is tested to be compatible with Corning tissue culture-treated plates (3570) and may not be suitable for use with other plates.
4. This cell line is Blasticidin resistant.

5. Assay Procedure

The following instructions outline the recommended procedure for monitoring TNFα-induced phosphorylation of GFP-IkB alpha at Ser32 using TR-FRET as the readout.

Note:

- We recommend using white, tissue-culture-treated, 384-well assay plates with low fluorescence background, and have optimized this assay for use with Corning Assay Plates (Part# 3570).
- Some solvents may affect assay performance. Assess the effect of a test compound solvent before screening. This cell line has been qualified for DMSO tolerance up to 1%. See validation packet at www.invitrogen.com/lanthascreen for the assay performance of this cell line in the presence of various DMSO concentrations. The cell stimulation described below is carried out in the presence of 0.1% DMSO to simulate the effect that a test compound solvent might have on the assay. If you use other solvents and/or solvent concentrations, change the following assay conditions and optimize appropriately.

5.1 Quick Reference Guide

For more detailed protocol information, see **Section 5.2**.

Agonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells (Positive control Agonist)	Cell-free Wells	Test Compound Wells
Step 1 Plate Cells	32 µL cells in Assay Medium (20,000 cells/well)	32 µL cells in Assay Medium (20,000 cells/well)	32 µL Assay Medium (no cells)	32 µL cells in Assay Medium (20,000 cells/well)
Step 2 Incubate Cells	Incubate cells for 16–20 hours in a humidified 37ºC/5% CO_2 incubator			
Step 3 Prepare Complete Lysis Buffer	Before stimulating the cells, prepare a suitable volume of incomplete lysis buffer (1.2 × # of wells × 20 µL) and supplement with protease/phosphatase inhibitor cocktails and Tb-Ab			
Step 4 Add Agonist or Test Compounds	8 µL Assay Medium with 0.5% DMSO	8 µL 5X TNFα in Assay Medium with 0.5% DMSO	8 µL Assay Medium with 0.5% DMSO	8 µL 5X Test Compounds in 0.5% DMSO
Step 5 Incubate Cells	Incubate in a humidified 37°C/5% CO_2 incubator for 30 minutes; The optimal stimulation time may vary depending on the agonist			
Step 6 Lyse Cells	Add 30 µL/well of complete lysis buffer including terbium-labeled antibody (5 nM)			
Step 7 Equilibrate Plate	Incubate 1 hour at room temperature			
Step 8 Read Plate	See **Section 5.3**			

Antagonist Assay Quick Reference Guide

	Unstimulated Wells	Stimulated Wells	Positive Control Compound Wells	Cell-free Wells	Test Compound Wells
Step 1 Plate Cells	32 µL cells in Assay Medium (20,000 cells/well)	32 µL cells in Assay Medium (20,000 cells/well)	32 µL cells in Assay Medium (20,000 cells/well)	32 µL Assay Medium (no cells)	32 µL cells in Assay Medium (20,000 cells/well)
Step 2 Incubate Cells	Incubate cells for 16-20 hours in a humidified 37ºC/5% CO_2 incubator				
Step 3 Prepare complete lysis buffer	Before stimulating the cells, prepare a suitable volume of incomplete lysis buffer (1.2 x # of wells x 20 µL) and supplement with protease/phosphatase inhibitor cocktails and Tb-Ab				
Step 4 Add Antagonist or Test Compounds	4 µL Assay Medium with 1% DMSO	4 µL Assay Medium with 1% DMSO	4 µL 10X control compound in Assay Medium with 1% DMSO	4 µL Assay Medium with 1% DMSO	4 µL 10X Test Compounds in Assay Medium with 1% DMSO
Optional Step:	Incubate plate with Antagonist for 30 minutes before proceeding				
Step 5 Add Agonist	4 µL Assay Medium	4 µL 10X TNFα in Assay Medium	4 µL 10X TNFα in Assay Medium	4 µL 10X TNFα in Assay Medium	4 µL 10X TNFα in Assay Medium
Step 6 Incubate Cells	Incubate in a humidified 37 ºC/5% CO_2 incubator for 30 minutes				
Step 7 Lyse Cells	Add 30 µL/well of complete lysis buffer including terbium-labeled antibody (5 nM)				
Step 8 Equilibrate Plate	Incubate 1 hour at room temperature				
Step 9 Read Plate	See **Section 5.3**				

5.2 Detailed Assay Protocol

Plate layouts and experimental outlines will vary; in screening mode, we recommend using at least three wells for each control: Unstimulated Control, Stimulated Control, and Cell-free Control.

Note: Some solvents may affect assay performance. Assess the effects of solvent before screening.

5.2.1 Precautions

- Work on a dust-free, clean surface.
- If pipetting manually, you may need to centrifuge the plate briefly at room temperature (30 seconds at 14 × *g*) after additions to ensure all assay components are on the bottom of the wells.
- Cells should be grown to reach 80 to 95% confluency prior to assay.
- Complete lysis buffer may need to be prepared prior to stimulation of cells, in order to avoid exceeding the stimulation time for this assay.

5.2.2 Plate Cells

1. Harvest cells from culture in growth medium and suspend in assay medium at a density of 6.25×10^5 cells/mL.
2. Plate the cells into white tissue culture treated 384-well plates. Add 32 µL per well of Assay Medium to the cell-free control wells. Add 32 µL per well of the cell suspension to Unstimulated and Stimulated wells.
3. After plating, incubate the plates in a 37°C/5% CO_2 incubator for 16–20 hours.

5.2.3 Prepare Lysis buffer

1. Prepare lysis buffer: 20 mM Tris, pH 7.4, 5 mM EDTA, 5 mM NaF, 150 mM NaCl, 1% NP-40 (or equivalent). This incomplete lysis buffer can be prepared in large batches and stored at –20°C. The complete lysis buffer (consisting of phosphatase/protease inhibitor cocktails as well as the LanthaScreen® Tb-anti-IkB [pSer32] antibody) should be prepared on the day of the experiment before stimulation of cells.
2. Determine the volume of complete lysis buffer needed for the assay (assuming 30 µL/well using the standard "addition only" protocol and 20 µL/well for the optional two-step protocol with aspiration; see **Section 5.2.6**). Add 1/100 volume of each protease inhibitor and phosphatase inhibitor cocktail to the incomplete lysis buffer. Mix well by inversion several times.
3. Add LanthaScreen® Tb-anti-IkB [pSer32] antibody to the lysis buffer to a final concentration of 5 nM. Mix gently by inversion several times.

Note: Commonly used phosphatase inhibitors such as sodium orthovanadate (VO_4) and sodium pyrophosphate can interfere with the integrity of terbium chelate and should be not be added to the lysis buffer or used in place of any components described above.

5.2.4 Agonist Assay Plate Setup

Note: This subsection provides directions for performing an Agonist assay. See **Section 5.2.5** for directions for performing an antagonist assay.

Note: The positive agonist controls are run at the concentration of TNFα that gives the maximum stimulation (top of the dose response curve). We recommend running a dose response curve to determine the optimal concentration (EC_{100}) for your TNFα solution. See **Section 6** for a representative curve. From this example data, we determined the EC_{100} to be 5 ng/mL or 286 pM.

1. Prepare a stock solution of 0.5% DMSO in Assay Medium.
2. Prepare a 5X stock of Test Compounds in Assay Medium with 0.5% DMSO (or if test compound is dissolved in DMSO, make sure the DMSO concentration for the 5X solution is 0.5%).
3. Reconstitute TNFα (Tumor Necrosis Factor alpha) at 100 µg/mL according to the manufacturer's protocol. Avoid repeated freeze thaws or long-term storage above -20ºC.
4. Prepare a 5X stock of TNFα in Assay Medium containing 0.5% DMSO. We recommend running a dose response curve to determine the EC_{100} for your TNFα solution. See **Section 6** for a representative curve.
5. Add 8 µL of the stock solution of 0.5% DMSO in Assay Medium to the Unstimulated Control and Cell-free Control wells.
6. Add 8 µL of the 5X stock solution of TNFα to the Stimulated Control wells.
7. Add 8 µL of the 5X stock of Test Compounds to the Test Compound wells.
8. Incubate the agonist assay plate in a humidified 37°C/5% CO_2 incubator for 30 minutes and then proceed to **Section 5.2.6.**

5.2.5 Antagonist Assay Plate Setup

Note: This subsection provides directions for performing an antagonist assay. See **Section 5.2.4** for directions for performing an agonist assay.

1. Prepare a stock solution of 1% DMSO in Assay Medium.
2. Prepare a 10X stock of test compound in Assay Medium with 1% DMSO. Alternatively, if the test compound is dissolved in DMSO, prepare a 10X stock of test compound in Assay Medium and make sure the DMSO concentration for the 10X solution is 1.0%.
3. Reconstitute TNFα (Tumor Necrosis Factor alpha) at 100 µg/mL according to the manufacturer's protocol. Avoid repeated freeze/thaw cycles or long-term storage above –20ºC.
4. Prepare a 10X stock of TNFα in Assay Medium at an EC_{80} concentration. We recommend running a dose response curve to determine the EC_{80} for your TNFα solution. See **Section 6** for representative data. From this example data, we determined the EC_{80} to be 1.6 ng/mL or 91.4 pM.
5. Prepare a 10X stock of positive control inhibitor (control antagonist compound) in Assay Medium with 1% DMSO. We recommend running a dose response curve to determine the optimal inhibition concentration for the antagonist solution.

6. Add 4 µL of the 10X stock of test compound to each Test Compound well.
7. Add 4 µL of the stock solution of 1% DMSO to each Stimulated Control well, Unstimulated Control well, and Cell-free Control well.
8. Add 4 µL of the 10X stock of positive control inhibitor to each Antagonist Control well.
9. If desired, incubate the Test Compounds with the cells in a humidified 37 ºC/5% CO_2 incubator before proceeding. Typically, a 30-minute incubation is sufficient.
10. Add 4 µL of the 10X EC_{80} stock solution of TNFα prepared in step 4 to each Test Compound well, Stimulated Control well, Antagonist Control well, and Cell-free Control well.
11. Add 4 µL of Assay Medium to each Unstimulated Control well.
12. Incubate the antagonist assay plate in a humidified 37°C/5% CO_2 incubator for 30 minutes.

5.2.6 Cell Lysis

Note: See **Section 7** for an optional two-step protocol that includes an aspiration step.

1. Remove assay plate from the humidified 37°C/5% CO_2 incubator
2. Immediately add 30 µL of complete lysis buffer to each well and cover plate.
3. Incubate covered plate at room temperature for 1 hour.

Note: Alternately, cells may be lysed using a two-step protocol involving media aspiration (which requires less Tb-anti-IkB [pSer32] antibody). For the two-step protocol, first aspirate media using a multichannel aspirator (carefully avoid disrupting the cell monolayer). Next add 20µL/well of complete lysis buffer prepared as indicated above but including 2 nM Tb-anti-IkB [pSer32] antibody (instead of 5 nM final concentration). Assay performance should be roughly equivalent using either method.

5.3 Detection

All TR-FRET measurements are to be made at room temperature from the top of the wells, preferably in 384-well, low volume, white assay plates with low fluorescence background (e.g., Corning Product #3570).

5.3.1 Instrumentation, Filters, and Plates

The data presented in this document were generated using a BMG PHERAstar plate reader using the LanthaScreen® filter block available from BMG. The assay can be performed on a variety of plate readers including those from Tecan. If you are using a LanthaScreen® Cellular Assay, we do not recommend the use of monochromatic-based instruments, as the sensitivity of these instruments is not sufficient to adequately detect the endogenously expressed GFP fusion proteins.

Visit www.invitrogen.com/lanthascreen or contact Invitrogen Discovery Sciences Technical Support at 800-955-6288 (select option 3 and enter 40266), or email tech_support@invitrogen.com for more information on performing LanthaScreen® assays on your particular instrument.

Recommended filters for the fluorescence plate reader are:

Excitation filter:	337 nm
Donor Emission filter: 490 nm	
Acceptor Emission filter:	520 nm
Integration start	100 µsec
Integration time	200 µsec

5.3.2 Reading an Assay Plate

1. Set the fluorescence plate reader to top / time resolved-read mode.
2. Allow the lamp in the fluorescence plate reader to warm up for at least 10 minutes before making measurements.
3. Use the following filter selections:

	Scan 1	Scan 2
Purpose:	Measure Donor Signal (Terbium)	Measure Acceptor Signal (TR-FRET to GFP)
Excitation filter:	337 nm (30 nm bandwidth)	
Emission filter:	490 nm (10 nm bandwidth)	520 nm (25 nm bandwidth)
Dichroic Mirror	Variable, see above	
Delay Time	100 µs	
Integration Time	200 µs	

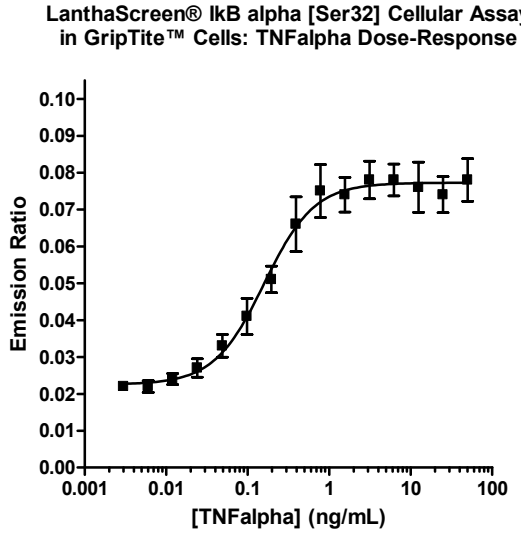
4. Calculate the acceptor/donor Emission Ratio (TR-FRET Ratio) for each well, by dividing the acceptor emission values (520 nm) by the donor emission values (490 nm).

6. Data Analysis

6.1 Background Subtraction (Optional) and Response Ratio

1. Use the assay plate layout to identify the location of the cell-free wells. These control wells are used for background subtraction.
2. Determine the average 520/490 nm emission ratio from the cell-free wells (average antibody background).
3. Subtract the average antibody background (520/490 nm ratio) from all of the emission ratio data.
4. Determine the average emission ratio from the unstimulated wells (cells receiving no agonist). Subtract the average antibody background from these data as well.
5. Divide (or normalize) all the background-subtracted emission ratio data by the average signal from unstimulated cells to generate a Response Ratio, which gives an indication of the "assay window".

6.2 Representative Data



EC_{50}	0.18 ng/mL (10.4 pM)
EC_{80}	~ 0.58 ng/mL (33 pM)
EC_{100}	~ 5 ng/mL (0.29 nM)
Z′ at EC_{100}	0.71

Figure 1. Dose-response of LanthaScreen® IkB alpha GripTite® cells to TNFα. LanthaScreen® IkB alpha GripTite™ cells were treated with TNFα over the indicated concentration range in a 384-well format. Cells were incubated for 30 minutes with TNFα and then lysed by addition of 30 µL lysis buffer (including 5 nM Tb-anti-IkB alpha [pSer32] detection antibody). Fluorescent emission values at 520 nm and 490 nm were obtained using a BMG LABTECH PHERAstar fluorescence plate reader, and the uncorrected 520/490 nm emission ratios (y-axis) were plotted against the concentration of the agonist (x-axis).

7. Optional Two-Step Protocol for Cell Lysis

This section provides directions for performing the two-step protocol with aspiration for cell lysis.

Note: This protocol requires the use of a smaller volume of lysis buffer (and therefore less antibody per well). Typically, this format can results in slightly higher Response Ratios than the "addition only" protocol (**Section 5.2.6**); however, the data quality (Z' factor) is similar using either method. One approach may be preferred over the other, depending on the HTS needs of the user.

1. Remove assay plate from the humidified 37°C/5% CO_2 incubator.
2. Carefully aspirate media from each well using a multichannel aspirator. In order to minimize cell loss, avoid direct contact with the adhered cells on the bottom of the well when aspirating. Cell loss is prevented by only contacting the side of the well with the tip of the aspirator.
3. Immediately add 20 µL of complete lysis buffer including 2 nM LanthaScreen® Tb-anti-IkB [pSer32] antibody to the stimulated cells in assay medium (different than 30 µL and 5 nM for "addition only").
4. Incubate covered plate at room temperature for 1 hour.
5. Proceed to **Section 5.3** for detection.

8. References

Robers, M. B.; Horton, R. A.; Bercher, M. R.; Vogel, K. W.; Machleidt, T., High-throughput cellular assays for regulated post-translational modifications. *Anal. Biochem*. **2008**, *372*, 189–197.

9. Purchaser Notification

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Use of Genetically Modified Organisms (GMO)

Information for European Customers: The LanthaScreen® IkB GripTite™ cell line is genetically modified with the plasmid pCDNA6.2/EmGFP-IkB. As a condition of sale, use of this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.