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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 (HTS) 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 from Invitrogen is unique in that it uses a long lifetime terbium chelate (Tb) as the donor species. The extended excited state for Tb is 1000-fold longer than the majority of fluorophores (milliseconds as opposed to nanoseconds). This unique feature allows the measurement of FRET between Tb and the acceptor molecule after a time delay (typically 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 the kinase target protein in living cells as a fusion with green fluorescent protein (GFP) or yellow fluorescent protein (YFP), which is a suitable TR-FRET acceptor for the donor molecule Tb, modification-specific antibodies labeled with Tb can be used to detect stimulus-induced post-translational modifications (e.g., phosphorylation or ubiquitination) in a lysed-cell assay format.

The LanthaScreen™ IGF-1R GripTite™ cellular assay allows the accurate monitoring of the growth factor-induced phosphorylation at multiple tyrosine residues on the insulin-like growth factor-1 receptor (IGF-1R). Following activation of the IGF-1R/PI3K/AKT pathway by insulin-like growth factor-1 (IGF-1), the change in phosphorylation state of IGF-1R-YFP fusion protein is detected in cell lysates using the general phosphospecific reagent – LanthaScreen™ Tb-pY20 antibody. This assay has been designed in 384-well format and is compatible for use in high-throughput screening applications.

2. Materials Supplied

Cell Line Name:	LanthaScreen™ IGF-1R GripTite™
Description:	LanthaScreen™ IGF-1R GripTite™ (HEK 293 MSR) cells contain a stably integrated expression vector encoding an insulin-like growth factor-1 receptor (IGF-1R) fusion with yellow fluorescent protein (YFP) under the control of a CMV promoter. The IGF-1R-YFP DNA expression construct was introduced into HEK 293 MSR cells using Lipofectamine™ LTX transfection, followed by selection with Blasticidin. This cell line is a clonal population isolated by flow cytometry using YFP fluorescence as sorting marker.
Product Number:	K1834
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:	~16,000,000 (1.6×10^7) cells/ml
Application:	Detection of phosphorylation of IGF-1R-YFP; initial activation step in IGF-1 signaling
Growth Properties:	Adherent
Cell Phenotype:	Epithelial
Selection Marker:	Blasticidin (5 µg/ml)
Vector Used:	pcDNA6-cYFP
***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
Dulbecco's Modified Eagle Medium (D-MEM) with GlutaMAX™-1	Invitrogen	10569-010
DMSO	Fluka	41647
Fetal bovine serum (FBS), dialyzed, tissue-culture grade (DO NOT SUBSTITUTE!)	Invitrogen	26400-044
D-MEM, low glucose, w/o L-glutamine or phenol red	Invitrogen	11054-020
Bovine Serum Albumin (BSA), 10% Ultrapure	Invitrogen	P2046 or P2489
Non-Essential Amino Acids (NEAA)	Invitrogen	11140-050
Penicillin/Streptomycin (antibiotic)	Invitrogen	15140-122
HEPES (pH = 7.3)	Invitrogen	15630-080
Phosphate-buffered saline without calcium and magnesium [PBS(-)]	Invitrogen	14190-136
Trypsin/EDTA	Invitrogen	25300-062
Blasticidin (antibiotic)	Invitrogen	R210-01
Insulin-like growth factor-1 (IGF-1), Recombinant Human	Invitrogen	PHG9074
LanthaScreen™ Tb-pY20 Antibody	Invitrogen	PV3552 or PV3553
LanthaScreen™ Cellular Assay Lysis Buffer	Invitrogen	PV5598
Protease Inhibitor cocktail	Sigma Aldrich	P8340
Phosphatase Inhibitor cocktail	Sigma Aldrich	P2850
Trypan blue	Invitrogen	15250-061

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

Equipment	Recommended Source
Fluorescence plate reader with top-read and TR-FRET capability	Various
Filters, if required for plate reader (see **Section 5.3**)	Various
Optional: Microplate centrifuge	Various

4. Cell Culture Conditions

4.1 Media Required

Component	Growth Medium	Assay Medium	Freezing Medium	Thaw Medium
Dulbecco's Modified Eagle Medium (D-MEM) with GlutaMAX™-1	90% (500 ml)	—	—	90% (500 ml)
D-MEM, low glucose, w/o L-glutamine or phenol red	—	99.9% (500 ml)	—	—
Dialyzed FBS	10% (50 ml)	—	—	10% (50 ml)
Bovine Serum Albumin (BSA)	—	0.1% (0.5 ml)	—	—
HEPES (pH 7.3)	25 mM (12.5 ml)	—	—	25 mM (12.5 ml)
NEAA	0.1 mM (5 ml)	—	—	0.1 mM (5 ml)
Penicillin (antibiotic)	100 U/ml (5 ml)	—	—	100 U/ml (5 ml)
Streptomycin (antibiotic)	100 µg/ml (5 ml)	—	—	100 µg/ml (5 ml)
Blasticidin (antibiotic)	5 µg/ml	—	—	—
Recovery™ Cell Culture Freezing Medium	—	—	100%	—

4.2 Detailed Cell Handling Procedures

4.2.1 Thawing Method

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.

1. Place 29-ml of Thaw Medium (without Blasticidin) into a T225 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 contents to the T225 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 20% 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 (1 ml for a T25 flask, 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 (3 ml for a T25 flask, 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 pre-warmed Growth Medium.

4.2.3 Freezing Method

1. Harvest and count the cells, then spin cells down and resuspend in 4°C Recovery™ Cell Culture
2. Freezing Medium at a desired cell density.
3. Dispense 1-ml aliquots into cryogenic vials.
4. Place in an insulated container for slow cooling and store overnight at –80°C.
5. Transfer to liquid nitrogen the next day for storage.

4.2.4 Special Considerations

1. This cell line is a clonal population isolated by FACS using YFP fluorescence as a sorting marker.
2. Assay performance can be expected to depend on the specified media described within this document as responsive cells have been selected based on these formulations.
3. Incorporating an additional wash step (with PBS or Assay Media) after harvesting the cells ensures complete removal of Growth Media and aids in the serum starvation step when running this assay.
4. The LanthaScreen™ cellular assay developed with this cell line has been tested with Corning 384-well white flat bottom polystyrene tissue culture-treated microplates (#3570) and may or may not be compatible with other assay plates.
5. Do not allow GripTite™ cells to become overconfluent. For additional information about the GripTite™ HEK 293 MSR cellular background, please contact Technical Support.
6. This cell line is resistant to Blasticidin.

5. Assay Procedure

The following instructions outline the recommended assay procedure for monitoring growth factor-induced phosphorylation of IGF-1R-YFP in GripTite™ cells using the LanthaScreen™ Tb-pY20 antibody and TR-FRET as the readout.

1. We recommend using Corning 384-well white tissue culture-treated assay plates with low fluorescence background.
2. 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 (DMSO only)	Stimulated (primary agonist)	Cell-free Wells	Test Compound
Step 1 Plate cells in 384-well format	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 Serum starve cells (16–20 h)	Incubate in a humidified 37°C / 5% CO_2 incubator overnight (16–20 hours)			
Step 3 Prepare lysis buffer	Prepare a suitable volume of complete lysis buffer (See **Section 5.2.3**). Immediately before stimulating cells, add protease/phosphatase inhibitor cocktails and Tb-labeled antibody.			
Step 4 Add Agonist or Test Compounds	8 µl Assay Medium with 0.5% DMSO	8 µl 5X IGF-1 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 Aspirate media	Aspirate Assay Medium from each well using a multi-channel aspirator			
Step 7 Lyse Cells	Add 20 µL/well of complete lysis buffer including Tb-labeled antibody (2 nM)			
Step 8 Equilibrate Reaction	Incubate for 60 minutes at room temperature			
Step 9 Read Plate	See **Sections 5.3**			

Antagonist Assay Quick Reference Guide

	Unstimulated (DMSO only)	Stimulated (primary agonist)	Control Compound)	Cell-free Wells	Test Compound
Step 1 Plate cells in 384-well format	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 Serum starve cells (16–20 h)	Incubate in a humidified 37°C / 5% CO_2 incubator overnight (16–20 hours)				
Step 3 Prepare lysis buffer	Prepare a suitable volume of complete lysis buffer (See **Section 5.2.3**). Immediately before stimulating cells, add protease/phosphatase inhibitor cocktails and Tb-labeled antibody.				
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 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 60 minutes before proceeding				
Step 5 Add Agonist	4 µl Assay Medium	4 µl 10X IGF-1 in Assay Medium	4 µl 10X IGF-1 in Assay Medium	4 µl 10X IGF-1 in Assay Medium	4 µl 10X IGF-1 in Assay Medium
Step 6 Incubate cells	Incubate in a humidified 37 °C / 5% CO_2 incubator for 30 minutes				
Step 7 Aspirate media	Aspirate Assay Medium from each well using a multi-channel aspirator				
Step 8 Lyse Cells	Add 20 µL/well of complete lysis buffer including Tb-labeled antibody (2 nM)				
Step 9 Equilibrate Reaction	Incubate for 60 minutes at room temperature				
Step 10 Read Plate	See **Sections 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 80–90% confluency.
- 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 with tryspin-EDTA. Assay performance has been shown to be enhanced with cells seeded 2–3 days prior to harvest.
2. Wash cells with Assay Medium or PBS to remove residual growth media.
3. Resuspend cells in assay medium at a density of 6.25×10^5 cells/ml.
4. 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 (final density = 20,000 cells/well).
5. After plating, incubate the plates in a 37°C / 5% CO_2 incubator for 16–20 hours.

5.2.3 Lysis Buffer Preparation

Note: Complete lysis buffer (including Tb-labeled antibody) should be made immediately before use (the next day after plating the cells and just prior to stimulating the cells).

1. The lysis buffer is available from Invitrogen as SKU# PV5598. Alternatively, it can be prepared as described below.
2. 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.
3. Determine the volume of lysis buffer needed for the assay (assuming 20 µl/well). We recommend 8 ml of lysis buffer per 384-well plate. Add 1/100 volume of each protease inhibitor and phosphatase inhibitor cocktail to the incomplete lysis buffer. Mix well by inversion several times. **Note**: Commonly used phosphatase inhibitors such as sodium orthovanadate (VO_4) and sodium pyrophosphate can interfere with the integrity of Tb-chelate and should be avoided.
4. Add LanthaScreen™ Tb-pY20 antibody to the lysis buffer to a final concentration of 2 nM. Mix gently by inversion several times.
5. The complete lysis buffer (consisting of phosphatase / protease inhibitor cocktails as well as the LanthaScreen™ Tb-pY20 antibody) should be prepared on the day of the experiment and stored on ice until use.

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 IGF-1 that gives the maximum stimulation (top of the dose response curve). We recommend running a dose-response curve to determine the optimal concentration for your agonist solution. See **Section 6** for representative data.

1. Prepare a solution of 0.5% DMSO in Assay Medium.
2. Insulin-like growth factor (IGF-1; SKU# PHG9074) is supplied as a lyophilized powder and should be reconstituted at a concentration of 0.2 mg/ml and should be stored in aliquots at –20°C for long-term storage (several months). Aliquots of IGF-1 are freeze/thaw sensitive, but once thawed it can also be stored at 4°C for up to 1 week.
3. Prepare a 5X solution of IGF-1 in Assay Medium containing 0.5% DMSO.
4. We recommend starting the 5X IGF-1 solution at 5 µg /ml (1:40 dilution from 0.2 mg/ml). Prepare a 2-fold serial dilution in Assay Medium with 0.5% DMSO and run a dose-response curve to determine the EC_{50} for your IGF-1 solution. See **Section 6** for representative data.

5. Add 8 µl of the solution of 0.5% DMSO in Assay Medium to the Unstimulated Control and Cell-free Control wells.
6. Add 8 µl of the 5X dilution series of IGF-1 to the Stimulated Control wells.
7. Incubate the Agonist assay plate in a humidified 37°C / 5% CO_2 incubator for 30 minutes. Then proceed to **Section 5.2.6** for instructions for cell lysis.

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 solution of 1% DMSO in Assay Medium.
2. Prepare a 10X solution of Test Compounds in Assay Medium with 1% DMSO (or if test compound is dissolved in DMSO, prepare a 10X solution of Test Compounds in Assay Medium and make sure the DMSO concentration for the 10X solution is 1.0%). Two-fold serial dilutions are recommended here.
3. IGF-1 (Invitrogen SKU# PHG9074) is supplied as a lyophilized powder and should be reconstituted at a stock concentration of 0.2 mg/ml and should be stored in aliquots at –20°C for long-term storage (several months). Aliquots of IGF-1 are freeze/thaw sensitive, but once thawed it can also be stored at 4°C for up to 1 week.
4. Prepare a solution of IGF-1 in Assay Medium corresponding to 10X the EC_{80} concentration. We recommend running a dose-response curve to determine the EC_{80} for your IGF-1 solution. See **Section 6** for a representative curve.
5. Prepare a dilution series (10X) 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 dilution series solution of Test Compounds to the Test Compound wells.
7. Add 4 µl of the 1% DMSO solution to the Stimulated Control wells, the Unstimulated Control wells, and the Cell-free Control wells.
8. Add 4 µl of the positive control inhibitor (10X dilution series solution) to the Antagonist Control wells.
9. Incubate the Test Compounds with the cells in a humidified 37°C / 5% CO_2 incubator before proceeding. Typically, 30–60 minutes incubation times are sufficient.
10. Add 4 µl of the EC_{80} solution of IGF-1 prepared in step 4 to the Test Compound wells, the Stimulated Control wells, and the Antagonist Control wells.
11. Add 4 µl of Assay Medium to the Unstimulated Control and Cell-free Control wells.
12. Incubate the Antagonist assay plate in a humidified 37°C / 5% CO_2 incubator for 30 minutes. Then proceed to **Section 5.2.6** for instructions for cell lysis.

5.2.6 Cell Lysis

1. Remove assay plate from the humidified 37°C / 5% CO_2 incubator.
2. Carefully aspirate Assay Media using multi-channel aspirator.
3. Add 20 µl of complete lysis buffer (prepared in **5.2.3**) to each well and cover plate to prevent evaporation.
4. Incubate covered plate at room temperature for 60 minutes.

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.

5.3.1 Instrumentation, Filters, and Plates

The data presented in this document were generated using a PHERAstar plate reader (BMG LABTECH) 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 monochromator-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 fluorescence plate reader:

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 (Terbium) Signal	Measure Acceptor (GFP) Signal
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	

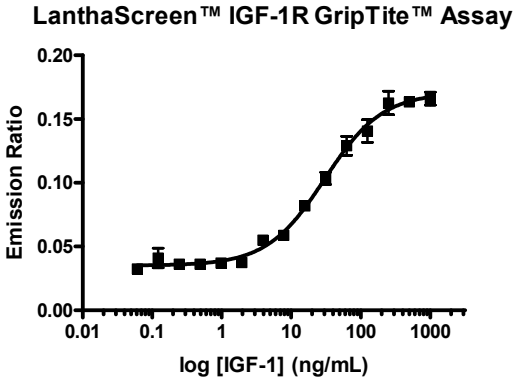
4. Calculate the acceptor/donor Emission Ratio (TR-FRET Ratio) for each well, by dividing the acceptor emission values (520 nm) by the donor emission values (490 nm).

6. Representative Data

6.1 Background Subtraction

1. Be sure to include ***cell-free*** control wells when performing the LanthaScreen™ assay. These wells should receive the appropriate amount of Assay Media and Lysis Buffer (including Tb-labeled antibody) and are used for background subtraction.
2. Determine the average 520 nm emission values from the cell-free control wells. This is the average acceptor background data coming from the Tb-antibody.
3. Subtract the average acceptor background data (the average 520 nm emission value determined above) from all of the 520 nm acceptor emission data in the cellular assay samples, thus providing background-subtracted acceptor data.
4. Calculate the acceptor/donor Emission Ratio for each well, by dividing the background-subtracted acceptor emission values by the donor emission values. That is, divide the background-subtracted 520 nm values (acceptor data) by the raw 490 nm values (donor data) to generate ratiometric TR-FRET data.
5. To obtain the Response Ratio, all TR-FRET emission ratios (520 / 490) are normalized against unstimulated values (the average of zero agonist or zero compound) for that particular experiment.

6.2 Representative Data



Average EC_{50} IGF-1 (ng/ml)	30.6
Average EC_{80} IGF-1 (ng/ml)	113.5
Max Stim Used	100 nM
Average Z' factor	0.76
Average Response Ratio	5.5

Figure 1. IGF-1 dose-response curve. LanthaScreen™ IGF-1R GripTite™ cells (20,000 cells/well in 32 µl of Assay Medium, 384-well format) were plated the day prior to the assay to serum starve overnight. On the day of the assay, cells were pretreated with 4 µl of 1% DMSO before treatment with the indicated concentrations of IGF-1 (4 µl addition) for 30 minutes. Following removal of the Assay Medium by aspiration, cells were lysed by addition of 20 µl of complete lysis buffer (which included both 2 nM of LanthaScreen™ Tb-pY20 antibody and protease + phosphatase inhibitor cocktails). The plate was incubated for 60 minutes at room temperature. Fluorescence emission values at 520 nm and 490 nm were obtained using a PHERAstar plate reader (BMG LABTECH) set to TR-FRET mode. The 520/490 nm emission ratios (no background subtraction) are plotted for each experiment, with n≥8 replicates per data point.

7. References

Carlson, C.B., Robers, M. B., Vogel, K. W., and Machleidt, T. (2009) **Development of LanthaScreen cellular assays for key components within the PI3K/AKT/mTOR pathway.** *J Biomol Screen 14*, 121-132

Robers, M. B., Horton, R. A., Bercher, M. R., Vogel, K. W., and Machleidt, T. (2008) **High-throughput cellular assays for regulated posttranslational modifications.** *Anal Biochem 372*, 189-197

8. Purchaser Notification

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Use of Genetically Modified Organisms (GMO)

Information for European Customers The LanthaScreen™ IGF-1R GripTite™ cell line is genetically modified with a pcDNA6-cYFP plasmid. 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.