Screening Protocol and Assay Conditions

Revised 07-Jun-16

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Overview and Assay Theory

The LanthaScreen Cellular Assay Custom Profiling Service from Life Technologies offers a method for the identification of compounds that target a cell signaling pathway by inhibiting an intermediate intracellular signaling event (e.g., phosphorylation). This assay platform uses time-resolved resonance energy transfer (TR-FRET) between a terbium-labeled phosphorylation-site specific antibody (PSSA) and a green fluorescent protein (GFP) fusion of a particular kinase substrate to provide an assay readout that is ratiometric, robust, and amenable to high-throughput screening (HTS) applications. Moreover, the use of a GFP fusion of the target along with a single detection antibody simplifies the assay protocol, eliminates the need for beads or additional reagents, and simplifies the assay relative to other two-antibody "sandwich" approaches (see **Figure 1**).

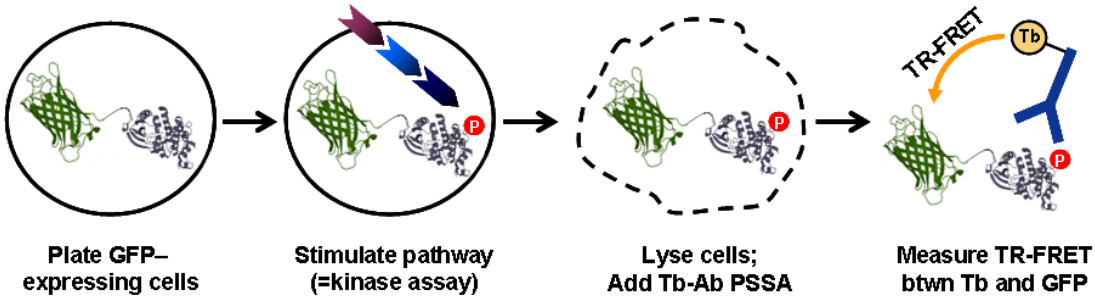

Figure 1 – LanthaScreen cellular assay schematic. Stable cell lines are generated that express the kinase substrate of interest as a GFP-fusion protein. Cells are plated in 384-well format and then treated (in the presence or absence of an inhibitor) to activate the signaling pathway to trigger phosphorylation by the endogenous kinase. The resulting modification is detected upon lysis of the cells and addition of a Tb-labeled phosphor-site specific antibody (Tb-PSSA) in the same step. The TR-FRET signal is subsequently measured on a fluorescence plate reader.

We ensure the consistency, reliability and performance of each cell line. Attributes of LanthaScreen Cellular Assays:

- Provide ready-to-screen, ratiometric assays for disease relevant targets
- Are functionally validated to ensure high-quality results consistently

These assays are shown to meet the following specifications:

- Z′-factor of 0.5 or greater for activator assays and Z′-factor of 0.4 or greater for inhibitor assays
- Appropriate EC_{50}/IC_{50} responses to known activators and inhibitors

Any assay results not meeting these specifications are automatically repeated until the results pass our QC criteria.

LanthaScreen Cellular Assay Conditions

Test Compounds

All Test Compounds are initially prepared at a 1000X concentration in 100% DMSO. Serial dilutions (½ log) of the Test Compounds are prepared in DMSO. The Known Inhibitor is prepared in this same manner.

Assay Plate

Corning 384-well white, flat bottom, polystyrene, tissue-culture treated assay plate (Corning #3570).

Assay Media

LanthaScreen Cellular Assays are typically run in low-serum (or serum-free media) in order to lower pathway activation and provide a baseline for subsequent analyses.

Lysis Buffer

The complete LanthaScreen Cellular Assay Lysis Buffer consists of 20 mM Tris-HCl, pH 7.4, 5 mM EDTA, 5 mM NaF, 150 mM NaCl, 1% NP-40 (or equivalent), protease inhibitor cocktail (Sigma #P8340), phosphatase inhibitor cocktail (Sigma #P2850), and 2 to 5 nM of the appropriate Tb-PSSA.

Agonist Assay Protocol (General)

1. 32 µL of cells diluted in Assay Media to appropriate cell density are added to the assay plate. If needed, cells are incubated at 37°C/5% CO_2 for 0 to 24 hours (depending upon cell line specifics) before compound is added.
2. 40 nL of 1000X compound or known activator titration is added to the cells in the assay plate. 8 µL of Assay Medium is added to these wells.
3. 8 µL of Assay Medium is added to remaining control wells to bring the volume up to 40 µL.
4. The assay plate is incubated at 37ºC/5% CO_2 in a humidified incubator for a pre-determined length of time (cell line/assay specific).
5. Cells are lysed by the addition of lysis buffer with a pre-determined concentration of Tb-labeled Ab (assay specific).
6. The assay plates are incubated in the dark at room temperature for a pre-determined length of time (assay specific).
7. Read plate on a fluorescence plate reader.

Antagonist Assay Protocol (General)

1. 32 µL of cells diluted in Assay Media to appropriate cell density are added to the assay plate. If needed, cells are incubated at 37°C/5% CO_2 for 0 to 24 hours (depending upon cell line specifics) before compound is added.
2. 40 nL of 1000X compound or known inhibitor titration plus 4 µL of assay media is added to the cells in the assay plate and incubated for 30 minutes at 37°C/5% CO_2 in a humidified incubator.
3. 4 µL of the EC_{80} concentration of activator, as determined in an Activator assay, is added to all wells containing test compound and known inhibitor to bring the final assay volume to 40 µL.
4. 4 µL of Assay Medium is added to remaining control wells to bring the volume up to 40 µL.
5. The assay plate is incubated at 37ºC/5% CO_2 in a humidified incubator for a pre-determined length of time (cell line/assay specific).

6. Cells are lysed by the addition of lysis buffer with a pre-determined concentration of Tb-labeled Ab (assay specific).
6. The assay plates are incubated in the dark at room temperature for a pre-determined length of time (assay specific).
7. Read plate on a fluorescence plate reader.

Assay Controls

The following controls are made for each individual assay, on every assay plate:

MAX STIM Control (If Applicable)
The maximum TR-FRET signal (Emission Ratio; 520 nm/490 nm) is established by the MAX STIM Control (or the 0% Inhibition Control). These control wells contain GFP+ cells stimulated with an EC_{100} concentration of agonist in the presence of 0.1% DMSO.

UNSTIM Control
The minimum TR-FRET signal is established by the UNSTIM Control. These control wells contain unstimulated cells in the presence of 0.1% DMSO.

EC_{80} Control (inhibitor mode only)
The EC_{80} control is a concentration of the known activator in assay media that has been determined through an activator experiment. In inhibitor mode, the EC_{80} control is used to determine the actual baseline of activation or 0% inhibition.

0% Inhibition
The TR-FRET signal obtained from wells containing cells stimulated with an EC_{80} concentration of agonist in the presence of 0.1% DMSO (no compound present).

Known Inhibitor Titration (If Applicable)
A Known Inhibitor control standard curve (10-point titration) is run on each assay plate to ensure that the assay is inhibited within an expected IC_{50} range.

Data Analysis

The following equations are used for each set of data points:

	Equation
Emission Ratio	**GFP Emission (520 nm) / Terbium Emission (490 nm)**
% Activation – Activator Assays	$\left\{ \frac{\text{Emission Ratio}_{\text{Compound}} - \text{Emission Ratio}_{\text{No Act. Ctrl}}}{\text{Emission Ratio}_{\text{Full Act. Ctrl}} - \text{Emission Ratio}_{\text{No Act. Ctrl}}} \right\} \text{X } 100$
% Inhibition – Inhibitor Assays	$\left\{ 1 - \frac{\text{Emission Ratio}_{\text{Compound}} - \text{Emission Ratio}_{\text{No Act. Ctrl}}}{\text{Emission Ratio}_{\text{EC80 Ctrl}} - \text{Emission Ratio}_{\text{No Act. Ctrl}}} \right\} \text{X } 100$
Z' - Activator Assays (using Emission Ratio values)	$1 - \frac{3\text{xStd Dev}_{\text{Full Act. Ctrl}} + 3\text{xStd Dev}_{\text{No Act. Ctrl}}}{\text{Mean}_{\text{Full Act. Ctrl}} - \text{Mean}_{\text{No Act. Ctrl}}}$
Z' - Inhibitor Assays (using Emission Ratio values)	$1 - \frac{3\text{xStd Dev}_{\text{EC80 Ctrl}} + 3\text{xStd Dev}_{\text{No Act. Ctrl}}}{\text{Mean}_{\text{EC80 Ctrl}} - \text{Mean}_{\text{No Act. Ctrl}}}$

Graphing Software

The Custom Profiling Service uses either Prism 4 from GraphPad or XL*fit* from IDBS to plot the data. Each dose-response curve is fit to a sigmoidal dose-response (variable slope) model. Data is plotted as the Response Ratio (with background subtraction and normalized to untreated cells).

LanthaScreen Cell Lines Available for Screening

Assay	Cell Line	Tech-nology	Activator	EC50 (nM)	Inhibitor	IC50 (nM)	Act. Mode	Inh. Mode
AKT [pSer473]	AKT GFP HEK293E	LS	IGF-1	0.193	PI-103	67.7	Yes	Yes
AKT [pThr308]	AKT GFP HEK293E	LS	IGF-1	0.400	PI-103	51.6	Yes	Yes
ATF2	ATF2 (19-106) A549	LS	Anisomycin	8.28	JNK Inhibitor II	6,550	Yes	Yes
ATF2	ATF2 (19-106) A549	LS	TNF-alpha	0.001	JNK Inhibitor II	5,100	Yes	Yes
c-jun	c-jun HeLa	LS	Anisomycin	15.5	JNK Inhibitor II	4,860	Yes	Yes
c-jun	c-jun HeLa	LS	TNF-alpha	0.010	JNK Inhibitor II	5,140	Yes	Yes
ERK2	ERK2 GFP U2OS	LS	EGF	0.035	U0126	54.1	Yes	Yes
ERK2	ERK2 GFP U2OS	LS	PDGF	0.105	U0126	48.6	Yes	Yes
ERK2 (BRAF V600E)	ERK2 A375	LS	None	---	Raf1 Kinase Inhibitor	389	No	Yes
IGF-1R	IGF-1R GripTite	LS	IGF-1	1.27	PQ401	7,330	Yes	Yes
IkB-alpha	IkBalpha GripTite	LS	TNF-alpha	0.049	BMS345541	5,570	Yes	Yes
PDCD4	PDCD4 HEK293E	LS	IGF-1	0.010	Rapamycin	0.568	Yes	Yes
PDCD4	PDCD4 HEK293E	LS	Insulin	0.098	Rapamycin	0.332	Yes	Yes
PRAS40 [pSer183]	PRAS40 HEK293	LS	IGF-1	0.008	PI-103	43.9	Yes	Yes
PRAS40 [pThr246]	PRAS40 HEK293	LS	IGF-1	0.005	PI-103	64.5	Yes	Yes
PRAS40 [pSer183]	PRAS40 HEK293	LS	Insulin	0.038	PI-103	32.7	Yes	Yes
PRAS40 [pThr246]	PRAS40 HEK293	LS	Insulin	0.018	PI-103	39.7	Yes	Yes
STAT1	STAT1 U2OS	LS	IFN-alpha	0.080	JAK Inhibitor I	10.6	Yes	Yes
STAT1	STAT1 U2OS	LS	IFN-gamma	0.059	JAK Inhibitor I	16.1	Yes	Yes
STAT3	STAT3 GripTite	LS	IFN-alpha	0.044	JAK Inhibitor I	8.73	Yes	Yes
STAT3	STAT3 GripTite	LS	IL-6	0.054	JAK Inhibitor I	18.0	Yes	Yes
STAT5	STAT5 TF-1	LS	EPO	0.010	JAK Inhibitor I	16.0	Yes	Yes
STAT5	STAT5 TF-1	LS	GM-CSF	0.003	JAK Inhibitor I	37.3	Yes	Yes
STAT5 (JAK2 V617F)	STAT5 (JAK2 V617F) U2OS	LS	None	---	JAK Inhibitor I	55.2	No	Yes

*EC_{50} and IC_{50} values are representative

Cell Line-Specific Assay Conditions

AKT [pSer473] - LanthaScreen AKT GFP HEK293E - Activator Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IGF-1 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-AKT [pSer473] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

AKT [pSer473] - LanthaScreen AKT GFP HEK293E - Inhibitor Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PI-103 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IGF-1 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-AKT [pSer473] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

AKT [pThr308] - LanthaScreen AKT GFP HEK293E - Activator Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IGF-1 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-AKT [pThr308] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

AKT [pThr308] - LanthaScreen AKT GFP HEK293E - Inhibitor Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PI-103 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IGF-1 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-AKT [pThr308] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ATF2 [pThr71] - LanthaScreen ATF2 (19-106) A549 - Activator Screen, Anisomycin Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator Anisomycin or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ATF2 [pThr71] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ATF2 [pThr71] - LanthaScreen ATF2 (19-106) A549 - Inhibitor Screen, Anisomycin Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JNK Inhibitor II or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator Anisomycin at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ATF2 [pThr71] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ATF2 [pThr71] - LanthaScreen ATF2 (19-106) A549 - Activator Screen, TNF-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator TNF-alpha or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ATF2 [pThr71] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ATF2 [pThr71] - LanthaScreen ATF2 (19-106) A549 - Inhibitor Screen, TNF-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JNK Inhibitor II or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator TNF-alpha at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ATF2 [pThr71] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

c-jun [pSer73] - LanthaScreen c-jun HeLa - Activator Screen, Anisomycin Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator Anisomycin or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-c-jun [pSer73] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

c-jun [pSer73] - LanthaScreen c-jun HeLa - Inhibitor Screen, Anisomycin Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JNK Inhibitor II or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator Anisomycin at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-c-jun [pSer73] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

c-jun [pSer73] - LanthaScreen c-jun HeLa - Activator Screen, TNF-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator TNF-alpha or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-c-jun [pSer73] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

c-jun [pSer73] - LanthaScreen c-jun HeLa - Inhibitor Screen, TNF-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 312,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (10,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JNK Inhibitor II or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator TNF-alpha at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-c-jun [pSer73] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ERK2 [pThr185/pTyr187] - LanthaScreen ERK2 GFP U2OS - Activator Screen, EGF Stimulation
Cells are harvested and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator EGF or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 6 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ERK2 [pThr185/pTyr187] antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ERK2 [pThr185/pTyr187] - LanthaScreen ERK2 GFP U2OS - Inhibitor Screen, EGF Stimulation
Cells are harvested and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor U0126 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator EGF at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 6 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ERK2 [pThr185/pTyr187] antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ERK2 [pThr185/pTyr187] - LanthaScreen ERK2 GFP U2OS - Activator Screen, PDGF Stimulation
Cells are harvested and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator PDGF or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 6 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ERK2 [pThr185/pTyr187] antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ERK2 [pThr185/pTyr187] - LanthaScreen ERK2 GFP U2OS - Inhibitor Screen, PDGF Stimulation
Cells are harvested and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor U0126 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator PDGF at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 6 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ERK2 [pThr185/pTyr187] antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

ERK2 [pThr185/pTyr187] - LanthaScreen ERK2 A375 - Inhibitor Screen, Constitutively Activated
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 468,750 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (15,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor Raf1 Kinase Inhibitor or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-ERK2 [pThr185/pTyr187] antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

IGF-1R [pY20] - LanthaScreen IGF-1R GripTite - Activator Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IGF-1 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-pY20 antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

IGF-1R [pY20] - LanthaScreen IGF-1R GripTite - Inhibitor Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PQ401 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IGF-1 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 2 nM of LanthaScreen Tb-anti-pY20 antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

IkBalpha [pSer32] - LanthaScreen IkBalpha GripTite - Activator Screen, TNF-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator TNF-alpha or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-IkBalpha [pSer32] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

IkBalpha [pSer32] - LanthaScreen IkBalpha GripTite - Inhibitor Screen, TNF-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor BMS345541 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator TNF-alpha at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-IkBalpha [pSer32] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PDCD4 [pSer457] - LanthaScreen PDCD4 HEK293E - Activator Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 937,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (30,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IGF-1 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PDCD4 [pSer457] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PDCD4 [pSer457] - LanthaScreen PDCD4 HEK293E - Inhibitor Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 937,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (30,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor Rapamycin or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IGF-1 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PDCD4 [pSer457] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PDCD4 [pSer457] - LanthaScreen PDCD4 HEK293E - Activator Screen, Insulin Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 937,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (30,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator Insulin or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PDCD4 [pSer457] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PDCD4 [pSer457] - LanthaScreen PDCD4 HEK293E - Inhibitor Screen, Insulin Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 937,500 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (30,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor Rapamycin or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator Insulin at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PDCD4 [pSer457] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pSer183] - LanthaScreen PRAS40 HEK293 - Activator Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IGF-1 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pSer183] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pSer183] - LanthaScreen PRAS40 HEK293 - Inhibitor Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PI-103 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IGF-1 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pSer183] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pThr246] - LanthaScreen PRAS40 HEK293 - Activator Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IGF-1 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pThr246] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pThr246] - LanthaScreen PRAS40 HEK293 - Inhibitor Screen, IGF-1 Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PI-103 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IGF-1 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pThr246] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pSer183] - LanthaScreen PRAS40 HEK293 - Activator Screen, Insulin Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator Insulin or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pSer183] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pSer183] - LanthaScreen PRAS40 HEK293 - Inhibitor Screen, Insulin Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PI-103 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator Insulin at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pSer183] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pThr246] - LanthaScreen PRAS40 HEK293 - Activator Screen, Insulin Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator Insulin or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pThr246] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

PRAS40 [pThr246] - LanthaScreen PRAS40 HEK293 - Inhibitor Screen, Insulin Stimulation
Cells are thawed and resuspended in Assay Media (DMEM phenol red free, 0.1% BSA, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor PI-103 or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator Insulin at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-PRAS40 [pThr246] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT1 [pTyr701] - LanthaScreen STAT1 U2OS - Activator Screen, IFN-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IFN-alpha or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 60 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT1 [pTyr701] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT1 [pTyr701] - LanthaScreen STAT1 U2OS - Inhibitor Screen, IFN-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IFN-alpha at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 60 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT1 [pTyr701] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT1 [pTyr701] - LanthaScreen STAT1 U2OS - Activator Screen, IFN-gamma Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IFN-gamma or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 60 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT1 [pTyr701] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT1 [pTyr701] - LanthaScreen STAT1 U2OS - Inhibitor Screen, IFN-gamma Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IFN-gamma at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 60 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT1 [pTyr701] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT3 [pTyr705] - LanthaScreen STAT3 GripTite - Activator Screen, IFN-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IFN-alpha or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT3 [pTyr705] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT3 [pTyr705] - LanthaScreen STAT3 GripTite - Inhibitor Screen, IFN-alpha Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IFN-alpha at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT3 [pTyr705] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT3 [pTyr705] - LanthaScreen STAT3 GripTite - Activator Screen, IL-6 Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator IL-6 or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT3 [pTyr705] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT3 [pTyr705] - LanthaScreen STAT3 GripTite - Inhibitor Screen, IL-6 Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 625,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (20,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator IL-6 at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen Tb-anti-STAT3 [pTyr705] antibody is added to the wells. The assay plate is incubated for 60 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT5 A/B [pTyr694/699] - LanthaScreen STAT5 TF-1 - Activator Screen, EPO Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 3,125,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (100,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator EPO or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen anti-STAT5 A/B [pTyr694/699] antibody and 10 nM of Tb-anti-Mouse antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT5 A/B [pTyr694/699] - LanthaScreen STAT5 TF-1 - Inhibitor Screen, EPO Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 3,125,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (100,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator EPO at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen anti-STAT5 A/B [pTyr694/699] antibody and 10 nM of Tb-anti-Mouse antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT5 A/B [pTyr694/699] - LanthaScreen STAT5 TF-1 - Activator Screen, GM-CSF Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 3,125,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (100,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control activator GM-CSF or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen anti-STAT5 A/B [pTyr694/699] antibody and 10 nM of Tb-anti-Mouse antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT5 A/B [pTyr694/699] - LanthaScreen STAT5 TF-1 - Inhibitor Screen, GM-CSF Stimulation
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 0.5% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 3,125,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (100,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 4 µL of Assay Media. The assay plate is incubated for 30-60 minutes at 37ºC/5% CO2 in a humidified incubator. 4 µL of 10X control activator GM-CSF at the pre-determined EC80 concentration is added to wells containing the control inhibitor or compounds. The assay plate is incubated for 30 minutes at 37ºC/5% CO2 in a humidified incubator. 30 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen anti-STAT5 A/B [pTyr694/699] antibody and 10 nM of Tb-anti-Mouse antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.

STAT5 A/B [pTyr694/699] - LanthaScreen STAT5 (JAK2 V617F) U2OS - Inhibitor Screen, Constitutively Activated
Cells are thawed and resuspended in Assay Media (OPTI-MEM, 1% csFBS, 0.1 mM NEAA, 1 mM Sodium Pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 375,000 cells/mL. 32 µL of the cell suspension is added to each well of a white TC-Treated assay plate (12,000 cells/well) and incubated for 16-24 hours at 37 ºC / 5% CO2 in a humidified incubator. 40 nL of the control inhibitor JAK Inhibitor I or test compound is added to the appropriate assay wells followed by an addition of 8 µL of Assay Media. The assay plate is incubated for 120 minutes at 37ºC/5% CO2 in a humidified incubator. The Assay Media is aspirated from the assay wells. 20 µL of LanthaScreen Cellular Assay Lysis Buffer containing 5 nM of LanthaScreen anti-STAT5 A/B [pTyr694/699] antibody and 10 nM of Tb-anti-Mouse antibody is added to the wells. The assay plate is incubated for 120 minutes at room temperature. The assay plate is read with a fluorescent plate reader.