PhosphoSens®-Lysate Continuous Fluorescent Intensity (FI) Kinase Assay

Basic Lysate Assay Protocol to Test Lysate Activity:

The assay will be testing the activity of the target kinase in a lysate of choice. The protocol will use heat-inactivated lysates as a negative control. Perform the assay to establish the following –

A. The target kinase activity is lost when the lysates are heat-inactivated, thereby establishing a negative control.

B. The recombinant target kinase exhibits phosphorylation activity in the presence of the sensor peptide substrate, thereby establishing a positive control.

C. Lysates with the target kinase exhibit phosphorylation activity in the presence of the sensor peptide substrate.

D. The target kinase activity in the lysates is inhibited by the tool compound.

STEP	PROCESS		
0	Prepare 1X EDB to be used in the assay.		
1	Prepare '1.72X Master Mix' by combining each of the components listed to the right. *Include other components as required.*	Component:	For 1 Well:
		Enzyme Reaction Buffer (10X)	2.52 µL
		ATP (10 mM)	2.52 µL
		DTT solution (10 mM)	2.52 µL
		EGTA Solution (5.5 mM)	2.52 µL
		Ultrapure deionized H2O	4.42 µL
		Total volume	14.5 µL
2	Add 14.5 µL 'Master Mix' with Reaction Buffer, ATP, DTT, and EGTA to each well.		
3	Add 5 µL 50 µM Sensor Peptide (5X) to each well.		
4	Add 0.5 µL 50X inhibitor dilutions in 100% DMSO or 100% DMSO vehicle to each well.		
5	Seal the plate and incubate at 30 °C for 5 minutes to equilibrate the plate		
6	*Add 5 µL of any of the following depending on the wells 1.EDB, or 2.EDB with enzyme at 5X the final concentration, or 3. heat inactivated lysates (control at 0.2 mg/mL), 4. Or Lysates with the target activity (0.2 mg/mL).		
7	Add plate to reader and monitor kinase activity by collecting fluorescence intensity (RFU) readings (lExMax 360 nm/lEmMax ~492 nm [485-498 nm]) every 2.0 minutes at 30°C for 2 hours or for 4 hours.		

Basic Lysate Assay Data Analysis:

Subtract the background determined with the "blank buffer " (No kinase/No lysate/Heat-inactivated lysate) for each time point from the total signals to obtain corrected Relative Fluorescence Units (RFU) values. It is highly recommended to run " blank buffer " at each compound concentration to correct for tool compound absorbance or fluorescence, if any. The slope from the "Active Linear range" of the progress curve gives the rate of the reaction in RFU/min. Compare the RFU/min values for the samples to evaluate the activity of target kinase in the tested lysates.