

Kinase Assay	are read 3 minutes after the addition of the detection solution. The absorbance is measured at 690 nm by a microtiter dish reader. A phosphate standard curve is used to calculate the μMol of phosphate formed. Samples are measured in triplicate.
Cell Research	Cell viability is determined using a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye reduction method. Cells are harvested during logarithmic growth phase, and seeded in 96-well plates. The cells are then cultured for 72 hours in the presence of oncolytics with or without modulators. MCF-7 and MCF-7/ADR cells are incubated 24 hours before the addition of the drug with and without the LY335979. LY335979 is prepared as 2 mM DMSO stocks and added to wells to give final concentrations ranging from 0.05 to 5 μM . After 72 hours, 20 μL of freshly prepared 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg/mL in Dulbecco's PBS) is added to each well and incubated for 4 hours in a 37 °C incubator containing 5% CO ₂ . Cells are pelleted in a Sorvall RT6000B centrifuge, 70 μL of medium is carefully removed from each well, and 100 μL of 2-propanol/0.04 N HCl is added. Cells are resuspended 5-10 times with a Multipipettor or until no particulate matter is visible. Plates are immediately read on a Titertek Multiskan MCC/340 microplate reader Flow Laboratories with a test wavelength of 570 nm and a reference wavelength of 630 nm. Controls are measured in quadruplicate and modulators are measured in duplicate. Cytotoxicity analyses are also performed using the CeliTiter 96 Aqueous assay kit.(Only for Reference)

Solubility Information

Solubility	DMSO: 80.83 mg/mL (126.89 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
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Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.5699 mL	7.8494 mL	15.6988 mL
5 mM	0.314 mL	1.5699 mL	3.1398 mL
10 mM	0.157 mL	0.7849 mL	1.5699 mL
50 mM	0.0314 mL	0.157 mL	0.314 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

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