Description

The ROS1 Kinase Assay Kit is designed to measure ROS1 activity for screening and profiling applications using Kinase-Glo® MAX as a detection reagent. The ROS1 Kinase Assay Kit comes in a convenient 96-well format, with enough purified ROS1, substrate IGF-1Rtide, ATP, and kinase assay buffer for 96 enzyme reactions. The signal is measured using a luminometer or a microplate reader capable of reading chemiluminescence.

Background

ROS1 is a receptor tyrosine kinase playing a role in many human cancers, including non-small cell lung cancer and ovarian cancer. ROS1 undergoes genetic rearrangements, creating fusion proteins that drive cellular proliferation. Recent preclinical and clinical findings suggest that targeting this receptor kinase with crizotinib shows promise for cancer treatment.

Applications

Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40268	ROS1*	10 µg	-80°C
79334	5x Kinase assay buffer	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
	IGF-1Rtide (10 mg/ml)	50 µl	-20°C
79696	96-well plate, white	1	Room Temp.

*** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.**

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo® MAX	Promega #V6073
Dithiothreitol (DTT; 0.5 M)	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw 5x Kinase assay buffer, ATP (500 µM), and IGF-1Rtide (10 mg/ml). Add DTT (0.5 M) to 5x Kinase assay buffer to make a 10 mM concentration (e.g. add 20 µl of 0.5 M DTT to 1 ml 5x Kinase assay buffer.
2. Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl of distilled water. Three ml of 1x Kinase assay buffer is sufficient for 100 reactions.
3. Prepare the Master Mix (25 µl per well): N wells x (23.5 µl of 1x Kinase assay buffer + 1 µl of ATP (500 µM) + 0.5 µl of IGF-1Rtide (10 mg/ml)). Add 25 µl to every well.
4. Prepare test inhibitor by diluting the stock solution of test compound 1:10 with 1x kinase assay buffer. Add 5 µl to each well labeled as “Test Inhibitor.”
   Note: If inhibitor was dissolved in DMSO, keep DMSO concentration of the Test Inhibitor at ≤10% in the inhibitor solution, as the final DMSO concentration in the reaction should be ≤1%.
5. For the “Positive Control" and “Blank,” prepare a “Diluent Solution” that contains the same concentration of diluent as the test inhibitor in 1x Kinase assay buffer (for example 10% DMSO in 1x Kinase assay buffer if the inhibitor was dissolved in DMSO), but does not contain inhibitor. Add 5 µl of this diluent solution to “Blank” and “Positive Control”.
6. To the wells designated as "Blank," add 20 µl of 1x Kinase assay buffer.
7. Thaw ROS1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of ROS1 required for the assay and dilute enzyme to 5 ng/µl with 1x Kinase assay buffer. Store remaining undiluted enzyme in aliquots at -80°C.
   Note: ROS1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
8. Initiate reaction by adding 20 µl of diluted ROS1 enzyme to the wells designated “Positive Control” and "Test Inhibitor." Incubate at 30°C for 45 minutes.

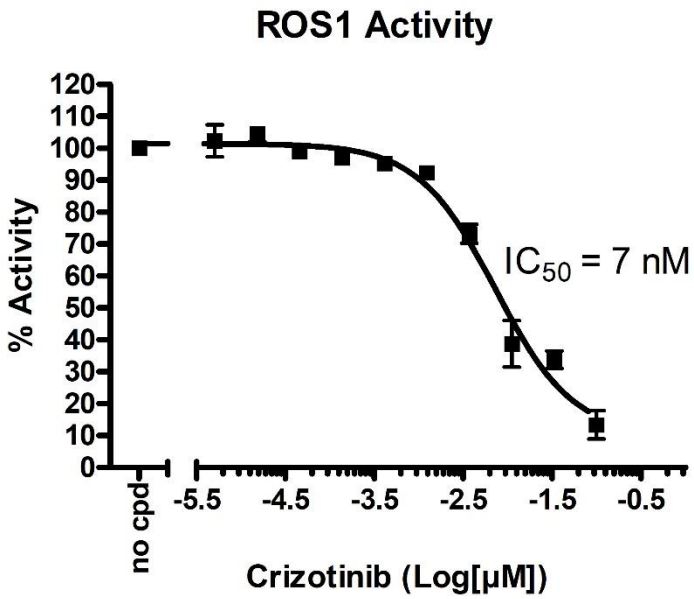
Component	Blank	Positive Control	Test Inhibitor
Master Mix	25 µl	25 µl	25 µl
Test inhibitor	-	-	5 µl
Diluent solution (no inhibitor)	5 µl	5 µl	
1x Kinase assay buffer	20 µl		-
ROS1 (5 ng/µl)	-	20 µl	20 µl
Total	50 µl	50 µl	50 µl

9. Thaw Kinase-Glo® MAX reagent.
10. After the 45 minutes reaction, add 50 µl of Kinase-Glo® MAX reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.
11. Read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry. To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results



*Inhibition of ROS1 by Crizotinib (Medchem Express #*HY-50878*), measured using the ROS1 kinase assay kit (BPS Bioscience, #78189). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

General considerations

"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

1. Davies, Kurtis D., and Doebele , Robert C.. "Molecular pathways: ROS1 fusion proteins in cancer." *Clinical Cancer Research* **19.15** (2013): 4040-4045.
2. Davies, Kurtis D., *et al*. "Identifying and targeting ROS1 gene fusions in non–small cell lung cancer." *Clinical Cancer Research* **18.17** (2012): 4570-4579.
3. Rimkunas, Victoria M., *et al*. "Analysis of receptor tyrosine kinase ROS1-positive tumors in non–small cell lung cancer: identification of a FIG-ROS1 fusion." *Clinical Cancer Research* **18.16** (2012): 4449-4457.

Related Products

Products	*Catalog #*	*Size*
ROS1, GST-tag	40268	10 µg
cKIT Assay Kit	79815	96 rxns.
cKIT (D816V) Assay Kit	79889	96 rxns.
c-Mer Kinase Assay Kit	79660	96 rxns.
c-Met Kinase Assay Kit	79559	96 rxns.
EGFR Kinase Assay Kit	40321	96 rxns.
EGFR(L858R) Kinase Assay Kit	40324	96 rxns.
EGFR(T790M/L858) Kinase Assay Kit	40322	96 rxns.
EGFR (T790M/C797S/ L858) Kinase Assay Kit	40326	96 rxns.