Chemi-Verse™ ROS1 Kinase Assay Kit

#82574
96 reactions

Description

The Chemi-Verse™ ROS1 Kinase Assay Kit is designed to measure ROS1 (ROS Proto-Oncogene 1) tyrosine kinase activity for screening and profiling applications using ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified ROS1 kinase domain (amino acids 1883-2347(end)), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

ROS1 (proto-oncogene ROS1), also known as c-ROS, is a type I tyrosine kinase receptor of the insulin receptor family. This protein is involved in cell differentiation and proliferation. ROS1 can act with multiple adaptor proteins involved in PI3K (phosphoinositide 3-kinase)/AKT, JAK (Janus kinase)/STAT3 (signal transducer and activator of transcription 3) and other signaling pathways. Mutations, rearrangements, and fusions of this gene can promote proliferation of tumor cells, such as in NSCLC (non-small cell lung cancer), with gene fusions being the highest driving force in tumor development and progression. For instance, fusion to CD74 is found in 38% of the $ROS1^+$ NSCLCs. The specific gene fusions localize the protein in different cellular compartments and thus leads to activation of different pathways. Overexpression was found in primary gliomas and meningiomas. The use of crizotinib and ceritinib, two ROS1-targeting TKIs (tyrosine kinase inhibitors), has been approved for use in $ROS1^+$ NSCLC. However, there are concerns with the development of drug resistance, due to mutation or activation of alternative signaling pathways, and side effects that range from edema to neutropenia, as ROS1 inhibitors are multi-kinase inhibitors. Next-generation inhibitors with higher affinity are currently in clinical trials and promise improved outcomes in the treatment of ROS1-related disorders.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40268	ROS1, GST-His-Tag*	4 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
79567	IGF-1Rtide (1 mg/ml)	250 µl	-20°C
82545	White 96-well plate	1	Room Temperature

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

Materials Required but Not Supplied

Name	Ordering Information
ADP-Glo™ Kinase Assay	Promega #V6930
DTT (Dithiothreitol), 1M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

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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 Principle

The **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The newly generated ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear to 1 mM ATP.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Crizotinib (#82583) or Staurosporine (#27002) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP,** and **IGF-1Rtide (1 mg/ml).**

   *Optional: If desired, make **5x Kinase Assay Buffer 1** with 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Assay Buffer 1** by mixing 600 µl of **5x Kinase Assay Buffer 1** with 2,400 µl of distilled water.

   *Note: Three (3 ml) of **1x Kinase Assay Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 2.5 µl of IGF-1Rtide (1 mg/ml) + 3.5 µl of distilled water).

4. Add 12.5 µl of Master Mix to every well.

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5. Prepare the **Test Inhibitor** (2.5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Kinase Assay Buffer 1, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x Kinase Assay Buffer 1 (Diluent Solution).

   OR

   5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer 1 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 1%.

6. Add 2.5 µl of Test Inhibitor to each well labeled “Test Inhibitor”.

7. Add 2.5 µl of Diluent Solution to the “Positive Control" and “Blank” wells.

8. Add 10 µl of 1x Kinase Assay Buffer 1 to the "Blank" wells.

9. Thaw **ROS1 Kinase** on ice. Briefly spin the tube to recover its full content.

10. Dilute the protein kinase (10 µl/well) to 4 ng/µl with **1x Kinase Assay Buffer 1**.

11. Initiate the reaction by adding 10 µl of diluted kinase to the wells designated “Positive Control” and "Test Inhibitor".

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
Diluted ROS1 (4 ng/µl)	-	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**

12. Incubate at 30°C for 45 minutes.

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13. Thaw the ADP-Glo™ reagent.
14. At the end of the 45 minute reaction, add 25 µl of ADP-Glo™ reagent to each well.
15. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
16. Thaw the Kinase Detection Reagent.
17. Add 50 µl of Kinase Detection reagent to each well.
18. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
19. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
20. The “Blank” value is subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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).

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Example Results

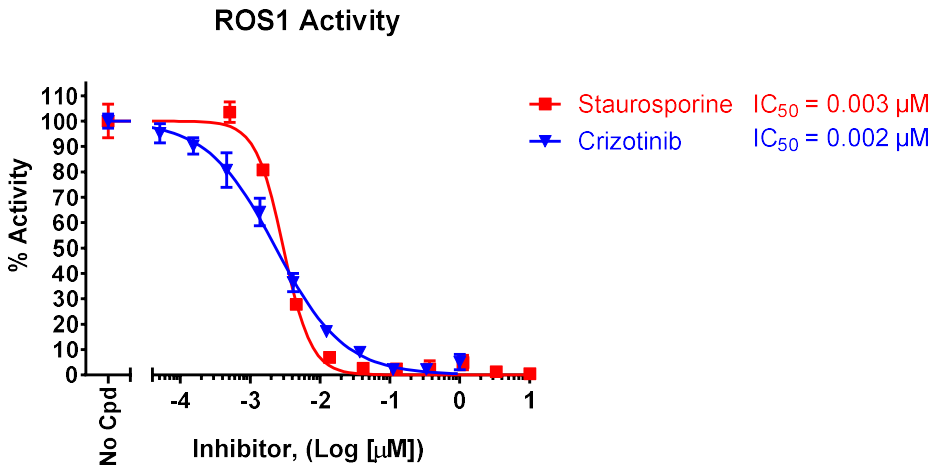

Figure 1: Inhibition of ROS1 kinase activity by the inhibitors Crizotinib and Staurosporine.
ROS1 kinase activity was measured in the presence of increasing concentrations of Crizotinib (#82583) or Staurosporine (#27002). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

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

References
Li S., *et al.*, 2024 *Cancer Med* 13(8):e7201.

Related Products

Products	*Catalog #*	*Size*
ROS1 (G2032R), GST-Tag Recombinant	101640	10 µg
ROS1 (G2101A), GST-Tag Recombinant	101641	10 µg
ALK1 (ACVRL1) Kinase Assay Kit	79549	96 reactions
ALK2 (ACVR1) Kinase Assay Kit	79605	96 reactions

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