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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Chemi-Verse™ ROCK2 Kinase Assay Kit is designed to measure ROCK2 (Rho-associated coiled-coil protein kinase 2) serine/threonine kinase activity for screening and profiling applications using ADP-Glo™ as a detection reagent. The ROCK2 Kinase Assay Kit comes in a convenient 96-well format, with enough purified ROCK2 (amino acids 5-554), substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

ROCK2 (Rho-associated coiled-coil protein kinase 2), an isoform of ROCK1, is a serine/threonine kinase target of small GTPase Rho that regulates the formation of actin stress fibers and focal adhesions, cytokinesis, and smooth muscle contraction. It is therefore important for proper vascular function and is involved in a number of fibrotic pathologies. The ROCK2 inhibitor Fasudil has been investigated for the treatment of cerebral cavernous malformation (CCM), which causes brain hemorrhages, and is in clinical trials for other applications such as Amyotrophic Lateral Sclerosis (ALS). Belumosudil, a newer ROCK2 inhibitor, was approved by the FDA for chronic graft versus host disease in 2021. The development of new inhibitors will continue to bring new treatments options to patients with ROCK2 related disorders.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40086	ROCK2, GST-tag*	1.5 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	50 µl	-20°C
79884	S6Ktide (10 mg/ml)	25 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
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 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 Azaindole 1 (Selleckchem #S6636) 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 Buffer 1**, **500 µM ATP** and **S6Ktide (10 mg/ml)**.

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

2. Prepare 3 ml of **1x Kinase Buffer 1** by mixing 600 µl of **5x Kinase Buffer 1** with 2400 µl of distilled water.

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

3. 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.

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

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

OR

3.2 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 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 Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase 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%.

4. Add 2.5 µl of Test inhibitor to the "Test Inhibitor" wells.

5. Add 2.5 µl of Diluent Solution to the "Blank" and "Positive Control" wells.

6. Thaw **ROCK2 Kinase** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

7. Dilute ROCK2 to 1.2 ng/µl with 1x Kinase Buffer 1 (10 µl/well).

8. Add 10 µl of diluted ROCK2 to wells designated "Positive Control" and "Test Inhibitor".

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

10. Preincubate for 30 minutes at Room Temperature (RT).

11. Prepare a **Master Mix** (12.5 µl per well): N wells x (6 µl of 5x Kinase Buffer 1+ 0.5 µl of 500 µM ATP + 0.25 µl of S6Ktide (10 mg/ml) + 5.75 µl of distilled water).

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

Component	Blank	Positive Control	Test Inhibitor
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Buffer 1	10 µl	-	-
Diluted ROCK2 (1.2 ng/ µl)	-	10 µl	10 µl
Master Mix	12.5 µl	12.5 µl	12.5 µl
Total	**25 µl**	**25 µl**	**25 µl**

13. Incubate at 30°C for 45 minutes.

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

Reading Chemiluminescence

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 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

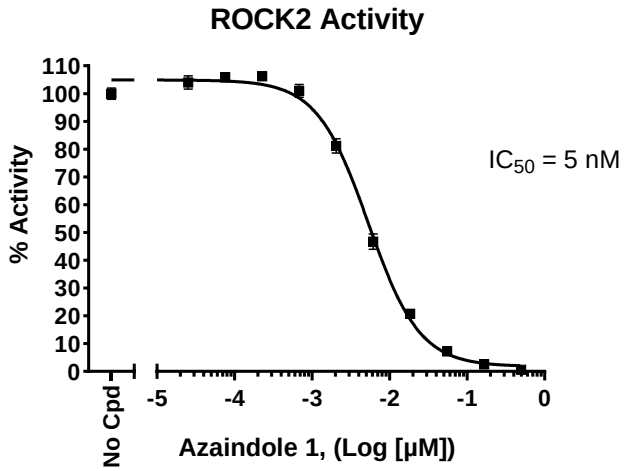

Figure 1: Inhibition of ROCK2 by Azaindole 1.
ROCK2 kinase activity was measured in the presence in the presence of increasing concentrations of Azaindole 1 (Selleckchem #S6636). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Lochhead P. A., *et al.,* 2010 *Oncogene* 29.17: 2591-2598.
Zheng Biqiang, *et al.,* 2011 *Clinical Cancer Research* 17.24: 7574-7583.

Related Products

Products	*Catalog #*	*Size*
ROCK1 Kinase Assay Kit	78386	96 reactions
ROCK1, GST-Tag Recombinant	40085	10 µg
Thiazovivin	78506	5 mg/ 10 mg
Fasudil	27029	250 mg

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