Description

The ROCK1 Kinase Assay Kit is designed to measure ROCK1 activity for screening and profiling applications using ADP-Glo® as a detection reagent. The ROCK1 Kinase Assay Kit comes in a convenient 96-well format, with enough purified ROCK1, substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

ROCK1 is a ubiquitously expressed serine/threonine kinase that is a downstream target of the small GTPase RhoA. ROCK1 is involved in diverse cellular functions, including smooth muscle contraction, actin cytoskeleton organization, cell adhesion and motility, and gene expression. ROCK1 contributes to the development of cardiac fibrosis and induction of fibrogenic cytokines in cardiomyocytes in response to pathological stimuli. ROCK1 knockout mice exhibit reduced perivascular and interstitial fibrosis which is associated with reduced expression of a variety of extracellular matrix (ECM) proteins and fibrogenic cytokines.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
40085	ROCK1*	10 µg	-80°C
79334	5x Kinase assay buffer	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
78387	S6K (1 mg/ml)	500 µl	-20°C
79696	96-well plate, white	1	Room Temperature

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

Materials Required but Not Supplied

Name	Catalog #
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.

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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. Prepare 3 ml of 1x Kinase assay buffer by mixing 600 µl of 5x Kinase assay buffer with 2400 µl water. Three (3) ml of 1x Kinase assay buffer is sufficient for 100 reactions.

2. 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.

    a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Kinase Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Kinase Assay Buffer (Diluent Solution).

    b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 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 to keep the concentration of DMSO constant.

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

       The final concentration of DMSO should not exceed 1%.

3. To the wells designated as "Blank," add 10 µl of 1x Kinase assay buffer.

4. Thaw ROCK1 on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. Calculate the amount of ROCK1 required for the assay and dilute to 10 ng/µl with 1x Kinase assay buffer.

   Notes: the concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

   Aliquot unused protein into 2-4 aliquots as may be necessary (single use aliquots) and store them at -80°C. ***Avoid multiple freeze/thaw cycles. Do not re-use the aliquots more than once or twice and do not re-use the diluted kinase.***

5. Add 10 µl of diluted ROCK1 to wells designated “Positive Control” and "Test Inhibitor."

Preincubate for 30 min at room temperature.

6. Thaw ATP (500 µM) and S6K (1 mg/ml). Prepare the Master Mix (12.5 µl per well): N wells x (7 µl of 1x Kinase assay buffer + 0.5 µl of ATP (500 µM) + 5 µl of S6K (1 mg/ml)). Add 12.5 µl to every well.

7. Incubate at 30°C for 45 minutes.

Component	Blank	Positive Control	Test Inhibitor
1x Kinase assay buffer	10 µl	-	-
Test inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
ROCK1 (10 ng/µl)	-	10 µl	10 µl
Master Mix	12.5 µl	12.5 µl	12.5 µl
Total	25 µl	25 µl	25 µl

8. During the incubation, thaw the ADP-Glo reagent. At the end of the 45-minute reaction, add 25 µl of ADP-Glo reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 45 minutes.

9. Thaw the Kinase Detection reagent. After 45 minutes, add 50 µl of Kinase Detection reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for another 45 minutes.

10. Immediately read samples in a luminometer or microtiter-plate reader capable of reading chemiluminescence. "Blank" value is subtracted from all 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).

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Example Results

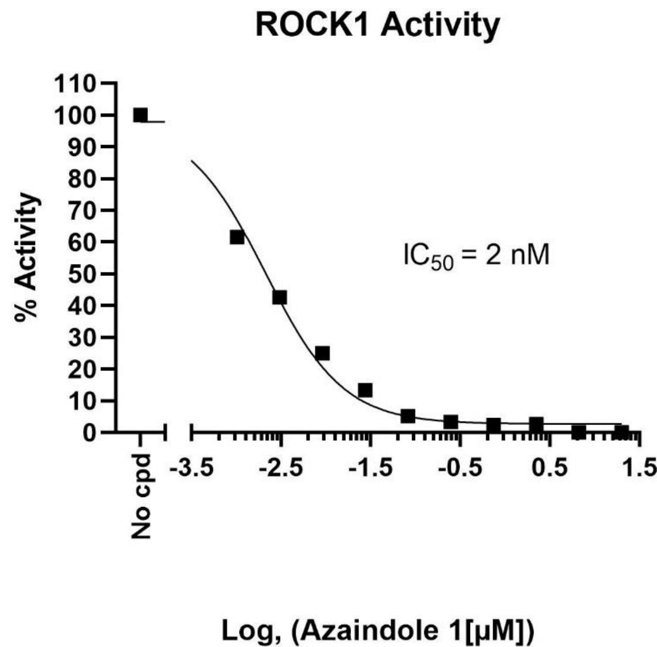

Figure 1: Inhibition of ROCK1 by Azaindole. The inhibition of ROCK1 kinase activity was measured in the presence of increasing Azaindole concentrations (Selleckchem #S6636) using the ROCK1 Kinase Assay Kit (BPS Bioscience #78386).

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. Lochhead, P. A., *et al.* "Activating ROCK1 somatic mutations in human cancer." Oncogene 2010; **29.17:** 2591-2598.
2. Zheng, Biqiang, *et al.* "MicroRNA-148a suppresses tumor cell invasion and metastasis by downregulating ROCK1 in gastric cancer." Clinical Cancer Research 2011; **17.24:** 7574-7583.

Related Products

Products	*Catalog #*	*Size*
ROCK1, GST-Tag	40085	10 µg
ROCK2, GST-Tag	40086	10 µg

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