Version 2a Last updated 12 March 2026

ab211175 ROCK Activity Assay Kit

For the detection and quantification of active ROCK1 (ROKβ) and ROCK2 (Rho Kinase)in human cell lysates and purified protein.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

ROCK Activity Assay Kit (ab211175) provides a non-isotopic, sensitive and specific method to monitor ROCK1 (ROKβ) and ROCK2 (Rho Kinase) using its physiological substrate MYPT1 (myosin phosphate target subunit 1). This kit can also be used in the screening of Rho kinase inhibitor molecules in purified protein samples.

This product detects the specific phosphorylation of MYPT1 by ROCK1 and ROCK2 at threonine 696 (T696) using an enzyme-based immunoassay. This substrate is initially incubated with the sample containing ROCK1 and ROCK 2 (such as purified kinase or cell lysate). Following the kinase reaction, the sample solution is transferred to a substrate capture plate where phosphorylated MYPT1 is detected by anti-phopsho-MYPT1-Thr696 antibody. A recombinant active ROCK2 is provided as a positive control. The sensitivity limit of this kit is 200 pg of active ROCK1 and ROCK2.

Members of the small GTPase Rho family are essential regulatory components of the signaling pathway that direct cell motility, adhesion, and cytokinesis through reorganization of actin cytoskeleton. Rho is activated by extracellular signals such as lysophosphatidic acid (LPA). The actions of Rho are mediated by downstream Rho effectors. One of these effectors is Rho-associated kinase (ROCK). Two ROCK isoforms have been identified: ROCK1 (also known as ROKβ) and ROCK2 (also known as Rho Kinase and ROKa). ROCK mediates Rho signaling and reorganizes actin cytoskeleton through phosphorylation of several substrates that contribute to the assembly of actin filaments and contractility. For example, ROCK inactivates myosin phosphatase through the specific phosphorylation of myosin phosphatase target subunit 1 (MYPT1) at Thr696, which results in an increase in the phosphorylated content of the 20-kDa myosin light chain (MLC20).

2. Protocol Summary

Incubate ROCK-containing sample in MYPT1-coated wells for 30 – 60 min at 30°C

Wash wells. Add primary antibody (anti-phospho-MYPT1 (Thr696) antibody) to wells. Incubate plate 1 hr at RT

Wash wells. Add HRP-conjugated secondary antibody. Incubate plate 1 hr at RT.

Add Substrate Solution and incubate for 10 – 20 min. Measure absorbance at OD 450 nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at 4°C in the dark (active ROCK2 should be stored at -80°C and ATP solution should be stored at -20°C) immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Avoid multiple freeze/thaw cycles.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity		Storage Condition (Before prep)	Storage Condition (After prep)
	96 tests	5 x 96 tests		
Rho Kinase Substrate Coated Plate	1 plate	5 plates	4°C	4°C
10X Kinase Buffer	20 mL	5 x 20 mL	4°C	4°C
10X Wash Buffer	100 mL	5 x 100 mL	4°C	4°C
Active ROCK2 (0.5 µg/mL)	20 µL	5 x 20 µL	-80°C	-80°C
Assay Diluent	50 mL	5 x 50 mL	4°C	4°C
ATP Solution (100 mM)	100 µL	5 x 100 µL	-20°C	-20°C
Anti-phospho-MYPT1 (Thr696) Antibody	20 µL	5 x 20 µL	4°C	4°C
Secondary Antibody, HRP conjugate	20 µL	5 x 20 µL	4°C	4°C
Substrate Solution	12 mL	5 x 12 mL	4°C	4°C
Stop Solution	12 mL	5 x 12 mL	4°C	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Checkpoint kinase sample (purified kinase, cell or tissue lysate)
- Microplate reader capable of measuring absorbance at OD 450 nm (620 nm as optional reference wavelength).
- MilliQ water or other type of double distilled water (ddH_2O)
- DTT 1M
- 0.5 M EDTA, pH 8.0
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom

For cell lysate preparation:

- Lysis Buffer: 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM 2- glycerophosphate, 1% Triton X-100 or 1% NP-40, 1 mM EDTA, 1 mM EGTA. Alternatively, you can use RIPA buffer or any commonly used lysis buffer, such as Mammalian Cell Lysis Buffer 5X (ab179835)
- Phosphatase (sodium orthovanadate) and protease inhibitors: we recommend Protease and Phosphatase Inhibitor Cocktail (ab201119)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Rho Kinase Substrate Coated Plate:

Ready to use as supplied. Store unused strips in the pouch provided at 4°C.

9.2 10X Kinase Buffer:

Ready to use as supplied.

Dilute the 10X Kinase Buffer in ddH_2O to create 1X Kinase Buffer. Stir the solution to homogenize.

Δ Note: Do not dilute the entire bottle of 10X Kinase Buffer. Kinase reaction requires 10X Kinase Buffer concentrate. Prepare only the minimum volume of 1X buffer required for the experiment.

Store 1X Kinase Buffer and remaining 10X Kinase Buffer at 4°C.

9.3 10X Wash Buffer:

Dilute the 10X Wash Buffer concentrate to 1X with ddH_2O. Mix thoroughly and gently. Equilibrate to room temperature before using. Store 1X Wash Buffer at 4°C.

9.4 Active ROCK2 (0.5 µg/mL):

Ready to use as supplied. Aliquot Active ROCK2 so that you have enough to perform the desired number of assays. Avoid multiple freeze/thaw cycles. Store at -80°C.

Immediately prior use, dilute active ROCK2 stock to 0.02 µg/mL in 1X Kinase Buffer – for example, 8 µL active ROCK2 into 182 µL 1X Kinase Buffer.

9.5 Assay Diluent:

Ready to use as supplied. Store at 4°C.

9.6 ATP Solution (100 mM):

Ready to use as supplied. Aliquot ATP Solution so that you have enough to perform the desired number of assays. Avoid multiple freeze/thaw cycles. Store at -20°C.

9.7 Anti-phospho-MYPT1 (T696) antibody:

Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C.

Immediately prior using, dilute anti-phospho-MYPT1 antibody 1:1000 dilution in Assay Diluent. Do not store diluted antibody solution.

9.8 Secondary antibody, HRP conjugate:

Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C. Immediately prior using, dilute secondary antibody 1:1000 in Assay Diluent. Do not store diluted antibody solution.

9.9 Substrate Solution:

Ready to use as supplied. Equilibrate to room temperature prior to use. Store at 4°C.

9.10 Stop Solution:

Ready to use as supplied. Equilibrate to room temperature prior to use. Store at 4°C.

10. Sample Preparation

- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before snap freezing your samples in liquid nitrogen and storing them immediately at -80°C. This is especially necessary if you have many samples. Do not store lysates for longer than 30 days.
- Add phosphatase and protease inhibitors to lysis buffer immediately prior use.

10.1 Cell lysate:

10.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 80% - 90% confluence).
10.1.2 Wash cells with cold PBS.
10.1.3 Resuspend cells in 100 µL of lysis buffer/RIPA buffer.
10.1.4 Homogenize cells quickly by pipetting up and down a few times.
10.1.5 Leave cells
10.1.6 Centrifuge 10 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
10.1.7 Collect supernatant and transfer to a clean tube.
10.1.8 Keep on ice.

10.2 Purified kinase (ROCK2) protein:

Dilute in appropriate buffer/solvent.

11. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Prepare all reagents as directed in the previous sections.
- We recommend that you assay all controls and samples in duplicate.
- 10X Kinase Reaction Buffer/DTT/ATP (Step 11.2.1) can be stored at 4°C for short term (1 – 2 weeks).

11.1 Plate Loading:

11.1.1 Add 90 µL of cell lysate or purified ROCK2 protein directly (or diluted with 1X Kinase Buffer as required) to wells.

11.1.2 Optional positive control (0.2 µg/mL ROCK2): add 90 µL of 0.02 µg/mL diluted active ROCK2 protein (Step 9.4) to wells.

11.2 Kinase Reaction:

11.2.1 Prepare 10X Kinase Reaction Buffer/DTT/ATP: add DTT to a final concentration of 10 mM and ATP to a final concentration of 2 mM to the 10X Kinase Buffer. For example, to prepare 1 mL, add 10 µL of DTT 1M and 20 µL of ATP 100 mM solution to 970 µL of 10X Kinase Buffer.

11.2.2 Initiate kinase reaction by adding 10 µL of 10X Kinase Reaction Buffer/DTT/ATP to the sample and positive control. Mix well by pipetting up and down.

11.2.3 Cover with a plate cover and incubate at 30°C for 30 – 60 minutes with gentle agitation.

11.2.4 Stop kinase reacting by emptying wells or by adding 50 µL 0.5 M EDTA pH 8.0 to each well.

11.3 Detection:

11.3.1 Remove plate cover and empty wells. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

11.3.2 Add 100 µL of diluted anti-phospho-MYPT1 (T696) antibody (Step 9.7) to each well.

11.3.3 Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

11.3.4 Remove plate cover and empty wells. Wash microwell strips 3 times as described in Step 11.3.5 above.

11.3.5 Add 100 µL of diluted HRP-conjugated secondary antibody (Step 9.8) to each well.

11.3.6 Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

11.3.7 Remove plate and empty wells. Wash microwell strips 5 times as described in Step 11.3.5 above. Proceed immediately to the next step.

11.3.8 Add 100 µL of Substrate Solution to each well, including blank wells. Incubate at room temperature for 5 – 20 minutes on an orbital shaker.

11.3.9 Stop enzymatic reaction by adding 100 µL of Stop Solution to each wells, including blank wells.

11.3.10 Measure immediately output on a microplate reader at OD=450 nm (primary wavelength).

Δ **Note:** color will fade over time so read immediately.

12. Typical Data

Typical data – data provided **for demonstration purposes only**.

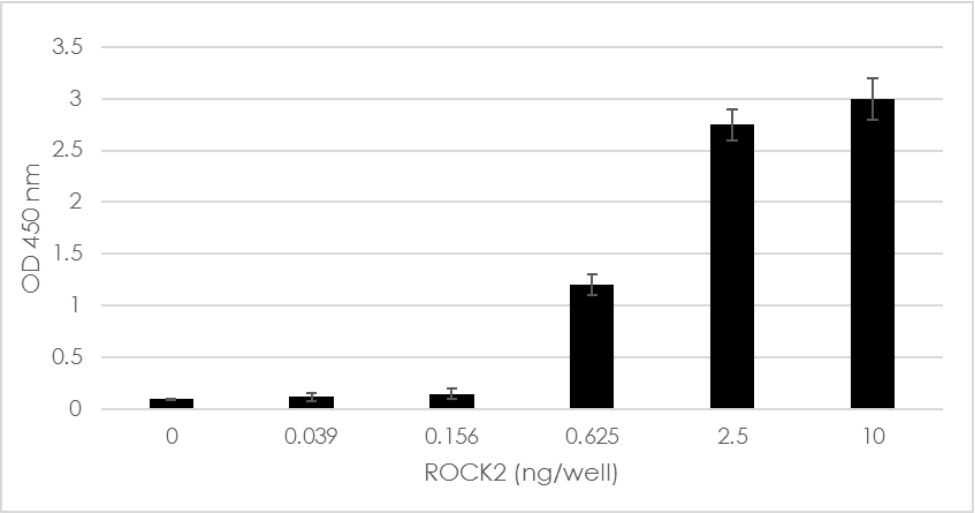

Figure 1. ROCK2 Kinase assay. Increasing amounts of ROCK2 were incubated for 60 minutes at 30°C. Phosphorylated MYPT1 was detected with specific anti-phospho-MYPT1 (T696) as described in the assay protocol.

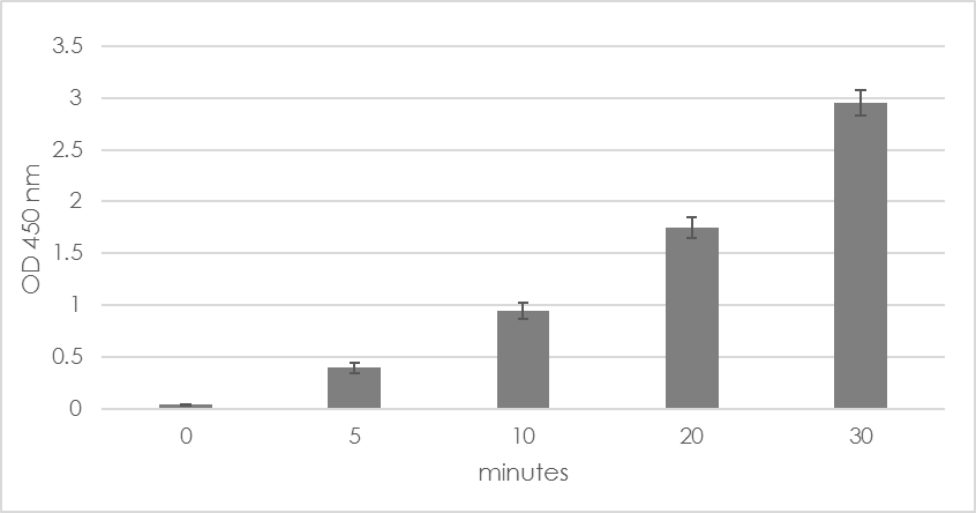

Figure 2. ROCK2 Kinase assay. Active ROCK2 (2.5 ng) was incubated at 30°C during 30 minutes. Measurements were taken at different times as shown. Phosphorylated MYPT1 was detected with specific anti-phospho-MYPT1 (T696) as described in the assay protocol.

13. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Unsuitable microplate for assay	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
Lower/higher readings in samples and controls	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

14. FAQs

15. Notes

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)