Store at -80°C

#7505

ROCK2 Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

new 03/06

Description: Purified recombinant human ROCK2 kinase (Pro6-Ser553), supplied as a GST fusion protein.

Background: ROCK (Rho-associated kinase), a family of serine/threonine kinases, is an important downstream target of GTPase Rho and plays an important role in Rho-mediated signaling pathway. Two isoforms of ROCK have been identified (ROCK1 and ROCK2). ROCK is composed of N-terminal catalytic, coiled-coil and C-terminal PH (pleckstrin homology) domains. The C-terminus of ROCK negatively regulates its kinase activity (1,2). Caspase-3-induced cleavage of ROCK1 and direct cleavage of ROCK2 by granzyme B (grB) activates ROCK and leads to phosphorylation of myosin light chain and inhibition of myosin phosphatase (3). This phosphorylation may account for the mechanism by which Rho regulates cytokinesis, cell motility, cell membrane blebbing during apoptosis and smooth muscle contraction (4,5,6).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing human ROCK2 (Pro6-Ser553) (GenBank Accession No. NM_004850) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

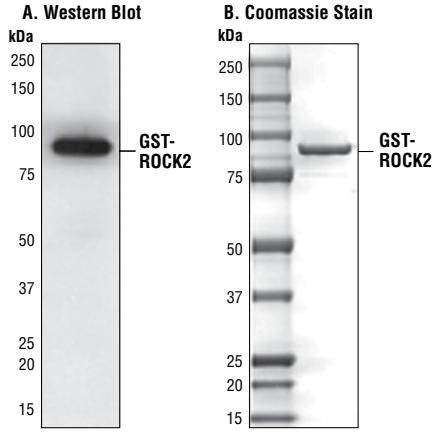

Figure 1. The purity of the GST-ROCK2 fusion protein was analyzed using SDS/PAGE followed by anti ROCK2 Western blot (A) or Coomassie stain (B).

Quality Control: The theoretical molecular weight of the GST-ROCK2 fusion protein is 92 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. ROCK2 kinase activitiy was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure ROCK2 activity using HTScan™ ROCK2 Kinase Assay Kit #7508 [Fig.3].

Background References:

(1) Nakagawa, O. et al. (1996) *FEBS Lett.* 392, 189–93.

(2) Lee, J.H. et al. (2004) *J. Cell. Biol.* 167, 327–37.

(3) Sebbagh, M. et al. (2005) *J. Exp. Med.* 201, 465–71.

(4) Amano, M. et al. (1996) *J. Biol. Chem.* 271, 20246–9.

(5) Kureishi, Y. et al. (1997) *J. Biol. Chem.* 272, 12257–60.

(6) Totsukawa, G. et al. (2000) *J. Cell Biol.* 150, 797–806.

Storage: Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ ROCK2 Kinase Assay Kit #7508

CREB (Ser133) Biotinylated Peptide #1331

Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624

Kinase Buffer (10X) #9802

ATP (10 mM) #9804

Staurosporine #9953

Serine/Threonine Kinase Substrate Screening Kit #7400

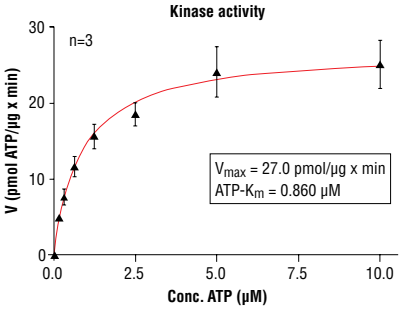

Figure 2. ROCK2 kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP, variable, 2.5 µg/50 µl PEG20,000, Substrate: Tetra (LRRWSLG), 5 µg/50 µl and recombinant ROCK2: 20 ng/50 µl.

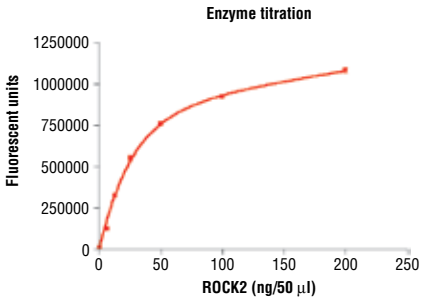

Figure 3. Dose dependence curve of ROCK2 kinase activity: DELFIA® data generated using Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624 to detect phosphorylation of substrate peptide (#1331) by ROCK2 kinase. In a 50 µl reaction, increasing amounts of ROCK2 and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for ROCK2 Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb #9624
5. Kinase Buffer (10X) #9802
6. ATP (10 mM) #9804
7. CREB (Ser133) Biotinylated Peptide #1331
8. DELFIA® Europium-labeled Anti-rabbit antibody (PerkinElmer Life Sciences #AD0105)
9. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
10. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

DELFIA® is a registered trademark of PerkinElmer Life Sciences

B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 1 ml 10X kinase buffer [250 mM Tris-HCl pH 7.5, 100 mM $MgCl_2$, 1 mM Na_3VO_4, 50 mM β-glycerophosphate, 20 mM dithiothreitol (DTT)] to 1.5 ml dH_20 to make 2.5 ml 4X reaction buffer.
5. Dilute enzyme in 1.25 ml of 4X reaction buffer to make 4X reaction cocktail ([enzyme]=4.0 ng/µl in 4X reaction cocktail).
6. Add 12.5 µl of the 4X reaction cocktail to 12.5 µl/well of prediluted compound of interest (usually around 10 µM) and incubate for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

25 mM Tris-HCl (pH 7.5)
10 mM $MgCl_2$
5 mM β-glycerophosphate
0.1 mM Na_3VO_4
2 mM DTT
200 µM ATP
1.5 µM peptide
50 ng ROCK2 Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction to a 96-well streptavidin-coated plate containing 75 µl dH_2O/well and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a CREB (Ser133) Biotinylated Peptide and Phospho-PKA Substrate (RRXS/T) (100G7) Rabbit mAb diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at 37°C for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled secondary antibody 1:1000 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com