

#7532 Store at -80°C

# ROCK1 Kinase

✓ 5 µg

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

**Description:** Purified recombinant human ROCK1 (Leu17-Leu535) kinase, 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 (4-6).

**Source/Purification:** The GST-Kinase fusion protein was produced using a baculovirus expression system using sf9 cells and a recombinant virus encoding human ROCK1 (Leu17-Leu535) (GenBank Accession No. NM\_005406) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using GSH-agarose.

**Quality Control:** The theoretical molecular weight of the GST-ROCK1 fusion protein is 85 kDa. The purity of the kinase was assessed using SDS-PAGE followed by Coomassie stain [Fig.1]. ROCK1 kinase activity was determined using a radiometric assay [Fig.2].

- Background References:**
- (1) Nakagawa, O. et al. (1996) *FEBS Lett.* 392, 189–193.
  - (2) Lee, J.H. et al. (2004) *J. Cell. Biol.* 167, 327–337.
  - (3) Sebbagh, M. et al. (2005) *J. Exp. Med.* 201, 465–471.
  - (4) Amano, M. et al. (1996) *J. Biol. Chem.* 271, 20246–20249.
  - (5) Kureishi, Y. et al. (1997) *J. Biol. Chem.* 272, 12257–12260.
  - (6) Totsukawa, G. et al. (2000) *J. Cell Biol.* 150, 797–806.

**Storage:** Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione. Store at -80°C.

Keep on ice during use.  
Avoid repeated freeze-thaw cycles.  
**Companion Products:**  
Kinase Buffer (10X) #9802  
ATP (10 mM) #9804  
Serine/Threonine Kinase Substrate Screening Kit #7400

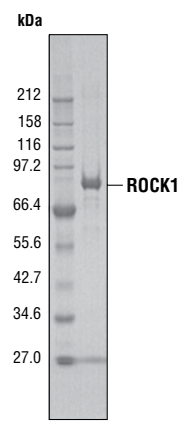


Figure 1. The purity of the GST-ROCK1 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

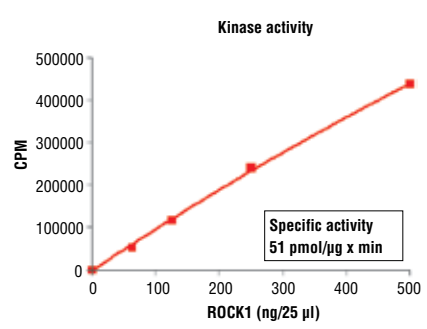


Figure 2. ROCK1 kinase activity was measured in a radiometric assay using the following reaction conditions: 5 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 1 mM EGTA, 0.4 mM EDTA, 5 mM MgCl<sub>2</sub>, 0.05 mM DTT, 50 µM ATP. Substrate: S6K substrate peptide (KRRRLASLR) 400 ng/µL, and recombinant ROCK1: variable.

## Protocol for ROCK1 Kinase Assay

**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. Kinase Buffer (10X)**  
50 mM MOPS, pH 7.2  
25 mM  $\beta$ -glycerophosphate  
10 mM EGTA  
4 mM EDTA  
50 mM  $MgCl_2$   
0.5 mM DTT
- 2.** ATP (10 mM) #9804
- 3.**  $^{32}P$ - $\gamma$ ATP
- 4.** S6K substrate peptide (1  $\mu$ g/ $\mu$ l)

### B Suggested Protocol

- 1.** Dilute 10 mM ATP with 3X assay buffer 1:40 to make 250  $\mu$ M ATP.
- 2.** Dilute [ $^{32}P$ ] ATP to 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP with 250  $\mu$ M ATP solution.
- 3.** Transfer enzyme from -80°C to ice. Allow enzyme to thaw on ice.
- 4.** Dilute ROCK1 protein to 25 ng/ $\mu$ l with 1X assay buffer followed by 2-fold serial dilutions.
- 5.** To start the reaction combine 10  $\mu$ l diluted ROCK1 kinase solution, 10  $\mu$ l S6K substrate peptide (1  $\mu$ g/ $\mu$ l), and 5  $\mu$ l 0.16  $\mu$ Ci/ $\mu$ l [ $^{32}P$ ] ATP solution.

### Final Assay Conditions

- 5 mM MOPS, pH 7.2
  - 2.5 mM  $\beta$ -glycerophosphate
  - 1 mM EGTA
  - 4 mM  $MgCl_2$
  - 0.05 mM DTT
  - 400 ng/ $\mu$ l S6K substrate peptide
- 6.** After 15 minutes terminate reaction by spotting 20  $\mu$ l of the reaction mixture onto phosphocellulose P81 paper.
  - 7.** Air dry the P81 paper then wash with 1% phosphoric acid 3 times.
  - 8.** Transfer P81 paper to 4 ml scintillation tube then add 3 ml scintillation cocktail.
  - 9.** Count samples in a scintillation counter.

Cell Signaling Technology offers a full line of protein kinases, substrates, and antibody detection reagents for high throughput screening. Please direct all inquiries to: [drugdiscovery@cellsignal.com](mailto:drugdiscovery@cellsignal.com).