



ROCK1 (Human), Active

CY-SPR10

## ROCK1 (Human), Active Recombinant protein expressed in Sf9 cells

Cat# CY-SPR10

Lot No.  
5 µg 0.1 µg/µl

### 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 (1). 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 (2).

### Product Description:

Recombinant human ROCK1 (17-535) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM\_005406.

### Gene Aliases:

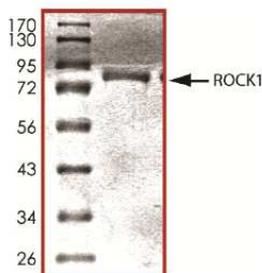
P160ROCK; ROCK-I; ROKbeta

### Formulation:

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM EGTA, 0.1mM EDTA, 0.1mM PMSF, 25% glycerol.

### Purity & Molecular Weight:

The purity of ROCK1 was determined to be >95% by densitometry. Approx. MW 85kDa



### Storage:

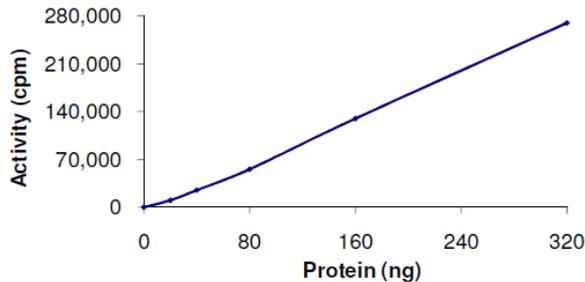
Store product at -70 °C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Stability:

1 year at -70 °C from date of shipment.

**Specific Activity:**

The specific activity was determined to be 64 nmol /min/mg as per Activity Assay Protocol.

**Activity Assay Protocol:**

Assay activity of the kinase in a 25  $\mu$ L reaction consisting of 5  $\mu$ L of 5 X Kinase Assay Buffer, 5  $\mu$ L of 1 mg/ml the Substrate Solution, 10  $\mu$ L of diluted kinase and 5  $\mu$ L of 250  $\mu$ M ATP solution containing [ $\gamma$ - $^{32}$ P] ATP (0.167  $\mu$ Ci/ $\mu$ L). Start the reaction by adding the ATP solution. Incubate for 15 minutes at 30°C. Terminate the reaction by spotting 20  $\mu$ L of the reaction mixture onto phosphocellulose P81 paper. Air-dry the P81 paper and sequentially wash 4 times for approximately 10 minutes each in 1% phosphoric acid with constant gentle stirring. Count the P81 paper in a liquid scintillation counter.

**Substrate Solution:**

S6K synthetic peptide substrate (KRRRLASLR) diluted in distilled H<sub>2</sub>O to a final concentration of 1mg/ml.

**5 X Kinase Assay Buffer:**

25mM MOPS, pH 7.2, 12.5mM  $\beta$ -glycerol-phosphate, 25mM MgCl<sub>2</sub>, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

**References:**

- 1.Zhao, Y M. et al: Rho-associated kinases play a role in endocardial cell differentiation and migration. Dev Biol. 2004 Nov 1;275(1):183-91.
- 2.Zhang, C. et al: Targeted deletion of ROCK1 protects the heart against pressure overload by inhibiting reactive fibrosis. FASEB J. 2006 May;20(7):916-25.

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