## ROCK1, GST-tag Recombinant

Catalog: 40085 Lot: 230112

**Product Information** 

**Description:** Recombinant human ROCK1 (Rho associated coiled-coil containing protein kinase 1),

encompassing amino acids 17-535. This construct contains an N-terminal GST tag. The

recombinant protein was affinity purified and kinase active.

Species: Human

Construct: ROCK1 (GST-17-535)

**Concentration:** 0.10 mg/ml

**Expression System:** Sf9 **Purity:** 80%

**Format:** Aqueous buffer solution.

Formulated In: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM

DTT, 0.1 mM PMSF, 25% glycerol

**MW:** 85 kDa

**Genbank Accession:** NM\_005406

**Stability:** At least 6 months at -80°C.

Storage: -80°C

**Instructions for Use:** Thaw on ice and gently mix prior to use. DO NOT VORTEX. Perform a quick spin before

opening. Aliquot into small volumes and flash freeze for long term storage. Avoid

multiple freeze/thaw cycles.

**Specific Activity:** 65 pmol/min/μg

Assay Conditions: Kinase activity was measured using the ADP-Glo™ Kinase Assay kit (Promega; Cat#

V9101) which quantifies the amount of ADP produced. The ADP-Glo™ Reagent is added to terminate the reaction and deplete the remaining ATP. The Kinase Detection Reagent is then added to convert ADP to ATP and to measure the newly synthesized ATP using

a luciferase reaction.

ROCK1 activity was measured by using S6K substrate (KRRRLASLR) diluted in water to a final concentration of 1 mg/ml. Reaction was initiated by mixing increasing amounts of the ROCK1 with 25  $\mu$ M ATP in 40 mM Tris-HCl, pH 7.4, 20 mM MgCl<sub>2</sub>, 0.1 mg/ml BSA

prepared with 250  $\mu$ M fresh DTT and the 20  $\mu$ g/mL substrate.

After a 40-minute incubation at 37°C, the reaction was terminated by addition of the AMP-Glo™ Reagent followed by a subsequent 40 minute incubation at room temperature. Kinase Detection Reagent was then added and incubated for another 30 minutes. Detection of luminescence was measured using the Luminescence Module Protocol on GloMax®-Multi Microplate reader. The corrected activity (RLU) was calculated by removing the blank value for each sample divided by the (specific activity of ADP in RLU/pmol)\*(Reaction time in min)\*(Enzyme amount in µg or mg). The blank was determined from a "no kinase" sample by replacing the enzyme working solution

with an equal volume of Kinase Dilution Buffer IX (1X).

**Applications:** Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.



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**Quality Control Data** 

## 4-20% SDS-PAGE Coomassie Staining

## Specific Activity





