

Certificate of Analysis

ROCK-II (11-552), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-451, 14-451-K, 14-451M

Parent Lot # 25703U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged recombinant human ROCK-II residues 11–552, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 82% by SDS-PAGE and Coomassie blue staining. MW = 63.3kDa.

Specific Activity (Parent lot# 25703U): 814U/mg, where one unit of ROCK-II, active activity is defined as 1nmol phosphate incorporated into 30μM long S6 substrate peptide per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.05mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

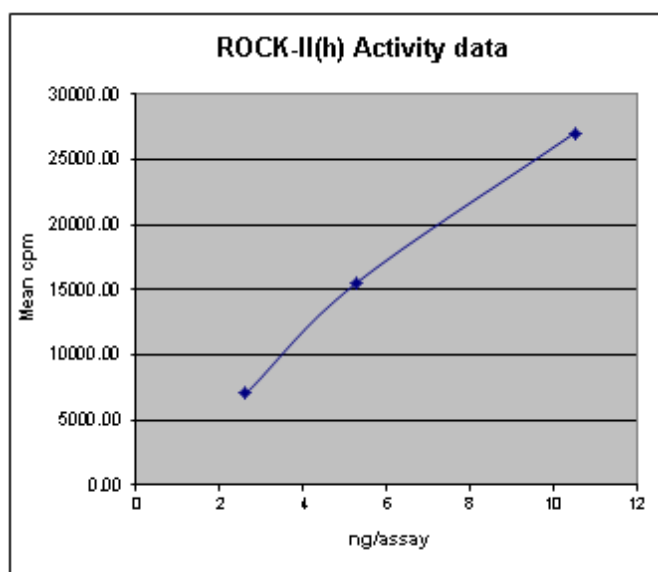
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

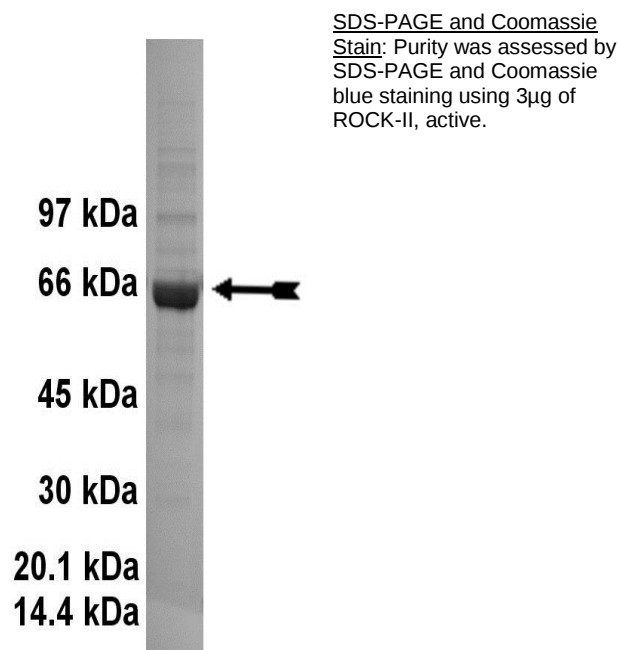
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.6–10.5ng of this lot of enzyme phosphorylated 30μM long S6 substrate in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as ROCK-II with the translated sequence listed on page three.



Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA.
2. **Long S6 substrate:** Use at a final assay concentration of 30mM. Prepare a 300mM stock and add 2.5µl of stock per assay point.
3. **ROCK-II, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.6–10.5ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5µl of 10 x reaction buffer to wells.
2. Add 2.5µl of **long S6 substrate**.
3. Add **2.5µl (2.6–10.5ng) of RockII, active**.
4. Add 7.5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

ROCK-II Sequence Information

Protein	human ROCK-II
Tags	N-terminal 6His
Native sequence	P8 of the recombinant protein is equivalent to P11 of human ROCK-II
Accession number	GenBank 3327051

Recombinant ROCK-II amino acid sequence:

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1  MHHHHHPGA  PETAPGDGAG  ASRQRKLEAL  IRDPRSPINV  ESLLDGLNSL  VLDLDFPALR
61  KNKNIDNFLN  RYEKIVKKIR  GLQMKAEYD  VVKVIGRGAF  GEVQLVRHKA  SQKVYAMKLL
121 SKFEMIKRSD  SAFFWEERDI  MAFANSPWV  QLFYAFQDDR  YLYMVMEMP  GGDLVNLMSN
181 YDVPEKWAKF  YTAEVVLALD  AIHSMGLIHR  DVKPDNMLLD  KHGHLKLADF  GTCMKMDETG
241 MVHCDTAVGT  PDYISPEVLK  SQGGDGFYGR  ECDWWSVGVF  LYEMLVGDT  FYADSLVGTY
301 SKIMDHKNSL  CFPEDAISK  HAKNLICAF  TDREVLGRN  GVEEIRQHPF  FKNDQWHWDN
361 IRETAAPVVP  ELSSDIDSSN  FDDIEDDKG  VETFPPIKAF  VGNQLPFI  G  TYRENLLLS
421 DSPSCRENDS  IQSRKNEESQ  EIQKLYTLE  EHLSNEMQAK  EELEQKCKSV  NTRLEKTAKE
481 LEEEITLRKS  VESALRQLER  EKALLQHNA  EYQRKADHEA  DKRNLENDV  NSLKDQLEDL
541 KKRQNQSQI

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Recombinant ROCK-II nucleotide sequence:

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1  atgcatcatc  accatcacca  tcccggcgcc  cccgagaccg  cgccggggga  cggggcaggc
61  gcgagccgcc  agaggaagct  ggaggcgctg  atccgagacc  ctcgctcccc  catcaacgtg
121 gagagcttgc  tggatggctt  aaattccttg  gtccttgatt  tagattttcc  tgctttgagg
181 aaaaacaaga  acatagataa  tttcttaaat  agatatgaga  aaattgtgaa  aaaaatcaga
241 ggtctacaga  tgaaggcaga  agactatgat  gttgtaaaag  ttattggaag  aggtgctttt
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421 atggcctttg  ccaatagccc  ctgggtgggt  cagctttttt  atgcctttca  agatgatagg
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1621 aaaaaaagaa  atcaaaactc  tcaaatataa

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