

## 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 Ni<sup>2+</sup>/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 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.

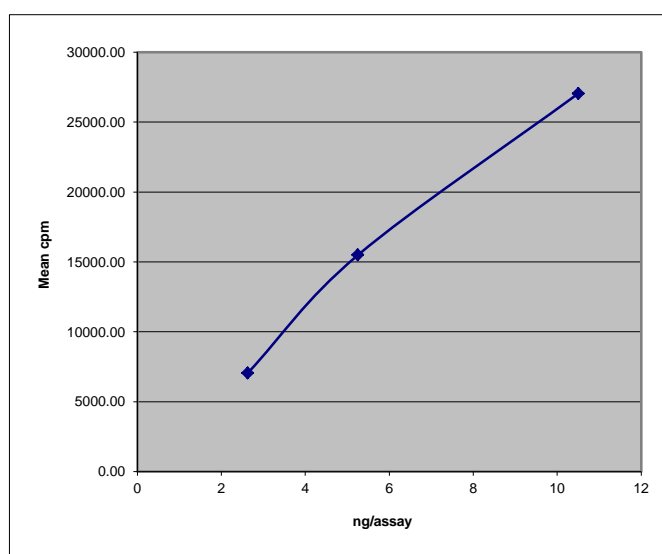
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

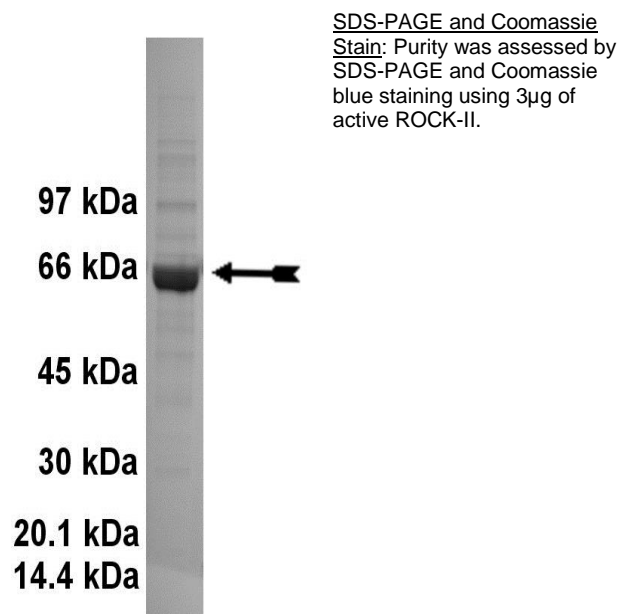
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### 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.



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## 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. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5 x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]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 dH<sub>2</sub>O.
5. Add 10µl of diluted [ $\gamma$ -<sup>33</sup>P]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.

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## ROCK-II Sequence Information

<b><u>Protein</u></b>	human ROCK-II
<b><u>Tags</u></b>	N-terminal 6His
<b><u>Native sequence</u></b>	P8 of the recombinant protein is equivalent to P11 of human ROCK-II
<b><u>Accession number</u></b>	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 VLFYAFQDDR YLYMVMEMP GGDLVNLMNS
181 YDVPEKWAKF YTAEVVLALD AIHSMGLIHR DVKPDNMLLD KHGHLKLADF GTCMKMDETG
241 MVHCDTAVGT PDYISPEVLK SQGGDGFYGR ECDWWSVGVF LYEMLVGDTP FYADSLVGTY
301 SKIMDHKNSL CFPEDAISK HAKNLICAFI TDREVRIGRN GVEEIRQHPF FKNDQWHWDN
361 IRETAAPVVP ELSSDIDSSN FDDIEDDKGD VETFPKIPAF VGNQLPFIGF TYYRENNLLS
421 DSPSCRENDS IQSRKNEESQ EIQKKLYTLE EHLSNEMQAK EELEQKCKSV NTRLEKTAKE
481 LEEEITLRKS VESALRQLER EKALLQHKNA EYQRKADHEA DKKRNLNDV NSLKDQLEDL
541 KKRNNQNSQI
  
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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 tagatthtcc tgctttgagg
181 aaaaacaaga acatagataa tttcttaaat agatatgaga aaattgtgaa aaaaatcaga
241 ggtctacaga tgaaggcaga agactatgat gttgtaaaag ttattggaag aggtgctttt
301 ggtgaagtgc agttggttcg tcacaaggca tcgcagaagg tttatgctat gaagcttctt
361 agtaagtttg aaatgataaa aagatcagat tctgcctttt tttgggaaga aagagatatt
421 atggcctttg ccaatagccc ctgggtgggt cagctttttt atgcctttca agatgatagg
481 tatctgtaca tgtaaatgga gtacatgcct ggtggagacc ttgtaaacct tatgagtaat
541 tatgatgtgc ctgaaaaatg ggccaaatth tacactgctg aagttgttct tgctctggat
601 gcaatacact ccatgggttt aatacacaga gatgtgaagc ctgacaacat gctcttggat
661 aaacatggac atctaaaatt agcagattht ggcacgtgta tgaagatgga tgaacaggc
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1081 ataagagaaa cggcagctcc tgtagtacct gaactcagca gtgacataga cagcagcaat
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1441 ctagaagagg agattacctt acggaaaagt gtggaatcag cattaagaca gttagaaaga
1501 gaaaaggcgc ttcttcagca caaaaatgca gaatatcaga ggaaagctga tcatgaagca
1561 gacaaaaaac gaaatttggg aaatgatgtt aacagcttaa aagatcaact tgaagatttg
1621 aaaaaaagaa atcaaaactc tcaaatataa
  
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