

Certificate of Analysis

ROCK-I, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-601, 14-601-K, 14-601M

Parent Lot # D8NN032U

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-I amino acids 17–535, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Autoactivated by incubating with Mg/ATP and redialysed to remove excess ATP. Purity 71.1% by SDS-PAGE and Coomassie blue staining. MW = 61.4kDa.

Specific Activity (Parent lot# D8NN032U): 63U/mg, where one unit of ROCK-I, active activity is defined as 1nmol phosphate incorporated into 30μM Long S6 peptide per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.367mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

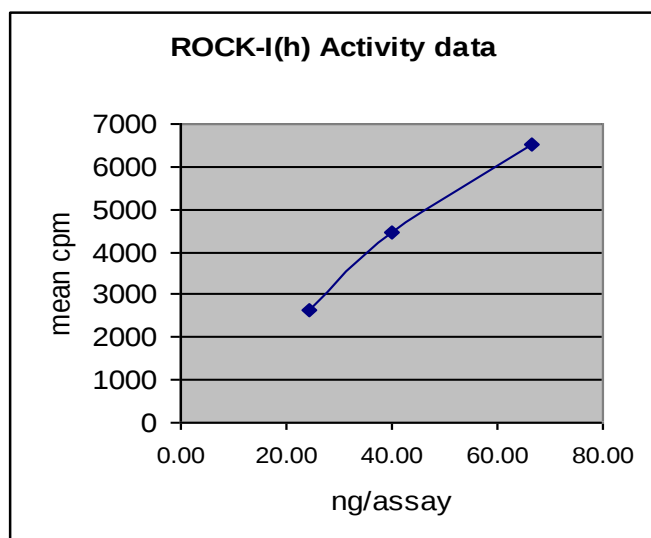
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

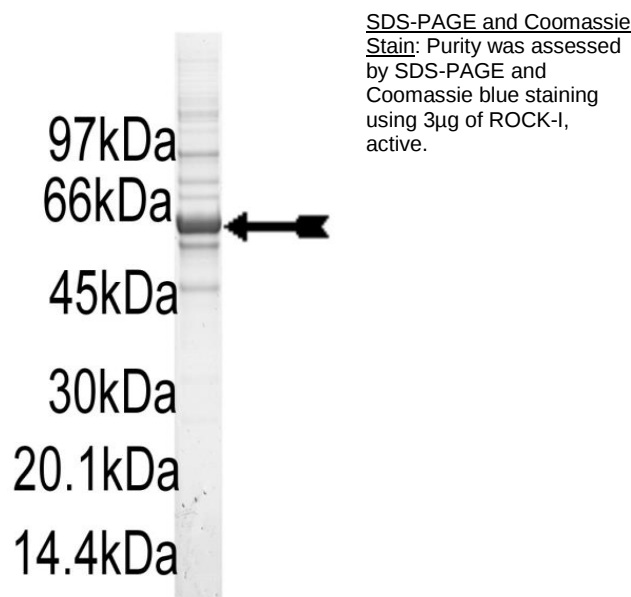
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 24.2–66.6ng of this lot of enzyme phosphorylated 30μM Long S6 peptide 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 identity as ROCK-I with the translated native sequence listed on page three.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Long S6 substrate peptide:** Use at a final assay concentration of 30 μ M. Make up a 300 μ M stock. Add 2.5 μ l of stock per assay point.
3. **ROCK-I, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 24.2–66.6ng 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **Long S6 substrate peptide**.
3. Add **2.5 μ l (24.2–66.6ng) ROCK-I, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l 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 4 ml 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 1 μ l of 30% phosphoric acid.

ROCK-I Sequence Information

Protein	human ROCK-I
Tags	N-terminal 6His
Native sequence	L8 of the recombinant protein is equivalent to L17 of human ROCK-I
Accession number	GenBank NM_005406

Recombinant ROCK-I amino acid sequence:

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1  MHHHHHLLLR DPKSEVNSDC LLDGLDALVY DLDFPALRKN KNIDNFLSRY KDTINKIRDL
61  RMKAEDYEVV KVIGRGAFGE VQLVRHKSTR KVIYAMKLLSK FEMIKRSDSA FFWEERDIMA
121 FANSPWVVL FYAFQDDRYL YMVMEYMPGG DLVNLMSNYD VPEKWARFYT AEVVLALDAI
181 HSMGFIHRDV KPDNMLLDKS GHLKLADFGT CMKMNKEGMV RCDTAVGTPD YISPEVLKSQ
241 GGDGYYGREC DWWSVGVFY EMLVGDTPFY ADSLVGTYSK IMNHKNSLTF PDDNDISKEA
301 KNLICAFSLD REVLGRNGV EEIKRHLFFK NDQWAWETLR DTVAPVVPDL SSDIDTSNFD
361 DLEEDKGEET TFPIPKAFVG NQLPFVGFY YSNRRYLSSA NPNDNRTSSN ADKSLQESLQ
421 KTIYKLEEQH HNEMQLKDEM EQKCRTSNIK LDKIMKELDE EGNQRRNLES TVSQIEKEKM
481 LLQHRINEYQ RKAEQENEKR RNVEVEVSTL KDQLEDLKKV SQNSQL

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Recombinant ROCK-I nucleotide sequence:

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1  atgcatcacc atcaccatca tctgctgcgg gatcccaaatt cggaagtga ttcgattgtt
61  ttgctggatg gattggatgc tttggtatat gatttggatt ttcctgcctt aagaaaaaac
121 aaaaatattg acaacttttt aagcagatat aaagacacaa taaataaaat cagagattta
181 cgaatgaaag ctgaagatta tgaagtagtg aaggtgattg gtagagggtg atttggagaa
241 gttcaattgg taaggcataa atccaccagg aaggtatatg ctatgaagct tctcagcaaa
301 tttgaaatga taaagagatc tgattctgct tttttctggg aagaaagggg catcatggct
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421 tacatggtga tgggaatacat gcctggtgga gatcttgtaa acttaatgag caactatgat
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1501 agaaatgtag aaaatgaagt ttctacatta aaggatcagt tggaagactt aaagaaagtc
1561 agtcagaatt cacagctttaa

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Reviewed and approved by site quality representative.

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