eurofins | Discovery Services

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 Ni^{2+}/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 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.

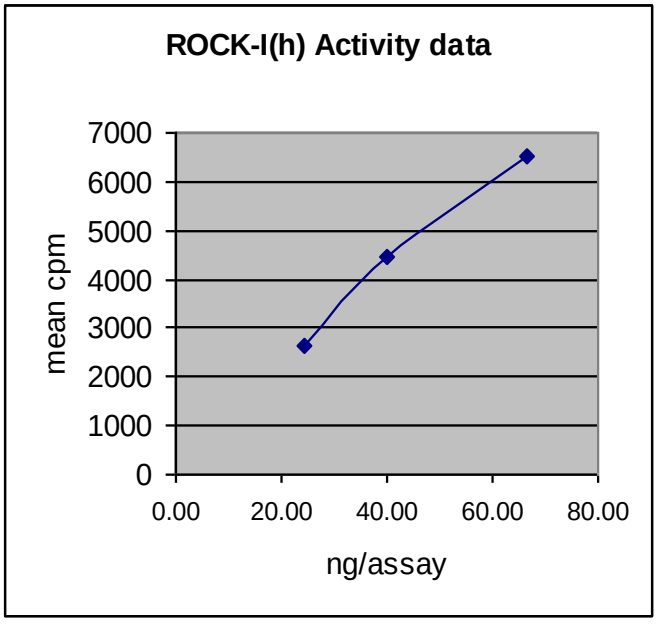
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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°C.

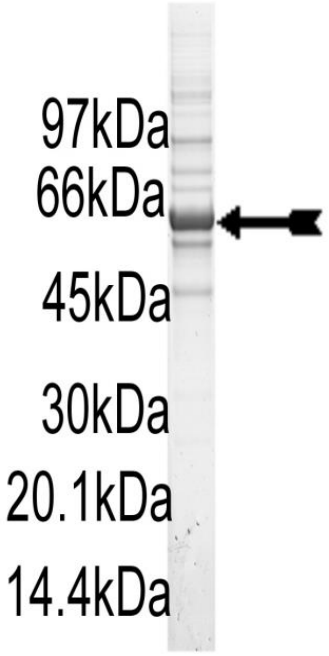
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of ROCK-I, active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

Certificate of Analysis

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 30µM. Make up a 300µM stock. Add 2.5µl 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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **Long S6 substrate peptide**.
3. Add **2.5µl (24.2–66.6ng) ROCK-I, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}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 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 1µl of 30% phosphoric acid.

eurofins | Discovery Services

Certificate of Analysis

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:

```
   1 MHHHHHHLLR DPKSEVNSDC LLDGLDALVY DLDFPALRKN KNIDNFLSRY KDTINKIRDL
  61 RMKAEDYEVV KVIGRGAFGE VQLVRHKSTR KVYAMKLLSK FEMIKRSDSA FFWEERDIMA
 121 FANSPWVVQL FYAFQDDRYL YMVMEYMPGG DLVNLMSNYD VPEKWARFYT AEVVLALDAI
 181 HSMGFIHRDV KPDNMLLDKS GHLKLADFGT CMKMNKEGMV RCDTAVGTPD YISPEVLKSQ
 241 GGDGYYGREC DWWSVGVFLY EMLVGDTPFY ADSLVGTYSK IMNHKNSLTF PDDNDISKEA
 301 KNLICAFLTD REVRLGRNGV EEIKRHLFFK NDQWAWETLR DTVAPVVPDL SSDIDTSNFD
 361 DLEEDKGEEE TFPIPKAFVG NQLPFVGFTY YSNRRYLSSA NPNDNRTSSN ADKSLQESLQ
 421 KTIYKLEEQL HNEMQLKDEM EQKCRTSNIK LDKIMKELDE EGNQRRNLES TVSQIEKEKM
 481 LLQHRINEYQ RKAEQENEKR RNVENEVSTL KDQLEDLKKV SQNSQL
```

Recombinant ROCK-I nucleotide sequence:

```
   1 atgcatcacc atcaccatca tctgctgcgg gatcccaaat cggaagtgaa ttcggattgt
  61 ttgctggatg gattggatgc tttggtatat gatttggatt ttcctgcctt aagaaaaaac
 121 aaaaatattg acaacttttt aagcagatat aaagacacaa taaataaaat cagagattta
 181 cgaatgaaag ctgaagatta tgaagtagtg aaggtgattg gtagaggtgc atttggagaa
 241 gttcaattgg taaggcataa atccaccagg aaggtatatg ctatgaagct tctcagcaaa
 301 tttgaaatga taaagagatc tgattctgct tttttctggg aagaaaggga catcatggct
 361 tttgccaaca gtccttgggt tgttcagctt ttttatgcat tccaagatga tcgttatctc
 421 tacatggtga tggaatacat gcctggtgga gatcttgtaa acttaatgag caactatgat
 481 gtgcctgaaa aatgggcacg attctatact gcagaagtag ttcttgcatt ggatgcaatc
 541 cattccatgg gttttattca cagagatgtg aagcctgata acatgctgct ggataaatct
 601 ggacatttga agttagcaga ttttggtact tgtatgaaga tgaataagga aggcatggta
 661 cgatgtgata cagcggttgg aacacctgat tatatttccc ctgaagtatt aaaatcccaa
 721 ggtggtgatg gttattatgg aagagaatgt gactggtggt cggttggggt atttttatac
 781 gaaatgcttg taggtgatac acctttttat gcagattctt tggttggaac ttacagtaaa
 841 attatgaacc ataaaaattc acttaccttt cctgatgata atgacatatc aaaagaagca
 901 aaaaacctta tttgtgcctt ccttactgac agggaagtga ggttagggcg aaatggtgta
 961 gaagaaatca aacgacatct cttcttcaaa aatgaccagt gggcttggga aacgctccga
1021 gacactgtag caccagttgt acccgattta agtagtgaca ttgatactag taattttgat
1081 gacttggaag aagataaagg agaggaagaa acattcccta ttcctaaagc tttcgttggc
1141 aatcaactac cttttgtagg atttacatat tatagcaatc gtagatactt atcttcagca
1201 aatcctaatg ataacagaac tagctccaat gcagataaaa gcttgcagga aagtttgcaa
1261 aaaacaatct ataagctgga agaacagctg cataatgaaa tgcagttaaa agatgaaatg
1321 gagcagaagt gcagaacctc aaacataaaa ctagacaaga taatgaaaga attggatgaa
1381 gagggaaatc aaagaagaaa tctagaatct acagtgtctc agattgagaa ggagaaaatg
1441 ttgctacagc atagaattaa tgagtaccaa agaaaagctg aacaggaaaa tgagaagaga
1501 agaaatgtag aaaatgaagt ttctacatta aaggatcagt tggaagactt aaagaaagtc
1561 agtcagaatt cacagctttaa
```

Reviewed and approved by site quality representative.