

Certificate of Analysis

ROK α /ROCKII, active, rat

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-338, 14-338-K, 14-338M

Parent Lot # 28217U

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 and N-terminal HA tagged, recombinant, rat ROK α /ROCKII, amino acids 2–543. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 63% by SDS-PAGE and Coomassie blue staining. MW = 67.2kDa.

Specific Activity (Parent lot# 28217U): 1649U/mg, where one unit of ROK α /ROCKII activity is defined as 1nmol phosphate incorporated into 30 μ M long S6 substrate peptide per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 4.35mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 2mM benzamidine, 0.4mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

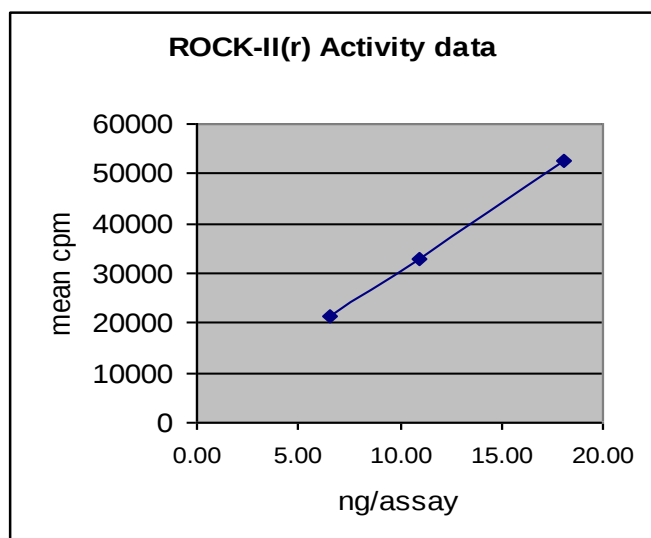
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

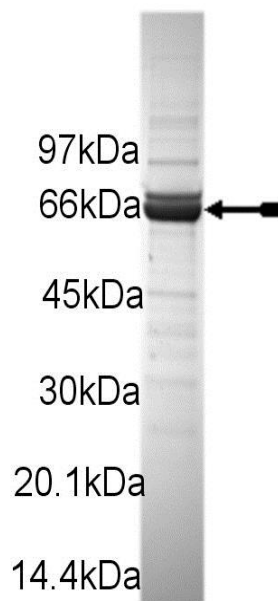
Quality Control Testing

Kinase Assay: 6–18ng of this lot of enzyme phosphorylated 30 μ M long S6 substrate 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 ROK α /ROCKII 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 3 μ g of ROK α /ROCKII active.



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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 30 μ M. Prepare a 300 μ M stock and add 2.5 μ l of stock per assay point.
3. **ROK α /ROCK-II, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 6–18ng 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 2.5 μ l of 10 x reaction buffer to wells.
2. Add 2.5 μ l of long **S6 substrate**.
3. Add **2.5 μ l (6–18ng) ROK α /ROCK-II, active**.
4. Add 7.5 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 twice 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 containing all components plus 1 μ l of 30% phosphoric acid.

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ROKα/ROCKII Sequence Information

Protein	Rat ROKα/ROCKII
Tags	N-terminal 6His and N-terminal HA tags
Native sequence	P43 of the recombinant protein is equivalent to P2 of rat ROKα/ROCKII
Accession number	GenBank NM_013022. The recombinant protein contains the amino acid substitution A9P with respect to GenBank NM_013022. This conflict is reported in the human GenBank entries D87931 and AB014519, and also in the murine GenBank entries NM_009072 and U58513. The recombinant protein also contains the amino acid substitutions S341Q, A342H and S343P with respect to GenBank NM_013022. These are reported in the human GenBank entries D87931, 6633807, 14736226 and AB014519: and also in murine GenBank entries NM_009072 and GenBank U58513.

Recombinant ROKα/ROCKII amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMGSEF ATMPYDVPD YAPGAPEAAP GDGAGAGRQR
61 KLEALIRDPR SPINVESLLD GLNSLVLDLD FPALRKNKNI DNFLNRYEKI VKKIRGLQMK
121 AEDYDVVKVI GRGAFGEVQL VRHKASQKVY AMKLLSKFEM IKRSDSAFFW EERDIMAFAFAN
181 SPWVVLFCFQ QDDRYLYMV MEYMPGGDLV NLMSNYDVPE KWAKFYTAEV VLALDAIHSM
241 GLIHRDVKPD NMLLDKHGHL KLADFGTCMK MDETGMVHCD TAVGTPDYIS PEVLKSQGGD
301 GYGRECDWW SVGVFLFEML VGDTPFYADS LVGTYSKIMD HKNSLCFPED TEISKHAKNL
361 ICAFLTDREV RLGRNGVEEI KQHPFFKNDQ WNWDNIRETA APVPELSSD IDSSNFDDIE
421 DDKGDEVETFP IPKAFVGNQL PFIGFTYFRE NLLLSDSPPC RENDAIQTRK SEESQEIQKK
481 LYALEEHLSS EVQAKEELEQ KCKSINTRLE KTAKELEEEI TFRKNVESTL RQLEREKALL
541 QHKNAEYQRK ADHEADKKRN LENDVNSLKD QLEDLKKRNQ SSQI

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Recombinant ROKα/ROCKII nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatggg atccgaattc gccaccatgt acccatacga tgtgccagat
121 tacgcccccg gcgccccgga ggccgcgcgc ggggacgggg cgggcgcggg tcgtcagagg
181 aagctggagg cgctcatccg agaccctcgc tcgccatca acgtggaaag cctgctggat
241 ggcttaaatt ccttggtcct tgatttgat tttcctgctt tgaggaaaaa taaaaatata
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361 gcagaagact atgatgttgt aaaagttatt ggaagagggt cttttggtga agttcagttg
421 gttcgtcata aggcatacga gaaggtttat gcaatgaagc ttcttagtaa atttgaaatg
481 ataaaaagat cagattctgc tttttctggt gaagaaagag atattatggc ttttgctaac
541 agtccgtggg tggttcagct cttttgtgcc ttccaagatg acaggatat gtacatgggtg
601 atggagtaca tgccagggtg agacctgtgt aacctgatga gcaattatga tgtgcctgag
661 aagtgggcca agttttatac tgctgaagtc gtgcttgac tggtatgcaat acactccatg
721 ggcttaatac acagagatgt gaagcccgat aacatgctat tggataaaca cggacatcta
781 aaattagcag attttggcac atgtatgaaa atggatgaaa caggcatggt gcatttgtat
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961 gtaggggaca ctccatttta tgcagattca cttgtaggaa cctacagcaa aattatggat
1021 cataaaaatt cactgtgttt tcctgaagat acagaaattt ctaaactatg gaagaatctc
1081 atatgtgcct tcttaacaga caggagggtg cgacttgga gaaatggggg agaagaaatc
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1261 gatgacaaag gcgatgtaga gaccttccc attcctaaag cttttgtggg aaatcagctg
1321 ccttttatag gatttaccta ctttagagaa aatttggtac taagtgattc tccacctgtg
1381 agagaaaatg atgcaatata aacaaggaaa agtgaagaaa gtcaggagat tcagaaaaaa
1441 ttatatgcac tagaagaaca ctttagcagt gaggtacaag ccaaggagga actggaacag

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1501 aagtgcaagt ctattaatac tcgtctagag aaaacagcaa aggaactaga agaagagatt
1561 accttttagga aaaatgtgga atcaacatta agacagctag aaagagaaaa ggcccttctt
1621 cagcacaaaa atgcagaata tcaacggaaa gctgatcatg aagctgacaa gaaacggaat
1681 ttggaaaaatg atgttaacag cttaaaagat caacttgaag atttgaagaa aagaaaccag
1741 agctctcaga tataaa
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