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Certificate of Analysis

RSK1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-509, 14-509-K, 14-509M
Parent Lot # 1765772

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 full-length human RSK1 expressed in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Activated with PDK1 (cat# 14-280) and MAPK2 (cat# 14-173) and repurified using glutathione-Sepharose, heparin-Sepharose and Ni^{2+}/NTA agarose. Purity 94.6% by SDS-PAGE and Coomassie blue staining. MW = 84kDa.

Specific Activity (Parent lot# 1765772): 3145U/mg, where one unit of RSK1 activity is defined as 1nmol phosphate incorporated into 30µM (KKKNRTLSVA) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.632mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl pH7.5, 0.03% Brij-35, 0.1mM EGTA, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

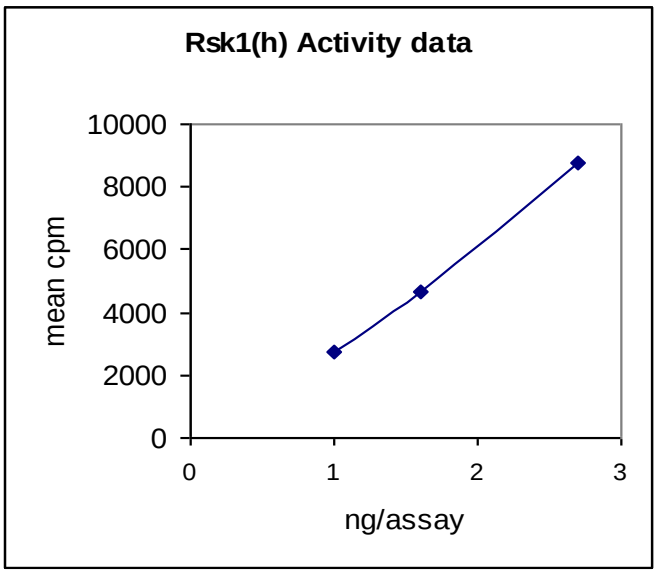
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

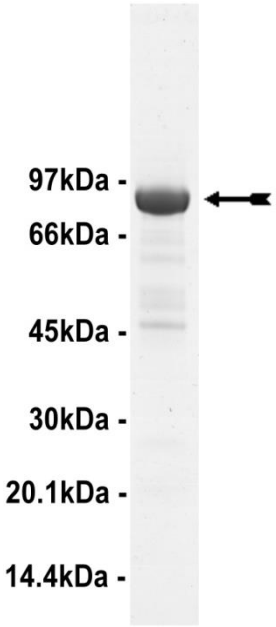
Quality Control Testing

Kinase Assay: 1.0–2.7ng of this lot of enzyme phosphorylated 30µM (KKKNRTLSVA) 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 RSK1 with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active RSK1.



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Kinase Assay Protocol

Stock Solutions:

1. **5x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKNRTLSVA):** Use at a final assay concentration of 30µM. Prepare a 300µM stock. Add 2.5µl of stock per assay point.
3. **RSK1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.0–2.7ng 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 **(KKKNRTLSVA)**.
3. Add **2.5µl (1.0–2.7ng) RSK1, 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 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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RSK1 Sequence Information

Protein human RSK1

Tags *N*-terminal 6His

Native sequence M10 of the recombinant protein is equivalent to M1 of human RSK1

Accession number GenBank NM_002953

Recombinant RSK1 amino acid sequence:

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   1 MHHHHHHEFM PLAQLKEPWP LMELVPLDPE NGQTSGEEAG LQPSKDEGVL KEISITHHVK
  61 AGSEKADPSH FELLKVLGQG SFGKVFLVRK VTRPDSGHLY AMKVLKKATL KVRDRVRTKM
 121 ERDILADVNH PFVVKLHYAF QTEGKLYLIL DFLRGGDLFT RLSKEVMFTE EDVKFYLAEL
 181 ALGLDHLHSL GIIYRDLKPE NILLDEEGHI KLTDFGLSKE AIDHEKKAYS FCGTVEYMAP
 241 EVVNRQGHSH SADWWSYGVL MFEMLTGSLP FQGKDRKETM TLILKAKLGM PQFLSTEAQS
 301 LLRALFKRNP ANRLGSGPDG AEEIKRHVFY STIDWNKLYR REIKPPFKPA VAQPDDTFYF
 361 DTEFTSRTPK DSPGIPPSAG AHQLFRGFSF VATGLMEDDG KPRAPQAPLH SVVQQLHGKN
 421 LVFSDGYVVK ETIGVGSYSE CKRCVHKATN MEYAVKVIDK SKRDPSEEIE ILLRYGQHPN
 481 IITLKDVYDD GKHVYLVTEL MRGGELLDKI LRQKFFSERE ASFVLHTIGK TVEYLHSQGV
 541 VHRDLKPSNI LYVDESGNPE CLRICDFGFA KQLRAENGLL MTPCYTANFV APEVLKRQGY
 601 DEGCDIWSLG ILLYTMLAGY TPFANGPSDT PEEILTRIGS GKFTLSGGNW NTVSETAKDL
 661 VSKMLHVDPH QRLTAKQVLQ HPWVTQKDKL PQSQLSHQDL QLVKGAMAAT YSALNSSKPT
 721 PQLKPIESSI LAQRRVRKLP STTL
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Recombinant RSK1 nucleotide sequence:

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   1 atgcatcatc accatcacca tgaattcatg ccgctcgccc agctcaagga gccctggccg
  61 ctcatggagc tagtgccgct ggacccggag aatggacaga cctcagggga agaagctgga
 121 cttcagccgt ccaaggatga gggcgtcctc aaggagatct ccatcacgca ccacgtcaag
 181 gctggctctg agaaggctga tccatcccat ttcgagctcc tcaaggttct gggccaggga
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 361 gagagagaca tcctggctga tgtaaatcac ccattcgtgg tgaagctgca ctatgccttc
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 961 gcagaggaaa tcaagcggca tgtcttctac tccaccattg actggaataa gctataccgt
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1561 gccagctttg tcctgcacac cattggcaaa actgtggagt atctgcactc acaggggttt
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1621 gtgcacaggg acctgaagcc cagcaacatc ctgtatgtgg acgagtccgg gaatcccgag
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2221 tccaccaccc tgtga
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Reviewed and approved by site quality representative.