

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 Ni²⁺/NTA agarose. Activated with PDK1 (cat# 14-280) and MAPK2 (cat# 14-173) and repurified using glutathione-Sepharose, heparin-Sepharose and Ni²⁺/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, active 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.

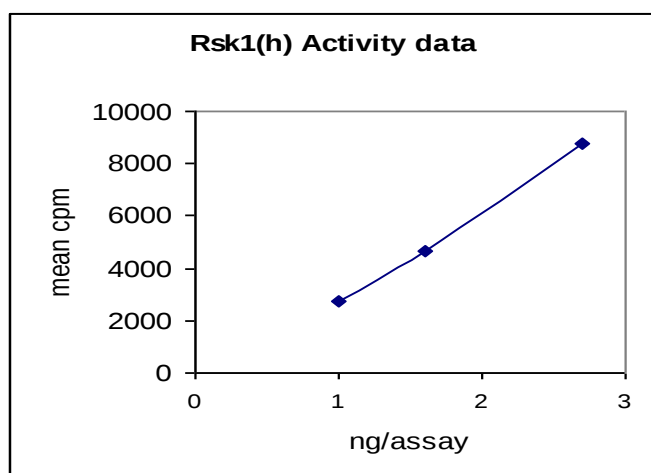
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

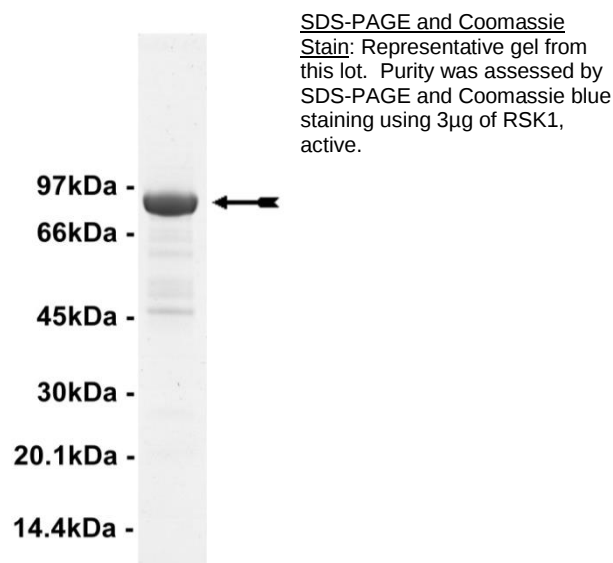
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 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.



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Kinase Assay Protocol

Stock Solutions:

1. **5x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKNRTLVA):** 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 -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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **(KKKNRTLVA)**.
3. Add **2.5µl (1.0–2.7ng) RSK1, active**.
4. Add 5µl of dH₂O.
5. Add 10µl of diluted [γ -³³P]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

<u>Protein</u>	human RSK1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M10 of the recombinant protein is equivalent to M1 of human RSK1
<u>Accession number</u>	GenBank NM_002953

Recombinant RSK1 amino acid sequence:

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1  MHHHHHHEFM  PLAQLKEPWP  LMELVPLDPE  NGQTSGEEAG  LQPSKDEGVL  KEISITHHVK
61  AGSEKADPSH  FELLKVLGQG  SFGKVFLVRK  VTRPDSGHL  Y  AMKVLKKATL  KVRDRVRTKM
121  ERDILADVNH  PFVVKLHYAF  QTEGKLYLIL  DFLRGGDLFT  RLSKEVMFTE  EDVKFYLAEL
181  ALGLDHLHSL  GIIYRDLKPE  NILLDEEGHI  KLTDFGLSKE  AIDHEKKAYS  FCGTVEYMAP
241  EVVNRQGHSH  SADWWSYGVL  MFEMLTGSLP  FQKDRKETM  TLILKAKLGM  PQFLSTEAQS
301  LLRALFKRNP  ANRLGSGPDG  AEEIKRHVYF  STIDWNKLYR  REIKPPFKPA  VAQPDDTFYF
361  DTEFTSRTPK  DSPGIPPSAG  AHQLFRGFSF  VATGLMEDDG  KPRAPQAPLH  SVVQQLHGKN
421  LVFSDGYVVK  ETIGVGSYSE  CKRCVHKATN  MEYAVKVIDK  SKRDPSEEIE  ILLRYGQHPN
481  IITLKDVYDD  GKHVYLVTEL  MRGGEILLDK  LRQKFFSERE  ASFVLHTIGK  TVEYLHSQGV
541  VHRDLKPSNI  LYVDESGNPE  CLRICDFGFA  KQLRAENGLL  MTPCYTANFV  APEVLKRQGY
601  DEGCDIWSLG  ILLYTMLAGY  TPFANGPSDT  PEEILTRIGS  GKFTLSGGNW  NTVSETAKDL
661  VSKMLHVDPH  QRLTAKQVLQ  HPWVTQDKL  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
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1861 actccatttg ccaacggtcc cagtgaacac ccagaggaaa tcctaaccg gatcggcagt
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2221 tccaccaccc tgtga

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