

Certificate of Analysis

Rsk1/MAPKAP Kinase 1a, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-479, 14-479-K, 14-479M

Parent Lot # 31236U

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: Recombinant full-length rat RSK1 (residues 1–end), containing additional *N*-terminal 6His and HA (YPYDVPDYA) tags. Expressed in baculovirus in Sf21 insect cells, and purified using Ni²⁺/NTA-agarose. Activated with p42 MAPK and PDK1, and repurified by glutathione-, heparin-, and Ni²⁺/NTA-agarose chromatographies. Purity 87%. MW = 89.9kDa.

Formulation: 1.851mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM 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.

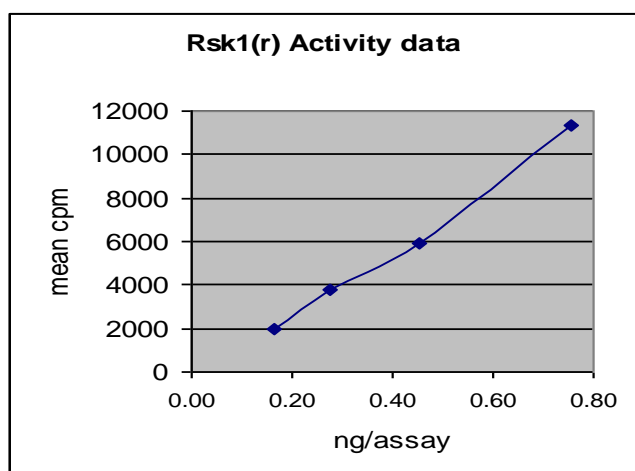
Specific Activity (Parent lot# 31236U): 7170U/mg, where one unit of RSK1, active activity is defined as 1nmol phosphate incorporated into 30µM Rsktide per minute at 30°C with a final ATP concentration of 100µM.

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.

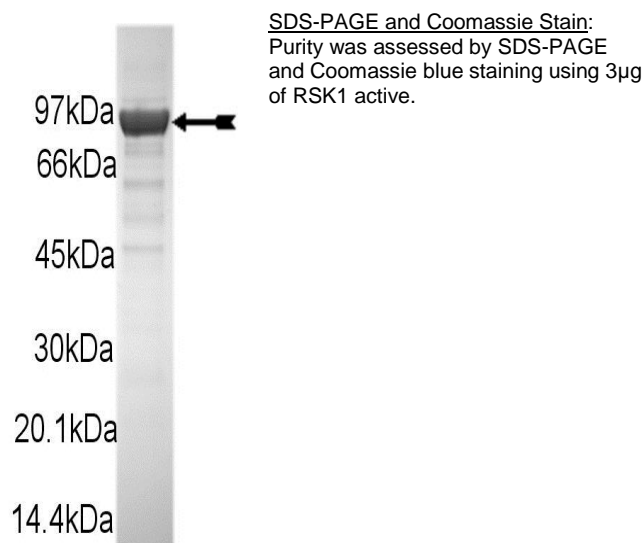
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.2–0.8ng of this lot of enzyme phosphorylated 30µM RSKtide 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 rat RSK1 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(KKKNRTLSVA):** Use at a final concentration of 30 μ M. Prepare a 300 μ M stock and add 2.5 μ l of stock per assay point.
3. **RSK1, active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.2–0.8ng 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.
2. Add 2.5 μ l of **(KKKNRTLSVA)**.
3. Add **2.5 μ l (0.2–0.8ng) of RSK1, 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 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 1 μ l of 30% phosphoric acid.

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RSK1 Sequence Information

<u>Protein</u>	Rat, RSK1
<u>Tags</u>	N-terminal 6His and N-terminal HA (YPYDVPDYA)
<u>Native sequence</u>	M47 of recombinant protein is equivalent to M1 of native sequence. Recombinant protein also contains two amino acids which are in conflict with M99169, these are S637N, and G697A, both of which are reported in EST BI285433. The residue coordinates in the native sequence have been given.
<u>Accession number</u>	GenBank M99169

Recombinant RSK1 amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF AAATMYPYDV PDYALEMPLA QLKEPWPLME
61 LVPLDPENQ ASGEEAGLQP SKDEGILKEI SITHHVKAGS EKADPSHFEL LKVLGQGSFG
121 KVFLVRKVTR PDNGHLYAMK VLKKATLKVR DRVRTKMERD ILADVNHPFV VKLHYAFQTE
181 GKLYLILDFL RGGDLFTRLS KEVMFTEEDV KFYLAELALG LDHLHSLGII YRDLKPENIL
241 LDEEGHIKLT DFGLSKEAID HEKKAYSFCG TVEYMAPEVV NRQGHTHSAD WWSYGVLMFE
301 MLTGSLPFQG KDRKETMTLI LKAKLGMPQF LSTEAQSLLR ALFKRNPANR LGSGPDGAE
361 IKRHIFYSTI DWNKLYRREI KPPFKPAVAQ PDDTFYFDTE FTSRTPRSDP GIPPSAGAHQ
421 LFRGFSFVAT GLMEDDSKPR ATQAPLHSV VQLHGKNLVF SDGYIVKETI GVGSYVCKR
481 CVHKATNMEY AVKVIDKSKR DPSEEIEILL RYGQHPNIIT LKDVIYDDSKH VYLVTLMRG
541 GELLDKILRQ KFFSEREASF VLYTISKTVE YLHSQGVVHR DLKPSNIIYV DESGNPECLR
601 ICDFGFAQL RAENGLLMT CYTANFVAPE VLKRQGYDEG CDIWSLGVLL YTMLAGYTPF
661 ANGPSDTPPE ILTRISSGKF TLNGGNWNTV SETAKDLVSK MLHVDPHQRL TAKQVLQHPW
721 ITQKDKLPQS QLSHQDLQLV KGAMAATYSA LSSSKPTPQL KPIESSILAQ RRVKRLPSTT
781 L

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Recombinant RSK1 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcccacatgga tccggaattc gcggccgcca ccatgtaccc atacgatgtt
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1201 ttcacgtcac gcacaccag ggattcgccg ggcatcccc ccagtgtctg tgcccatcag
1261 ctcttccgtg gcttcagctt cgtggccacc ggtctgatgg aggatgacag caagcctcgg
1321 gccaccagg ctccgctgca ctcggtggta cagcaactcc acgggaagaa cttggttttc

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1381 agc gatggct acatagtaaa ggagacgatc ggcg tgggct cctactctgt gtgtaagcgc
1441 tgtgtccaca aggccaccaa catggagtac gcagtcaaag tcatcgacaa aagcaaaaga
1501 gatccctccg aagagatcga gattcttctg cggtatggac agcaccctca catcatcacc
1561 ctgaaagatg tgtatgacga cagtaagcac gtatacctgg tgacagagct gatgaggggc
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2341 ctgtga
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