

Certificate of Analysis

RSK2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-480, 14-480-K, 14-480M

Parent Lot # WAE0475

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 RSK2 residues 2–end. Expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Activated with MAPK2 and PDK1, and re-purified by glutathione, heparin and Ni²⁺/NTA-agarose chromatographies. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 85kDa.

Specific Activity (Parent lot# WAE0475): 5067U/mg, where one unit of RSK2, active activity is defined as 1nmol phosphate incorporated into 30μM (KKKNRTLSVA) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 0.39mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol.

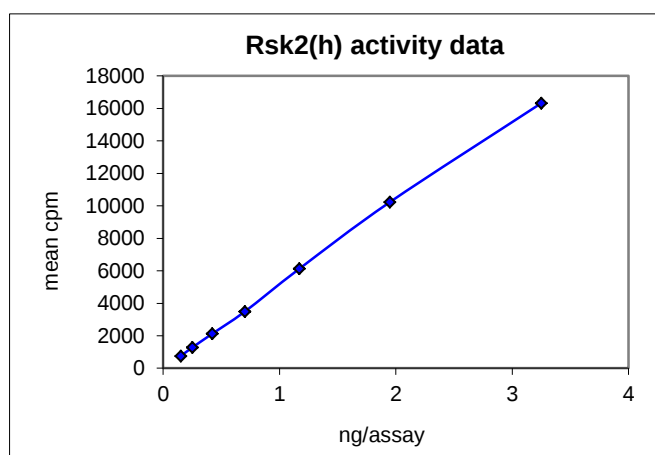
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

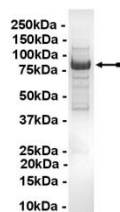
Quality Control Testing

Kinase Assay: 0.15–3.25ng 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 identity as RSK2 with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of RSK2, active.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH 7.0, 1mM EDTA.
2. **(KKKNRTLVA):** 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. **RSK2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 0.15–3.25ng 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 to wells.
2. Add 2.5 μ l of **(KKKNRTLVA)**.
3. Add **2.5 μ l (0.15–3.25ng) RSK2, 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.

Certificate of Analysis

RSK2 Sequence Information

<u>Protein</u>	human RSK2
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	P11 of the recombinant protein is equivalent to P2 of human RSK2
<u>Accession number</u>	The protein sequence is identical to that described in GenBank NM_004586 for human RSK2, over residues 2–end. The cDNA however is more closely related to the mouse sequence described in GenBank AY083469.

Recombinant RSK2 amino acid sequence:

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1 MAHHHHHHGS PLAQLADPWQ KMAVESPSDS AENGQQIMDE PMGEEEINPQ TEEVSIKEIA
61 ITHHVKEGHE KADPSQFELL KVLGQGSFGK VFLVKKISGS DARQLYAMKV LKKATLKVRD
121 RVRTKMERDI LVEVNHPFIV KLHYAFQTEG KLYLILDFLR GGDLFTRL SK EVMFTEEDVK
181 FYLAELALAL DHLHSLGIY RDLKPENILL DEEGHIKLT D FGLSKESIDH EKKAYSFCGT
241 VEYMAPEVVN RRGHTQSADW WSFGVLMFEM LTGTLPFQ GK DRKETMTMIL KAKLGMPQFL
301 SPEAQSLLRM LFKRNPANRL GAGPDGVEEI KRHSFFSTID WNKLYRREIH PPFKPATGRP
361 EDTFYFDPEF TAKTPKDSPG IPPSANAHQL FRGFSFVAIT SDDESQAMQT VGVHSIVQQL
421 HRNSIQFTDG YEVEDIGVG SYSVCKRCIH KATNMEFAVK IIDKSKRDPT EEIEILLRYG
481 QHPNIITLKD VYDDGKYVYV VTELMKG GEL LDKILRQKFF SEREASAVLF TITKTVEYLH
541 AQGVVHRDLK PSNILYVDES GNPESIRICD FGFAKQLRAE NGLLMTPCYT ANFVAPEVLK
601 RQGYDAACDI WSLGVLLYTM LTGYTPFANG PDDTPEEILA RIGSGKFSLS GGYWNSVSDT
661 AKDLVSKMLH VDPHQRLTAA LVLRHPWIVH WDQLPQYQLN RQDAPHLVKG AMAATYSALN
721 RNQSPVLEPV GRSTLAQRRG IKKITSTAL
  
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Recombinant RSK2 nucleotide sequence:

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1 atggcgcac accatcacca tcacggatcc ccgctggcgc agctggcgga cccgtggcag
61 aagatggctg tggagagccc ttccgacagc gcggagaatg gacagcaaat tatggatgaa
121 cctatgggag aggaggagat taaccacaaa actgaagaag tcagtatcaa agaaattgca
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301 gatgctagac agctttatgc catgaaagta ttaaagaagg ccacgctgaa agttcgagac
361 cgtgttcgga caaaaatgga acgtgatatc ttggtagaag tcaatcacc tttcattgtc
421 aaattgcatt acgcttttca aacggaaggg aagttgtatc ttattttgga ttttctcagg
481 ggcggagact tgtttacacg cttatccaaa gaggtgatgt tcacagagga agatgtcaaa
541 ttctacttgg ctgaacttgc acttgcttta gaccatcttc atagcctggg aataatctat
601 agagacttaa aaccagaaaa catacttctt gatgaagaag gtcacatcaa gttaactgat
661 tttggcttaa gtaaggaaatc tattgatcat gagaagaagg cttattcttt ttgtggcact
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781 tggtcctttg gtgtgttgat gtttgaaatg ctcactggta cactaccttt ccaaggaaaa
841 gatcgtaaag aaacaatgac tatgattctt aaagccaaac tcgggatgcc acagtttctg
901 agtctgaag cccagagtct tttacgaatg cttttcaaac ggaatcctgc aaacagatta
961 ggtgctggac cagatggagt tgaagaaatt aaaagacatt ctttttctc aacaatagac
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1261 cacagaaaca gtattcagtt tactgatgga tatgaagtaa aagaggatat tggcgttggc
1321 tcatactccg tttgtaagag atgtatacat aaagctacaa acatggagtt tgccgtgaag
1381 attattgata aaagcaagag agaccaaca gaagagattg aaattcttct tcgctatgga
1441 cagcatccaa acatcattac cctaaaggat gtgtatgatg atggaaaata tgtgtatgta
  
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Certificate of Analysis

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1501 gtaacagaac ttatgaaagg aggtgaattg ctggataaga ttcttagaca gaagtttttc
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1621 gcacaagggg tggttcacag agacttgaaa cctagcaaca ttctttatgt ggatgagtct
1681 ggtaatccag aatctattcg aatttgtgat tttggctttg caaaacaact gagagcagaa
1741 aatggtcttc tcatgactcc ttgttatact gcaaattttg ttgcaccaga ggttttaaaa
1801 cggcaagggt atgatgctgc ctgtgatata tggagtcctg gcgtcctcct ttatacaatg
1861 cttactgggt aactccatt tgcaaatggc cctgatgata ctccagagga aatactggca
1921 cgaataggta gtggaaaatt ctcactcagt ggtggttact ggaattctgt ttcagacaca
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2101 agacaggatg cgccgcatct cgtaaagggt gccatggcag ctacgtactc tgctttaaac
2161 cgcaatcagt ccccgatctt ggaaccagtg ggccgctcca ctcttgctca gcggagaggg
2221 attaaaaaaaa tcacctcaac agccctgtga

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