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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 Ni^{2+}/NTA-agarose. Activated with MAPK2 and PDK1, and re-purified by glutathione, heparin and Ni^{2+}/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.

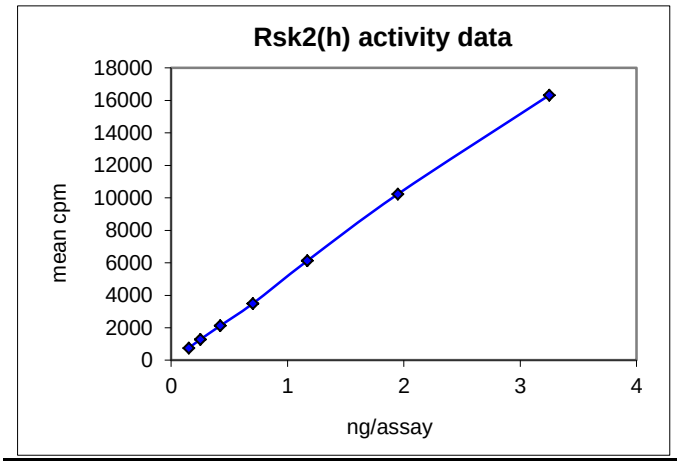
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

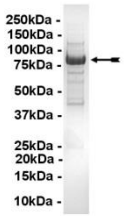
Quality Control Testing

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.



Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom

T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH .0, 1mM EDTA.
2. **(KKKNRTLSVA):** Use at a final concentration of 30µM. Prepare a 300µM stock and add 2.5µl 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-^{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 (0.15–3.25ng) RSK2, 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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RSK2 Sequence Information

Protein human RSK2

Tags *N*-terminal 6His

Native sequence P11 of the recombinant protein is equivalent to P2 of human RSK2

Accession number 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 GGDLFTRLSK EVMFTEEDVK
 181 FYLAELALAL DHLHSLGIIY RDLKPENILL DEEGHIKLTD FGLSKESIDH EKKAYSFCGT
 241 VEYMAPEVVN RRGHTQSADW WSFGVLMFEM LTGTLPFQGK DRKETMTMIL KAKLGMPQFL
 301 SPEAQSLLRM LFKRNPANRL GAGPDGVEEI KRHSFFSTID WNKLYRREIH PPFKPATGRP
 361 EDTFYFDPEF TAKTPKDSPG IPPSANAHQL FRGFSFVAIT SDDESQAMQT VGVHSIVQQL
 421 HRNSIQFTDG YEVKEDIGVG SYSVCKRCIH KATNMEFAVK IIDKSKRDPT EEIEILLRYG
 481 QHPNIITLKD VYDDGKYVYV VTELMKGGEL 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 atggcgcatc accatcacca tcacggatcc ccgctggcgc agctggcgga cccgtggcag
  61 aagatggctg tggagagccc ttccgacagc gcggagaatg gacagcaaat tatggatgaa
 121 cctatgggag aggaggagat taacccacaa actgaagaag tcagtatcaa agaaattgca
 181 atcacacatc atgtgaagga aggacatgaa aaggcagatc cttcccagtt tgaactttta
 241 aaagtattag ggcagggatc atttggaaag gttttcttag ttaaaaaaat ctcaggctct
 301 gatgctagac agctttatgc catgaaagta ttaaagaagg ccacgctgaa agttcgagac
 361 cgtgttcgga caaaaatgga acgtgatatc ttggtagaag tcaatcaccc 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 gtaaggaatc tattgatcat gagaagaagg cttattcttt ttgtggcact
 721 gtggaataca tggctccaga agtagttaac cgcagaggtc acactcagag tgcggactgg
 781 tggtcctttg gtgtgttgat gtttgaaatg ctcactggta cactaccttt ccaaggaaaa
 841 gatcgtaaag aaacaatgac tatgattctt aaagccaaac tcgggatgcc acagtttctg
 901 agtcctgaag cccagagtct tttacgaatg cttttcaaac ggaatcctgc aaacagatta
 961 ggtgctggac cagatggagt tgaagaaatt aaaagacatt cattttctc aacaatagac
1021 tggaataaac tatatagaag agagattcac ccacctttta agcctgcaac tggcagacct
1081 gaagatacat tttattttga tcctgagttt actgcaaaaa ctcccaaaga ttcaccgggc
1141 attccaccta gtgctaacgc acatcagctt tttcgggggt ttagttttgt tgctattacc
1201 tcagatgatg aaagccaagc tatgcagaca gttggtgtgc attcaattgt tcagcaatta
1261 cacagaaaca gtattcagtt tactgatgga tatgaagtaa aagaggatat tggcgttggc
1321 tcatactccg tttgtaagag atgtatacat aaagctacaa acatggagtt tgccgtgaag
1381 attattgata aaagcaagag agacccaaca gaagagattg aaattcttct tcgctatgga
1441 cagcatccaa acatcattac cctaaaggat gtgtatgatg atggaaaata tgtgtatgta
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1501 gtaacagaac ttatgaaagg aggtgaattg ctggataaga ttcttagaca gaagtttttc
1561 tcagagcgag aggccagcgc tgtcctgttt actataacca aaactgttga gtatcttcat
1621 gcacaagggg tggttcacag agacttgaaa cctagcaaca ttctttatgt ggatgagtct
1681 ggtaatccag aatctattcg aatttgtgat tttggctttg caaaacaact gagagcagaa
1741 aatggtcttc tcatgactcc ttgttatact gcaaattttg ttgcaccaga ggttttaaaa
1801 cggcaaggtt atgatgctgc ctgtgatata tggagtcttg gcgtcctcct ttatacaatg
1861 cttactggtt acactccatt tgcaaatggc cctgatgata ctccagagga aatactggca
1921 cgaataggta gtggaaaatt ctcactcagt ggtggttact ggaattctgt ttcagacaca
1981 gcaaaggacc tggtgtcaaa gatgcttcat gtagatcctc atcagagact gacggctgct
2041 ctggtgctca gacatccttg gattgtccac tgggaccaac taccacaata ccaactaaac
2101 agacaggatg cgccgcatct cgtaaagggt gccatggcag ctacgtactc tgctttaaac
2161 cgcaatcagt ccccagtctt ggaaccagtg ggccgctcca ctcttgctca gcggagaggg
2221 attaaaaaaa tcacctcaac agccctgtga
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Reviewed and approved by site quality representative.