

Certificate of Analysis

Rsk4, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-702, 14-702-K, 14-702M

Parent Lot # D8JN056U

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 Rsk4, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Activated using MAP Kinase 2 (cat# 14-173) and repurified using glutathione-agarose. Purity 80.9% by SDS-PAGE and Coomassie blue staining. MW = 85.2kDa.

Specific Activity (Parent lot# D8JN056U): 255U/mg, where one unit of Rsk4, active activity is defined as 1nmol phosphate incorporated into PAKtide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.206mg/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.

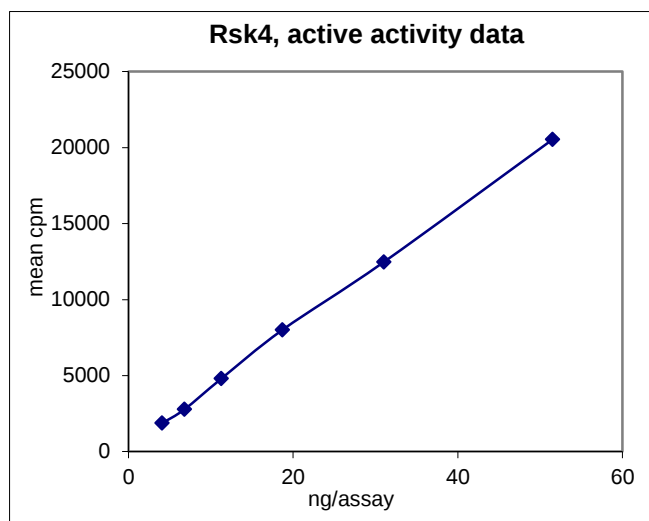
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

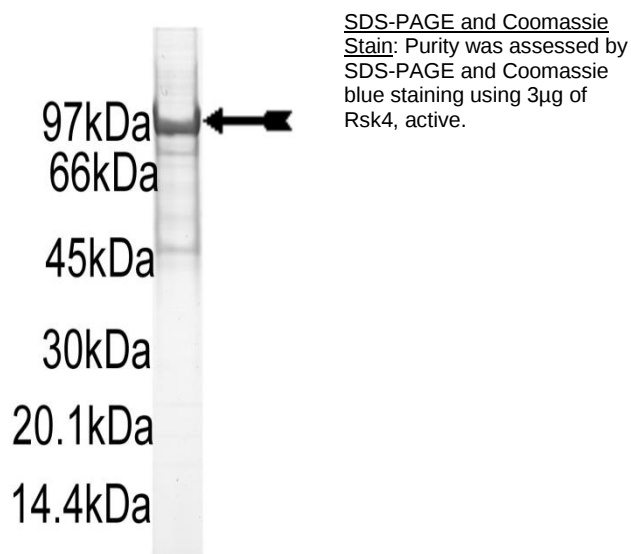
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4–52ng of this lot of enzyme phosphorylated 200µM PAKtide new peptide used in this assay 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 Rsk4 with the translated native 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. **PAKtide (RRRLSFAEPG):** Use at a final assay concentration of 200µM. Prepare 2mM stock and add 2.5µl of stock per assay point.
3. **Rsk4, active:** Dilute with 20mM MOPS-NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–52ng 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 **PAKtide**.
3. Add 2.5µl (**4–52ng**), **Rsk4 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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Rsk4 Sequence Information

<u>Protein</u>	Human Rsk4
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	GenBank NM_014496
<u>Accession number</u>	M10 of recombinant sequence is equivalent to M1 of human Rsk4

Recombinant Rsk4 amino acid sequence:

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1  MHHHHHHEFM LPFAPQDEPW DREMEVFSGG GASSGEVNGL KMVDEPMEEG EADSCHDEGV
61  VKEIPITHHV KEGYEKADPA QFELLKVLGQ GSFGKVFLVR KKTGPDAGQL YAMKVLKKAS
121 LKVRDRVRTK MERDILVEVN HPFIVKLHYA FQTEGKLYLI LDFLRGGDVF TRLSKEVLFT
181 EEDVKFYLAE LALALDHLHQ LGIVYRDLKP ENILLDEIGH IKLTDFGLSK ESVDQEKKAY
241 SFCGTVEYMA PEVVNRRGHS QSADWWSYGV LMFEMLTGTL PFQGKDRNET MNMILKAKLG
301 MPQFLSAEAQ SLLRMLFKRN PANRLGSEGV EEIKRHLFFA NIDWDKLYKR EVQPPFKPAS
361 GKPDDTFCFD PEFTAKTPKD SPGLPASANA HQLFKGFSFV ATSI AEEYKI TPITSANVLP
421 IVQINGNAAQ FGEVYELKED IGVGSYSVCK RCIHATTNME FAVKIIDKSK RDPSEEIEIL
481 MRYGQHPNII TLKDVFD DGR YVYLVDLMK GGELLDRIK QKCF SEREAS DILYVISKTV
541 DYLMQGVVH RDLKPSNILY MDESASADSI RICDFGFAKQ LRGENGLLLT PCYTANFVAP
601 EVLMQGGYDA ACDIWSLGLV FYTMLAGYTP FANGPNDTPE EILLRIGNGK FSLSGGNWDN
661 ISDGAKDLLS HMLHMDPHQR YTAEQILKHS WITHRDQLPN DQPKRNDVSH VVKGAMVATY
721 SALTHKTFQP VLEPVAASSL AQRSMKKRT STGL

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Recombinant Rsk4 nucleotide sequence:

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1  atgcatcatc accatcacca tgaattcatg ctaccattcg ctcctcagga cgagccctgg
61  gaccgagaaa tggaaagtgtt cagcggcgggc ggcgcgagca gcggcgaggt aaatggctct
121  aaaaatgggtg atgagccaat ggaagaggggc gaagcagatt cttgtcatga tgaaggagtt
181  gttaaagaaa tccctattac tcatcatgtt aaggaaggct atgagaaagc agatcctgca
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301  aagaagaccg gtcctgatgc tgggcagctc tatgcaatga aggtgttaaa aaaagcctct
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481  ctggattttc tcaggggagg agatgttttc acaagattat ccaaagaggt tctgtttaca
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661  atcaaattaa cagattttgg actcagcaag gtagtagtag atcaagaaaa gaaggcttac
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1501 tatgtttacc ttgttacgga ttaaatgaaa ggaggagagt tacttgaccg tattctcaaa
1561 caaaaatggt tctcggaaac ggaggctagt gatatactat atgtaataag taagacagtt

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1621 gactatcttc attgtcaagg agttgttcat cgtgatctta aacctagtaa tattttatac
1681 atggatgaat cagccagtgc agattcaatc aggatatgtg attttgggtt tgcaaaacaa
1741 cttcgaggag aaaatggact tctcttaact ccatgctaca ctgcaaactt tgttgcacct
1801 gaggttctta tgcaacaggg atatgatgct gcttgtgata tctggagttt aggagtcctt
1861 ttttacacaa tgttggctgg ctacactcca ttgctaata gcccgaatga tactcctgaa
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1981 atttcagacg gagcaaagga ttgctttcc catatgcttc atatggacc acatcagcgg
2041 tatactgctg aacaaatatt aaagcactca tggataactc acagagacca gttgccaaat
2101 gatcagccaa agagaaatga tgtgtcacat gttgttaagg gagcaatggt tgcaacatac
2161 tctgccctga ctcacaagac ctttcaacca gtcctagagc ctgtagctgc ttcaagctta
2221 gcccgcgac ggagcatgaa aaagcgaaca tcaactggcc tgtaa

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