

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.

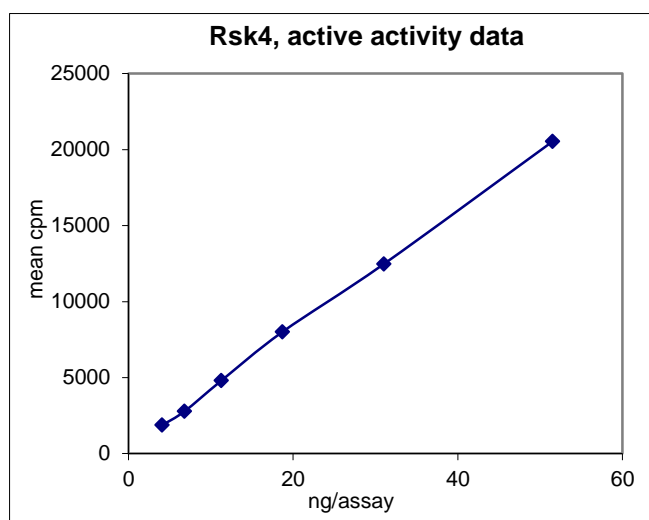
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

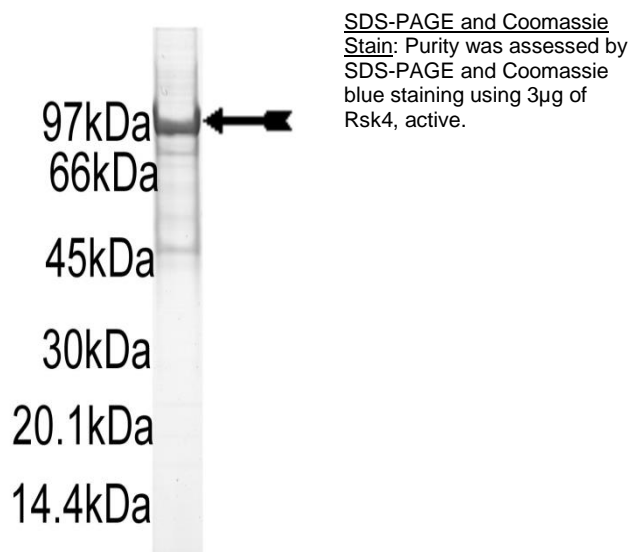
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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 200 μ M. Prepare 2mM stock and add 2.5 μ l 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 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PAKtide**.
3. Add 2.5 μ l (**4–52ng**), **Rsk4 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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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  MHHHHHEFM  LPFAPQDEPW  DREMEVFSGG  GASSGEVNGL  KMVDEPMEEG  EADSCHDEGV
61  VKEIPITHHV  KEGYEKADPA  QFELLKVLGQ  GSFQKVLVLR  KKTGPDAGQL  YAMKVLKKAS
121 LKVRDRVRTK  MERDILVEVN  HPFIVKLHYA  FQTEGKLYLI  LDFLRGGDVF  TRLSKEVLFT
181 EEDVKFYLAE  LALALDHLHQ  LGIVYRDLKP  ENILLDEIGH  IKLTFDGLSK  ESVDQEKKAY
241 SFCGTVEYMA  PEVVNRRGHS  QSADWWSYGV  LMFEMLTGTL  PFQGKDRNET  MNMILKAKLG
301 MPQFLSAEAQ  SLLRMLFKRN  PANRLGSEGV  EEIKRHFFA  NIDWDKLYKR  EVQPPFKPAS
361 GKPDDTFCFD  PEFTAKTPKD  SPGLPASANA  HQLFKGFSFV  ATSI AEEYKI  TPITSANVLP
421 IVQINGNAAQ  FGEVYELKED  IGVGSYSVCK  RCIHATTNME  FAVKIIDKSK  RDPSEEIEIL
481 MRYGQHPNII  TLKDVFD DGR  YVYLVTDLMK  GGELLDRIK  QKCF SEREAS  DILYVISKTV
541 DYLHCQGVVH  RDLKPSNILY  MDESASADSI  RICDFGFAKQ  LRGENGLLLT  PCYTANFVAP
601 EVLMQQGYDA  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  cagcggcggc  ggcgcgagca  gcggcgagggt  aaatggctct
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241  cagtttgagt  tgctcaaggt  tcttggtcag  gggtcatttg  gaaaggtttt  tcttgttaga
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361  ttaaaagtcc  gagacagagt  tcggacaaaag  atggagaggg  atatactggt  ggaagtaaat
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481  ctggattttc  tcaggggagg  agatgttttc  acaagattat  ccaaagaggt  tctgtttaca
541  gaggaagatg  tgaatttcta  cctcgcagaa  ctggcccttg  ctttgatca  tctgcaccaa
601  ttaggaattg  tttatagaga  cctgaagcca  gaaaacattt  tgcttgatga  aataggacat
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1561  caaaaatggt  tctcggaaac  ggaggctagt  gatatactat  atgtaataag  taagacagtt
    
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1741 cttcgaggag aaaatggact tctcttaact ccatgctaca ctgcaaactt tgttgcacct
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1861 ttttacacaa tgttggctgg ctacactcca tttgctaata gcccacatga tactcctgaa
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1981 attcagacg gagcaaagga tttgctttcc catatgcttc atatggacc acatcagcgg
2041 tatactgctg aacaatatatt aaagcactca tggataactc acagagacca gttgccaat
2101 gatcagccaa agagaaatga tgtgtcacat gttgttaagg gagcaatggt tgcaacatac
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2221 gccacgcgac ggagcatgaa aaagcgaaca tcaactggcc tgtaa
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