

## Certificate of Analysis

### RSK3, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-462, 14-462-K, 14-462M

Parent Lot # D7AN006U

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 RSK3, expressed in Sf21 insect cells. Purified using Ni<sup>2+</sup>/NTA agarose. Activated using MAP Kinase 2 (cat# 14-173) and PDK1 (cat# 14-452) and repurified using glutathione-agarose, heparin Sepharose and Ni<sup>2+</sup>/NTA-agarose. Purified to 27% by SDS-PAGE and Coomassie blue staining. MW = 87kDa.

**Specific Activity (Parent lot# D7AN006U):** 243U/mg, where one unit of RSK3, active activity is defined as 1nmol phosphate incorporated into 30µM (KKNRRTLSVA) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation:** 1.688mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

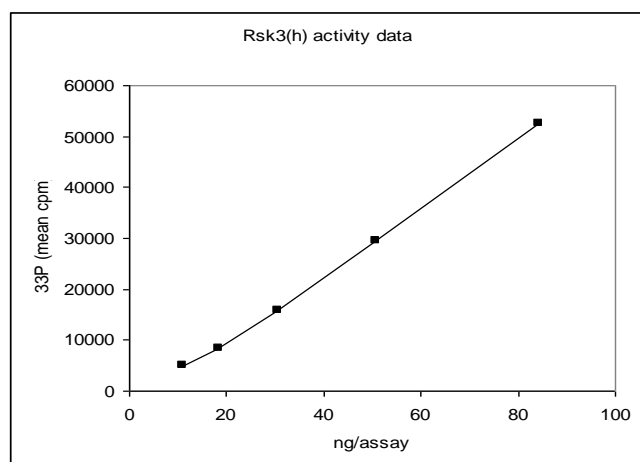
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

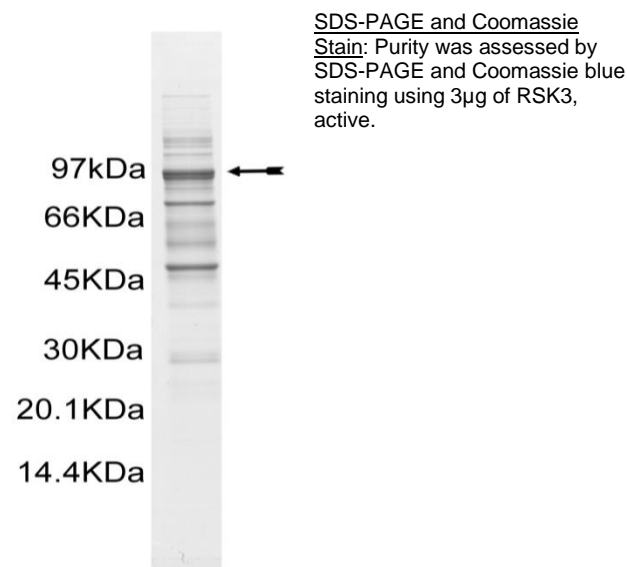
**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

### Quality Control Testing

**Kinase Assay:** 11–84ng of this lot of enzyme phosphorylated 30µM (KKNRRTLSVA) 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 RSK3 with the translated sequence listed on page three.



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### Kinase Assay Protocol

#### Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS pH7.0, 1mM EDTA.
2. **(KKNRSLVA):** Use at a final assay concentration of 30 $\mu$ M. Prepare a 300 $\mu$ M stock and add 2.5 $\mu$ l of stock per assay point.
3. **RSK3, active:** Dilute with 20mM MOPS pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 11–84ng per assay point.
4. **[ $\gamma$ -<sup>33</sup>P]ATP:** 2.5x magnesium acetate/[ $\gamma$ -<sup>33</sup>P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [ $\gamma$ -<sup>33</sup>P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

#### Assay Procedure (96 well plate format):

1. Add 5 $\mu$ l of 5 x reaction buffer per assay to wells.
2. Add 2.5 $\mu$ l of **(KKNRSLVA)**.
3. Add **2.5 $\mu$ l (11–84ng) RSK3, active**.
4. Add 5 $\mu$ l of dH<sub>2</sub>O.
5. Add 10 $\mu$ l of diluted [ $\gamma$ -<sup>33</sup>P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 $\mu$ l of 3% phosphoric acid.
8. Transfer a 10 $\mu$ 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 $\mu$ l of 30% phosphoric acid.

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## RSK3 Sequence Information

|                                |  |
|--------------------------------|--|
| <b><u>Protein</u></b>          | human RSK3   |
| <b><u>Tags</u></b>             | N-terminal 6His  |
| <b><u>Native sequence</u></b>  | M31 of the recombinant protein is equivalent to M1 of human RSK3 |
| <b><u>Accession number</u></b> | GenBank XM_004469  |

### Recombinant RSK3 amino acid sequence:

```

1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF MDLSMKKFAV RFFFSVYLRR KSRSKSSSL
61 RLEEEGVVKE IDISHHVKEG FEKADPSQFE LLKVLGQGSY GKVFLVRKVK GSDAGQLYAM
121 KVLKKATLKV RDRVRSKMER DILAENVHPF IVKLHYAFQT EGKLYLILDF LRGDDLFTL
181 SKEVMFTEED VKFYLAELAL ALDHLHSLGI IYRDLKPENI LLDEEGHIKI TDFGLSKEAI
241 DHDKRAYSFC GTIEYMAPEV VNRRGHTQSA DWWSFGVLMF EMLTGSLPFQ GKDRKETMAL
301 ILKAKLGMPQ FLSGEAQSL RALFKRNPENI RLGAGIDGVE EIKRHPFFVT IDWNTLYRKE
361 IKPPFKPAVG RPEDTFHFDP EFTARTPTDS PGVPPSANAH HLFGRGFSFVA SSLIQEPSQQ
421 DLHKVPVHPI VQQLHGNNIH FTDGYEIKED IGVGSYSVCK RCVHKATDTE YAVKIIDKSK
481 RDPSEEIEIL LRYGQHPNII TLKDVYDDGK FVYLMELMR GGELLDRI LR QRYFSEREAS
541 DVLCTITKTM DYLSHQGVVH RDLKPSNII RDESGSPESI RVCDFGFAKQ LRAGNGLLMT
601 PCYTANFVAP EVLKRQGYDA ACDIWSLGI LYTMLAGFTP FANGPDDTPE EILARIGSGK
661 YALSGGNWDS ISDAAKD VVS KMLHVDPHQR LTAMQVLKHP WVVNREYLS P NQLSRQDVHL
721 VKGAMAATYF ALNRTPOAPR LEPVLSNLA QRRGMKRLTS TRL
    
```

### Recombinant RSK3 nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc atggacctga gcatgaagaa gttcgcctg
121 cgcaggttct tctctgtgta cctgcgcagg aagtcgcgct ccaagagctc cagcctgagc
181 cggctcgagg aagaaggtgt cgtgaaggag atagacatca gccatcatgt gaaggagggc
241 tttgagaagg cagatccttc ccagtttgag ctgctgaagg ttttaggaca aggatcctat
301 ggaaaggtgt tcctggtgag gaaggtgaag ggggccgacg ctgggcagct ctacgccatg
361 aaggtcctta agaaagccac ctaaaaagtt cgggaccgag tgagatcgaa gatggagaga
421 gacatcttgg cagaagtga tcacccttc attgtgaagc ttcattatgc ctttcagacg
481 gaaggaaagc tctacctgat cctggacttc ctgccccgag gggacctct caccgcgctc
541 tccaaagagg tcatgttcac ggaggaggat gtcaagttct acctggctga gctggccttg
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721 gaccacgaca agagagcgta ctctctctgc gggacgatcg agtacatggc gcccgaggtg
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1201 cacctgttta gaggattcag ctttgtggcc tcaagcctga tccaggagcc ctcacagcaa
1261 gatctgcaca aagtcctcag tcaccaatc gtgcagcagt tacacgggaa caacatccac
1321 ttcaccgatg gctacgagat caaggaggac atcgggggtg gctcctactc agtgtgcaag
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1501 accctcaagg atgtctatga tgatggcaag tttgtgtacc tggtaatgga gctgatgcgt
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1621 gacgtcctgt gcaccatcac caagaccatg gactacctcc attcccagg ggtgtgtcat
1681 cgagacctga agccgagtaa catcctgtac agggatgagt cggggagccc agaatccatc
    
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```
1741 cgagtctgcg acttcggctt tgccaagcag ctgcgcgcgg ggaacgggct gtcctatgaca
1801 ccctgctaca cggccaatth cgtggccccg gaggtcctga agcgtcaagg ctatgatgcg
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1981 tatgcccttt ctgggggaaa ctgggactcg atatctgacg cagctaaaga cgtcgtgtcc
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2101 tgggtggtca acagagagta cctgtcccca aaccagctca gccgacagga cgtgcacctg
2161 gtgaagggcg cgatggccgc cacctacttt gctctaaaca gaacacctca ggccccgagg
2221 ctggagcccg tgctgtcatc caacctggct cagcgcagag gcatgaagag actcacgtcc
2281 acgcggctgt ag
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