

Certificate of Analysis

p70 S6 Kinase (1–421, T412E), active (Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-486, 14-486-K, 14-486M

Parent Lot # WAA0173

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 amino acids 1–421 of human p70 S6 kinase expressed in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose, activated by PDK1, and repurified on heparin-agarose. Purity 89.8% by SDS-PAGE and Coomassie blue staining. MW = 48.7kDa.

Specific Activity (Parent lot# WAA0173): 280U/mg, where one unit of p70 S6 kinase, active activity is defined as 1nmol phosphate incorporated into 100µM (KKRNRTLTV) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.032mg/ml of enzyme in 50mM Tris/HCl pH7.5, 270mM sucrose, 150mM NaCl, 1mM benzamidine, 0.2mM PMSF, 0.1mM EGTA, 0.1% 2-mercaptoethanol, 0.03% Brij-35. Frozen solution.

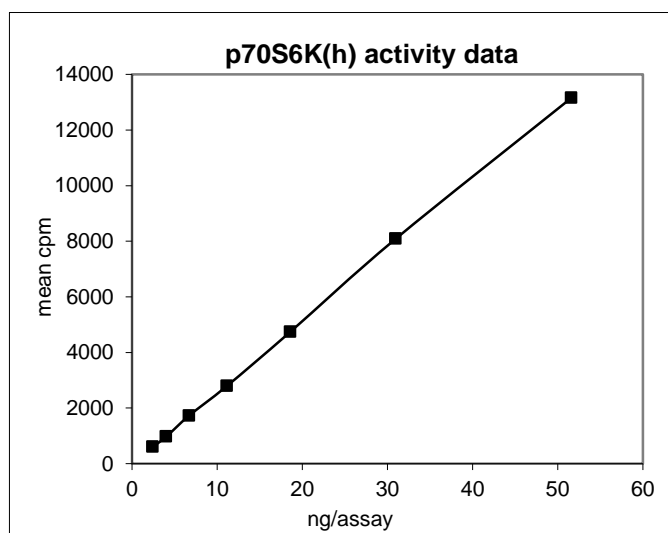
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

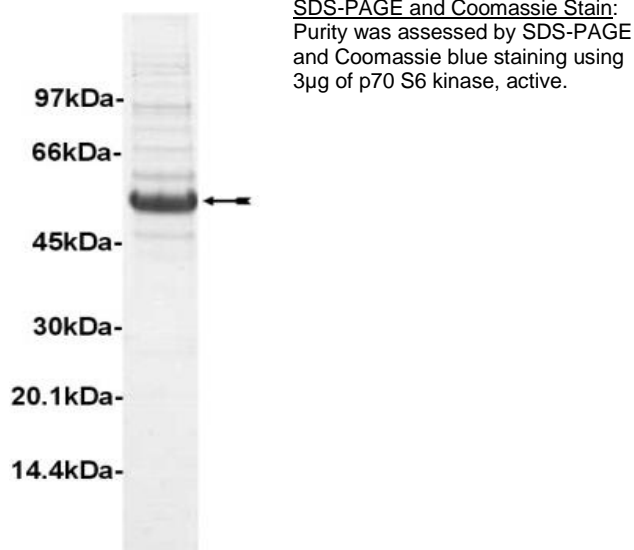
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.4–51.6ng of this lot of enzyme phosphorylated 100µM (KKRNRTLTV) 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 p70 S6 kinase with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
- 2. Substrate peptide (KKRNRTLTV):** Use at a final assay concentration of 100µM. Make up a 1mM stock. Add 2.5µl of stock per assay point.
- 3. p70 S6 kinase, (1-421, T412E), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.4–51.6ng 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 appropriate wells.
2. Add 2.5µl of (KKRNRTLTV).
3. Add **2.5µl (2.4–51.6ng) p70 S6 kinase, (1-421, T412E) 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 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 enzyme components plus 1µl 30% phosphoric acid.

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p70 S6 kinase Sequence Information

<u>Protein</u>	Human p70 S6 kinase
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the fusion protein is equivalent to M1 of human p70 S6 kinase. The recombinant protein encodes residues 1–421, only, of the native sequence.
<u>Accession number</u>	GenBank M60724. The recombinant protein contains an amino acid substitution, T412E, with reference to M60724. This mutation mimics phosphorylation of T412 and facilitates activation by PDK1.

Recombinant p70 S6 kinase amino acid sequence:

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1  MHHHHHMRR RRRRDGFYPA PDFRDREAED MAGVFDIDLQ QPEDAGSEDE LEEGGQLNES
61  MDHGGVGPYE LGMEHCEKFE ISETSVNRGP EKIRPECFEL LRVLGKGGYG KVFQVRKVTG
121 ANTGKIFAMK VLKAMIVRN AKDTAHTKAE RNILEEVKHP FIVDLIYAFQ TGGKLYLILE
181 YLSGGELFMQ LEREGIFMED TACFYLAEIS MALGHLHQKG IYRDLKPEN IMLNHQGHVK
241 LTDFGLCKES IHDGTVTHTF CGTIEYMAPE ILMRSGHNRA VDWWSLGALM YDMLTGAPPF
301 TGENRKKTID KILKCKLNL PYLTQEARDL LKLLKRNAA SRLGAGPGDA GEVQAHPPFR
361 HINWEELLAR KVEPPFKPLL QSEEDVSQFD SKFTRQTPVD SPDDSTLSES ANQVFLGFYE
421 VAPSVLES
  
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Recombinant p70 S6 kinase nucleotide sequence:

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1  atgcaccatc accatcacca tatgaggcga cgaaggaggc gggacggcctt ttaccagcgc
61  cctgacttcc gagacagggg agctgaggac atggcaggag tgtttgacat agacctggac
121 cagccagagg atgcaggctc tgaggatgag ctggaggagg ggggtcagtt aatgaaagc
181 atggaccatg ggggagttgg accatatgaa cttggcatgg aacattgtga gaaattttaa
241 atctcagaaa ctagtgtgaa cagagggcca gaaaaaatca gaccagaatg ttttgagcta
301 cttcgggtac ttggtaaagg gggctatgga aaggtttttc aagtacgaaa agtaacagga
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421 gctaaagata cagctcatalc aaaagcagag cggaatattc tggaggaagt aaagcatccc
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1201 agccccgatg actcaactct cagtgaaggt gcccaaccagg tctttctggg tttcgaatac
1261 gtggctccat ctgtacttga aagttga
  
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