

Certificate of Analysis

SAPK3, active

(Recombinant enzyme expressed in *E.coli* cells)

Item # 14-246, 14-246-K, 14-246M

Parent Lot # D8JN052U

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 GST tagged, recombinant full length human SAPK3, expressed in *E.coli* cells. Purified using glutathione sepharose. Activated using a constitutively active mutant of MKK6 and repurified using glutathione sepharose. Purity 89.7% by SDS-PAGE and Coomassie staining. MW = 68.6kDa.

Specific Activity (Parent lot# D8JN052U): 337U/mg, where one unit of SAPK3, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.1465mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 270mM sucrose, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

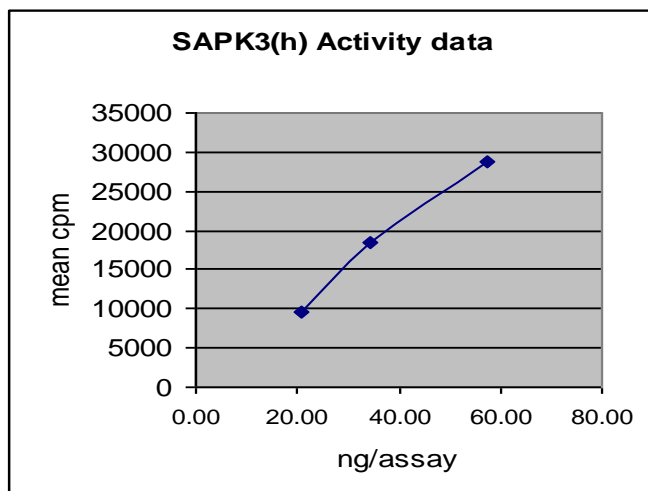
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

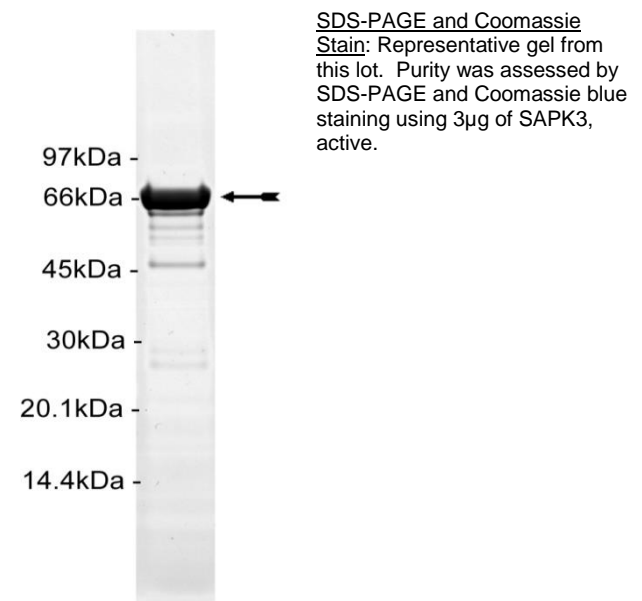
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 20.8–57.3ng of this lot of enzyme phosphorylated 0.33mg/ml myelin basic protein (MBP) 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 SAPK3 with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

- 1. 5 x Reaction Buffer:** 125mM Tris/HCl pH7.5, 0.1mM EGTA.
- 2. Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
- 3. SAPK3, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 20.8–57.3ng 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 **myelin basic protein (MBP)**.
3. Add **2.5µl (20.8–57.3ng) SAPK3, active**.
4. Add 10µl of diluted [γ -³³P]ATP mixture.
5. Add 5µl of dH₂O.
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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SAPK3 Sequence Information

<u>Protein</u>	Human SAPK3
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the fusion protein is equivalent to M1 of human SAPK3
<u>Accession number</u>	EMBL Y10487

Recombinant SAPK3 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPOID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFM SSPPPARSGF
241 YRQEVTKTAW EVRAVYRDLQ PVGSGAYGAV CSAVDGRTGA KVAIKKLYRP FQSELF AKRA
301 YRELRLKHM RHENVIGLLD VFTPDELDD FTDFYLVMPF MGTDLGKLMK HEKLGEDRIQ
361 FLVYQMLKGL RYIHAAGIIH RDLKPGNLAV NEDCELKILD FGLARQADSE MTGYVVTRWY
421 RAPEVILNWM RYTQTVDIWS VGCIMAEMIT GKTLFKGSDH LDQLKEIMKV TGTPPAEFVQ
481 RLQSDAEKNY MKGLPELEKK DFASILTNAS PLAVNLEKEM LVLDAEQRVT AGEALAHYPF
541 ESLHDTDEP QVQKYDDSDFD DVDRTLDEWK RVTYKEVLSF KPPRQLGARV SKETPL
  
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Recombinant SAPK3 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgatg agcgcgatga aggtgataaa
121 tggcgaaca aaaagtttga attgggtttg gagtttcca atcttccta ttatattgat
181 ggtgatgta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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1501 gattttgcct ctatcctgac caatgcaagc cctctggctg tgaacctcct ggagaagatg
1561 ctgggtgctgg acgcggagca gcggtgacg gcaggcgagg cgctggcca tccctacttc
1621 gagtccctgc acgacacgga agatgagccc caggtccaga agtatgatga ctctttgac
  
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1681 gacgttgacc gcacactgga tgaatggaag cgtgttactt acaaagaggt gctcagcttc  
1741 aagcctcccc ggcagctggg ggccagggc tccaaggaga cgcctctgtg a
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