

Certificate of Analysis

SAPK2b, active

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

Item # 14-253, 14-253-K, 14-253M

Parent Lot # D8PN046U

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, full length human SAPK2b, expressed in *E.coli* cells. Purified using glutathione sepharose. Activated using a constitutively active mutant of MKK6 and repurified using glutathione sepharose. Purity 80% by SDS-PAGE and Coomassie blue staining. MW = 71kDa.

Specific Activity (Parent lot# D8PN046U): 118U/mg, where one unit of SAPK2b 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.

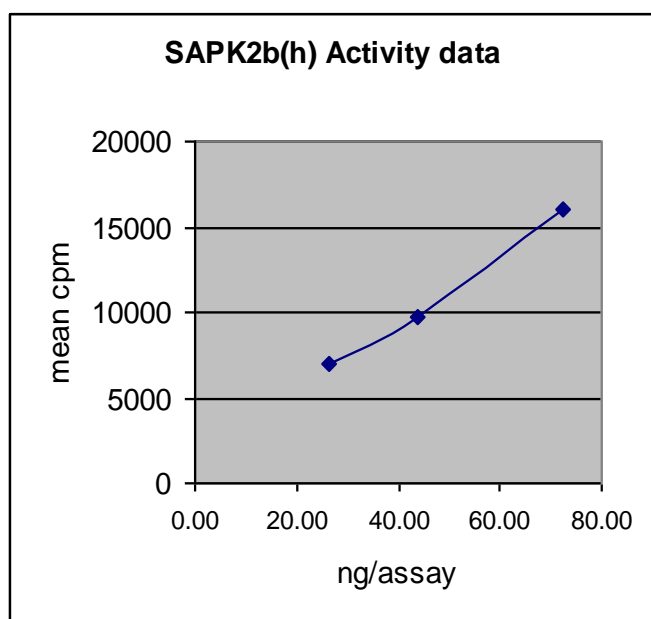
Formulation: 3.992mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Storage and Stability: On receipt of material store at -20°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.

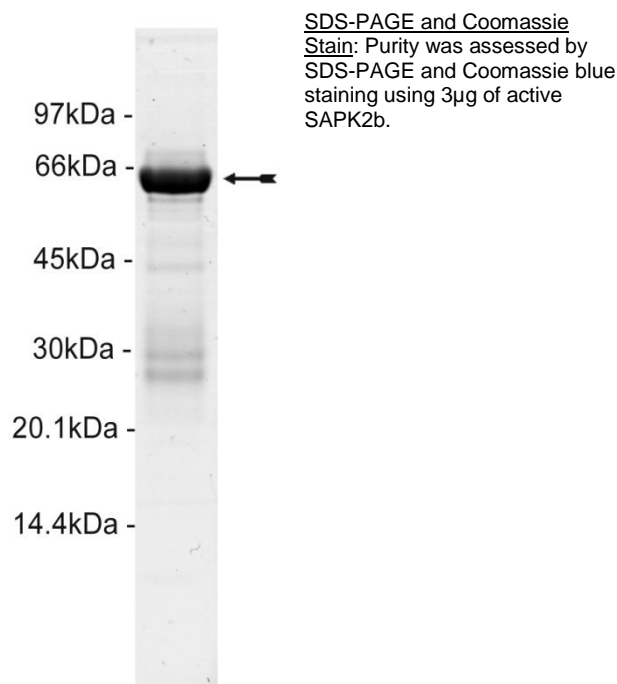
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 26–72ng of this lot of enzyme phosphorylated 0.33mg/ml 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 SAPK2b with the translated sequence listed on page three.



Certificate of Analysis

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. Prepare a 3.33mg/ml stock. Use 2.5µl of stock per assay point.
- 3. SAPK2b, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 26–72ng 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 to each well.
2. Add 2.5µl of **MBP**.
3. Add **2.5µl (26–72ng) SAPK2b, 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 to each well.
8. Using a multichannel pipette, spot 10µl 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.

Certificate of Analysis

SAPK2b Sequence Information

<u>Protein</u>	human SAPK2b
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the fusion protein is equivalent to M1 of human SAPK2b
<u>Accession number</u>	EMBL Y14440

Recombinant SAPK 2b amino acid sequence:

```

1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSR IA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPKSD LVPRGSPEFM SGPRAGFYRQ
241 ELNKT VWEVP QRLQGLRPVG SGAYGSVCSA YDARLRQKVA VKKLSRPFQS LIHARRTYRE
301 LRL LKHLKHE NVIGLLDVFT PATSIEDFSE VYLVTTLMGA DLNNIVKCQA LSDEHVQFLV
361 YQLLRGLKYI HSAGIIHRDL KPSNVAVNED CELRILDFGL ARQADEEMTG YVATRWYRAP
421 EIMLNWMHYN QTVDIWSVGC IMAELLQGKA LFPGSDYIDQ LKRIMEVVTG PSPEVLAKIS
481 SEHARTYIQS LPPMPQK DLS SIFRANPLA IDLLGRMLVL DSDQRVSAAE ALAHAYFSQY
541 HDPEDEPEAE PYDESVEAKE RTLEEWKELT YQEVLSFKPP EPPKPPGSLE IEQ
  
```

Recombinant SAPK2b nucleotide sequence:

```

1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaacaa aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
181 ggtgatgatta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
361 gattttctta gcaagctacc tgaatgctg aaaatgttcg aagatcgttt atgtcataaa
421 acatatttaa atggtgatca tgaaccat cctgacttca tgttgtatga cgctcttgat
481 gttgttttat acatggacc aatgtgcctg gatgcgttcc caaaattagt ttgttttaa
541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
601 tggcctttgc agggctggca agccacgtt ggtggtggcg accatcctcc aaaatcggat
661 ctggttccgc gtggatcccc ggaattcatg tggggccctc gcgccggctt ctaccggcag
721 gagctgaaca agaccgtgtg ggaggtgccg cagcggctgc aggggctgcg cccgggtggc
781 tccggcgcct acggctccgt ttgttcggcc tacgacgcc ggctgcgcca gaaggtggcg
841 gtgaagaagc tgtcgcgcc cttccagtcg ctgatccacg cgcgcagaac gtaccgggag
901 ctgcggctgc tcaagcacct gaagcacgag aacgtcatcg ggcttctgga cgtcttcacg
961 cggccacgt ccatcgagga cttcagcga gttgtacttg tgaccaccct gatgggcgcc
1021 gacctgaaca acatcgtcaa gtgccaggcg ctgagcgacg agcacgttca attcctgggt
1081 taccagctgc tgcgcgggct gaagtacatc cactcggccg ggatcatcca cgggacctg
1141 aagccagca acgtggctgt gaacgaggac tgtgagctca ggatcctgga tttcgggctg
1201 gcgcgccagg cggacgagga gatgaccggc tatgtggcca cgcgctggta cgggacact
1261 gagatcatgc tcaactggat gcattacaac caaacagtgg atatctggtc cgtgggctgc
1321 atcatggctg agctgctcca gggcaaggcc ctcttcccgg gaagcgacta cattgaccag
1381 ctgaagcgca tcatggaagt ggtgggcaca cccagccctg aggttctggc aaaaatctcc
1441 tcggaacacg cccggacata tatccagtc ctgccccca tgccccagaa ggacctgagc
1501 agcatcttcc gtggagccaa ccccctggcc atagacctcc ttggaaggat gctgggtctg
1561 gacagtgacc agagggtcag tgcagctgag gcactggccc acgcctactt cagccagtac
1621 cacgaccccg aggatgagcc agaggccgag ccatatgatg agagcgttga ggccaaggag
1681 cgcacgctgg aggatggaa ggagctcact taccaggaag tcctcagctt caagcccca
  
```

Certificate of Analysis

1741 gagccaccga agccacctgg cagcctggag attgagcagt ga

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 **Eurofins Pharma Discovery Services UK Limited** is an independent member of Eurofins Discovery Services.