

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.

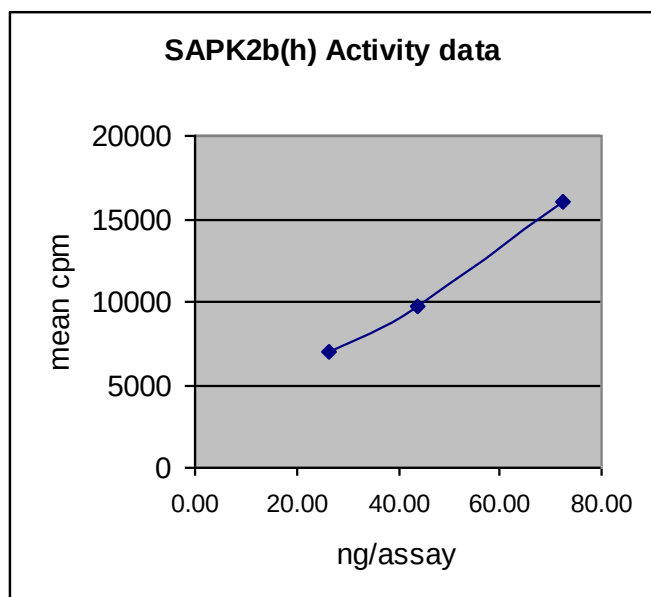
Formulation: 3.992mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

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.

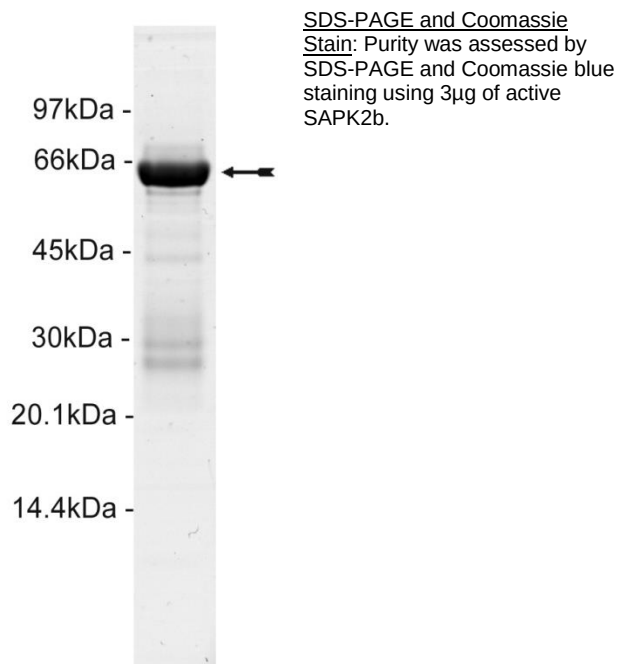
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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.



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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. 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.

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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:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSM IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFM SGPRAGFYRQ
241 ELNKTVEVP QRLQGLRPVG SGAYGSVCSA YDARLRQKVA VKKLSRPFQS LIHARRTYRE
301 LRLKHLKHE NVIGLLDVFT PATSIEDFSE VYLVTTLMGA DLNNIVKCQA LSDEHVQFLV
361 YQLLRGLKYI HSAGIHRDL KPSNVAVNED CELRILDFGL ARQADEEMTG YVATRWYRAP
421 EIMLNWMHYN QTVDIWSVGC IMAELLQGKA LFPGSDYIDQ LKRIMEVVG T PSPEVLAKIS
481 SEHARTYIQS LPPMPQKDLS SIFRANPLA IDLLGRMLVL DSDQRVSAE ALAHAYFSQY
541 HDPEDEPEAE PYDESVEAKE RTLEEWKELT YQEVLSFKPP EPPKPPGSLE IEQ

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Recombinant SAPK2b nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcatga aggtgataaa
121 tggcgaaaca aaaagtgtga attgggtttg gaggttccca atcttcctta ttatatgtat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
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541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
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1681 cgcacgctgg aggatggaa ggagctcact taccaggaag tcctcagctt caagccccc

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1741 gagccaccga agccacctgg cagcctggag attgagcagt ga

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