

Certificate of Analysis

P38 beta 2/SAPK2b, unactive (Recombinant enzyme expressed in *E.coli* cells)

Item # 14-244, 14-244-K, 14-244M

Parent Lot # 33213U

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 p38 beta 2/SAPK2b, unactive, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 97% by SDS-PAGE and Coomassie blue staining. MW = 71kDa.

Formulation: 2.74mg/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.

Specific Activity (Parent lot# 33213U): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with DDMKK6.

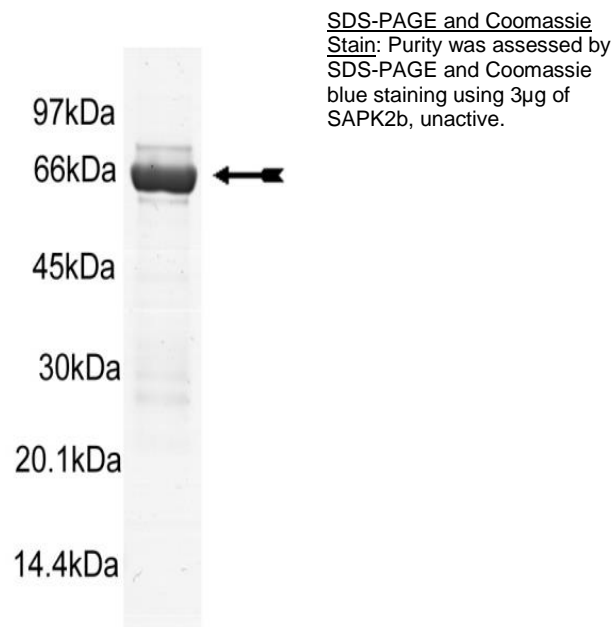
Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

Quality Control Testing

Activation Assay: 4µM unactive SAPK2a was activated using 300nM DDMKK6, diluted 10-fold and the increased activity against Myelin Basic Protein determined. The activation and subsequent assay are described on page two. Results of this assay are 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. **10 x SAPK2b activation buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
2. **5 x SAPK2b assay buffer:** 125mM Tris/HCl pH7.5, 0.1mM EGTA.
3. **Enzyme Dilution Buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
4. **Magnesium/ATP Cocktail (5 x stock):** 500µM cold ATP, and 50mM magnesium acetate
5. **[γ-³³P]ATP:** 2.5x magnesium acetate/[γ-³³P]ATP cocktail: 25mM magnesium acetate and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)
6. **DDMKK6, active:** Use at a final assay concentration of 300nM (0.024mg/ml). Prepare a 0.24mg/ml stock and add 2.5µl of stock per assay point.
7. **p38 beta/SAPK2a, unactive:** Use at a final concentration of 4µM (0.284mg/ml). Prepare a 1.42mg/ml stock and add 5µl of stock per assay point.
8. **Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and add 2.5µl of stock per assay point.

Assay Procedure:

Stage One: *Activation of SAPK2b by DDMKK6*

1. Add 2.5µl of SAPK2b activation buffer to a microcentrifuge tube.
2. Add 2.5µl of **DDMKK6, active**.
3. Add **5µl (7.1µg) SAPK2b, unactive**.
4. Add 10µl of dH₂O.
5. Add 5µl of magnesium/ATP Cocktail.
6. Incubate for 60 minutes at 30°C.
7. Stop reaction by diluting 10-fold and placing on ice.

Stage Two: *Phosphorylation of MBP by activated SAPK2b*

1. Add 5µl of SAPK2a assay buffer into a microcentrifuge tube.
2. Add 2.5µl of **MBP**.
3. Add 5µl of **Stage One** reaction product diluted 10-fold.
4. Add 2.5µl of dH₂O.
5. Add 10µl of the diluted [γ-³³P]ATP.
6. Incubate for 10minutes at 30°C.
7. Slowly transfer 20µl onto the centre of a 2cm x 2cm **P81** paper.
8. Wash assay squares twice for 5minutes with 75mM phosphoric acid
9. Wash assay squares once with acetone for 2 minutes.
10. Transfer assay squares to scintillation vials and add 1ml scintillation cocktail.
11. Read in scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all appropriate assay components plus 1µl of 30% phosphoric acid.

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SAPK2b Sequence Information

<u>Protein</u>	human p38 beta 2/SAPK2b
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human p38 beta 2/SAPK2b
<u>Accession number</u>	EMBL Y14440

Recombinant p38 beta 2/SAPK2b amino acid sequence:

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1 MSPILGYWKI KGLVQPTRL L LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQ SMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFSLKLP EML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAI PQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFM SGPRAGFYRQ
241 ELNKT VWEVP QRLQGLRPVG SGAYGSVCSA YDARLRQKVA VKKLSRPFQS LIHARRTYRE
301 LRL LKHLKHE NVIGLLDVFT PATSIEDFSE VYLVTTLMGA DLNNIVKCQA LSDEHVQFLV
361 YQLLRGL KYI HSAGIIHRDL KPSNVAVNED CELRILDFGL ARQADEEMTG YVATRWRYP
421 EIMLNW MHYN QTVDIWSVGC IMAELLQGKA LFPGSDYIDQ LKRIMEVVTG PSPEVLAKIS
481 SEHARTY IQS LPPMPQK DLS SIFRGANPLA IDLLGRMLVL DSDQRVSAAE ALAHAYFSQY
541 HDPEDEPEAE PYDESVEAKE RTLEEWKELT YQEVLSFKPP EPPKPPGSLE IEQ
    
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Recombinant p38 beta2/SAPK2b nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatac ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
121 tggcgaagaca 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
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541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
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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
    
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1501 agcatcttcc gtggagccaa ccccctggcc atagacctcc ttggaaggat gctggtgctg
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1621 cacgaccccc aggatgagcc agaggccgag ccatatgatg agagcgttga ggccaaggag
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1741 gagccaccga agccacctgg cagcctggag attgagcagt ga
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