

Certificate of Analysis

p38 alpha/SAPK2a, unactive (Recombinant enzyme expressed in *E.coli* cells)

Item # 14-252, 14-252-K, 14-252M

Parent Lot # 1633894

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 alpha/SAPK2a, expressed in *E.coli* cells. Purified using glutathione-agarose. Purity 97.4% by SDS-PAGE and Coomassie blue staining. MW = 67.7kDa.

Formulation: 1.934mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Specific Activity (Parent lot# 1633894): 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.

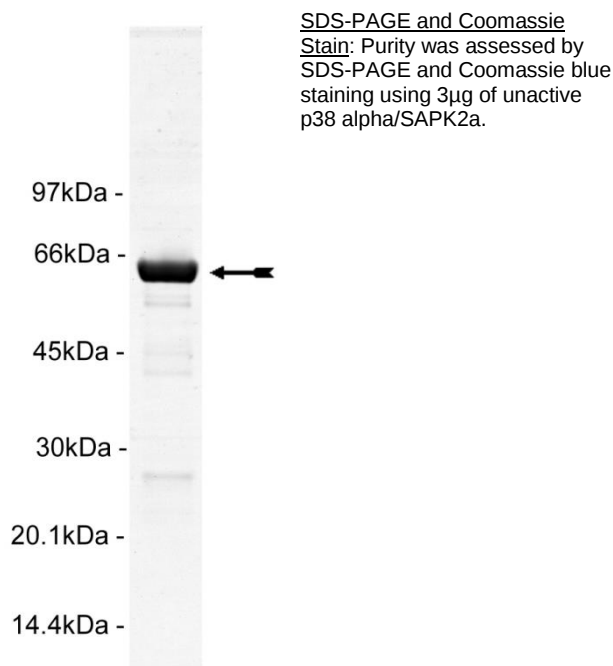
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Activation Assay: 4µM unactive SAPK2a was activated using 300nM DDMKK6, diluted 5–20 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 product identity as SAPK2a with the translated sequence listed on page three.

DDMKK6	Unactive SAPK2a	Mean cpm	Comments
0.6µg	270ng	11189	Kinase activity
None	1350ng	303	Background



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Kinase Assay Protocol

Stock Solutions:

- 10 x SAPK2a activation Buffer:** 500mM Tris/HCl, pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
- 5 x SAPK2a assay buffer:** 125mM Tris/HCl pH7.5, 0.1mM EGTA.
- Enzyme dilution buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
- Magnesium/ATP Cocktail (5 x stock):** 500μM cold ATP and 50mM magnesium acetate.
- [γ-³³P]ATP:** 2.5 x 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.)
- DD-MKK6, active:** Use at a final assay concentration of 300nM (0.024mg/ml). Prepare a 0.24mg/ml stock. Add 2.5μl of stock per assay point.
- p38 alpha/SAPK2a, unactive:** Use a final assay concentration of 4μM (0.271mg/ml). Prepare a 1.35mg/ml stock. Add 5μl of stock per assay point.
- Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock and use 2.5μl per assay point.

Assay Protocol:

Stage One: *DD-MKK6 dependant activation of SAPK2a.*

- Add 2.5 μl of SAPK2a activation buffer to a microcentrifuge tube (final reaction volume 25μl.)
- Add 2.5μl **DD-MKK6, active**.
- Add **5μl SAPK2a, unactive**.
- Add 10μl of dH₂O.
- Add 5μl of the 5 x cold Magnesium/ATP mixture.
- Incubate for 60 minutes at 30°C.
- Stop reaction by diluting 5-20 fold and incubate on ice.

Stage Two: *Phosphorylation of MBP (96 well plate format).*

- Add 5μl of SAPK2a assay buffer per assay to wells (final reaction volume 25μl).
- Add 2.5μl of **MBP**.
- Add **2.5μl of Stage One** reaction mixture.
- Add 5μl of dH₂O.
- Add 10μl of the diluted [γ-³³P]ATP mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5μl of 3% phosphoric acid.
- Spot 10μl onto the appropriate area of a P30 Filtermat.
- Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4ml scintillation cocktail.
- Read in 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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p38 alpha/SAPK2a Sequence Information

Protein	human p38 alpha/SAPK2a
Tags	N-Terminal GST
Native sequence	M227 of the recombinant protein is equivalent to M1 of human SAPK2a
Accession number	GenBank L35264

Recombinant SAPK2a amino acid sequence:

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1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPPYID
61  GDVKLTQSMa IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPIQID KYLKSSKYIA WPLQGWQATF GGGDHPKSD LVPRGSMSE RPTFYRQELN
241 KTIWEVPERY QNLSPVGSGA YGSVCAAFDT KTGLRVAVKK LSRPFQSIH AKRTYRELRL
301 LKHMKHENVI GLLDVFTPAR SLEEFNDVYL VTHLMGADLN NIVKCQKLT DHVQFLIYQI
361 LRLGKYIHSA DIIHRDLKPS NLAVNEDCEL KILDFGLARH TDDEMTGYVA TRWYRAPEIM
421 LNMWHYNQTV DIWSVGCIMA ELLTGRTLFP GTDHIDQLKL ILRLVGTPGA ELLKKISSES
481 ARNYIQSLTQ MPKMNFAVNF IGANPLAVDL LEKMLVLDSD KRITAAQALA HAYFAQYHDP
541 DDEPVADPYD QSFESRDLI DEWKSLEYDE VISFVPPPLD QEEMES

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Recombinant SAPK2a nucleotide sequence:

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1  atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61  ttggaatata ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatagctga caagcacaac
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1681 gatgagtgga aaagcctgac ctatgatgaa gtcacagctt ttgtgccacc accccttgac
1741 caagaagaga tggagtcctg a

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