

Certificate of Analysis

p38 alpha/SAPK2a, active
(Recombinant enzyme expressed in *E. coli* cells)
Item # 14-251, 14-251-K, 14-251M
Parent Lot # WAE0488

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 human full-length p38 alpha /SAPK2a, expressed in *E. coli* cells. Purified using glutathione-agarose. Activated using DD-MKK6 and repurified using glutathione- agarose. Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 67.7kDa.

Specific Activity (Parent lot# WAE0488): 24U/mg, where one unit of SAPK2a, 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.

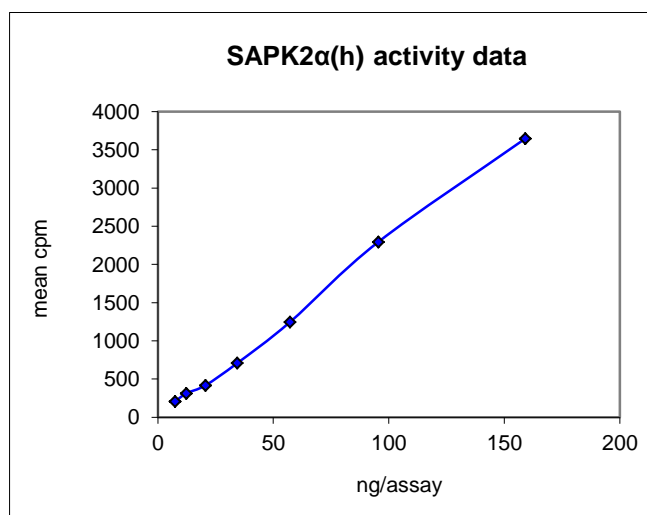
Formulation: 1.59mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid solution at -20°C.

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.

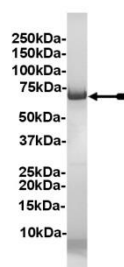
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 7.42–159ng 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 product identity as p38 alpha/SAPK2a with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of SAPK2a, active.

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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. SAPK2a, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7.42–159ng 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 (7.42–159ng) SAPK2a, 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 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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SAPK2a Sequence Information

Protein	Human 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  KGLVQPTRL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121 DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181 KRIEAIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGMSMQE  RPTFYRQELN
241 KTIWEVPERY  QNLSPVGSQA  YGSVCAAFDT  KTGLRVAVKK  LSRPFQSIIH  AKRTYRELRL
301 LKHMKHENVI  GLLDVFTPAR  SLEEFNDVYL  VTHLMGADLN  NIVKCQKLT  DHVQFLIYQI
361 LRGLKYIHS  A  DIHRDLKPS  NLAVNEDCEL  KILDFGLARH  TDEMTGYVA  TRWYRAPEIM
421 LNMWHYNQTV  DIWSVGCIMA  ELLTGRTLFP  GTDHIDQLKL  ILRLVGTPGA  ELLKKISSES
481 ARNYIQSLTQ  MPKMNFAVNF  IGANPLAVDL  LEKMLVLDS  KRITAAQALA  HAYFAQYHDP
541 DDEPVADPYD  QSFESRDLLI  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  catttggatg  agcgcgatga  aggtgataaa
121  tggcgaacaa  aaaagtttga  attgggtttg  gagtttcca  atcttcctta  ttatattgat
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1741 caagaagaga  tggagtctctg  a
    
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