

Certificate of Analysis

p38 α /SAPK2a (T106M) , active (Recombinant enzyme expressed in *E. coli* cells)

Item # 14-687, 14-687-K, 14-687M

Parent Lot # 1590325

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 α /SAPK2a containing the mutation T106M. Expressed in *E. coli* cells. Purified using glutathione-agarose. Activated using DD-MKK6 (cat# 14-537) and repurified using glutathione- agarose. Purity 92.9% by SDS-PAGE and Coomassie blue staining. MW = 67.7kDa.

Specific Activity (Parent lot# 1590325): 97U/mg, where one unit of p38 α /SAPK2a (T106M), 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 100 μ M.

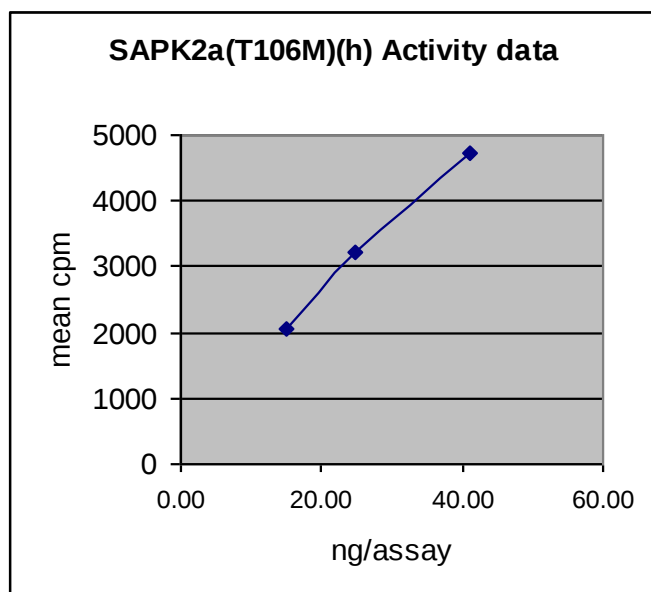
Formulation: 1.879mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid solution at -20°C.

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.

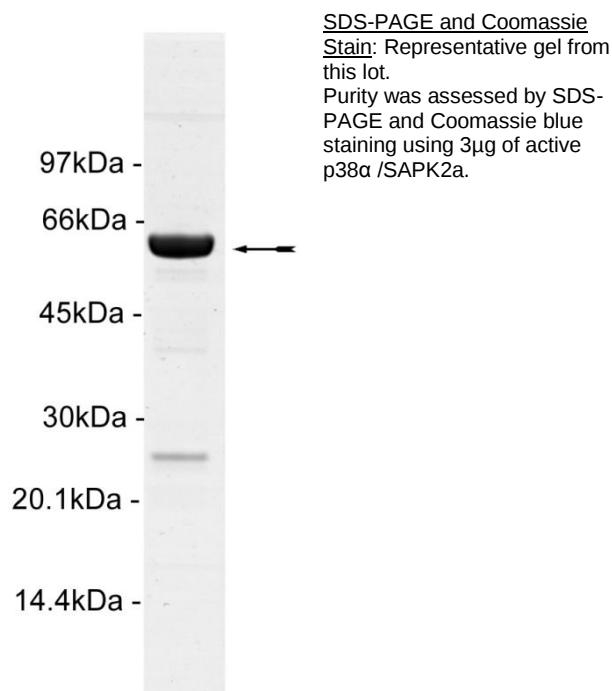
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 15–41ng 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 identity as p38 α /SAPK2a (T106M) with the translated native sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
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. **p38α/SAPK2a (T106M), active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 15–41ng 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 (15–41ng) p38α/SAPK2a (T106M), 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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p38α/SAPK2a (T106M) Sequence Information

Protein	Human p38α/SAPK2a (T106M)
Tags	N-terminal GST
Native sequence	M227 of the recombinant protein is equivalent to M1 of human p38α/SAPK2a
Accession number	GenBank L35264. The p38α/SAPK2a recombinant protein contains the amino acid substitution T106M with respect to GenBank L35764. This mutation confers resistance to SB203580.

Recombinant p38α/SAPK2a (T106M) 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  KRIEAIPIQID  KYLKSSKYIA  WPLQGWQATF  GGGDHPPKSD  LVPRGSMSQE  RPTFYRQELN
241  KTIWEVPERY  QNLSPVGSMA  YGSVCAAFDT  KTGLRVAVKK  LSRPFQSIH  AKRTYRELRL
301  LKHKHENV  GLLDVFTPAR  SLEEFNDVYL  VMHLMGADLN  NIVKCQKLT  DHVQFLIYQI
361  LRGLKYIHS  A  DIHRDLKPS  NLAVNEDCEL  KILDFGLARH  TDDEMTGYVA  TRWYRAPEIM
421  LNWVHYNQTV  DIWSVGCIMA  ELLTGRTLFP  GTDHIDQLKL  ILRLVGTPGA  ELLKKISSES
481  ARNYIQSLTQ  MPKMNFAVNF  IGANPLAVDL  LEKMLVLDS  KRITAAQALA  HAYFAQYHDP
541  DDEPVADPYD  QSFESRDLLI  DEWKSILTYDE  VISFVPPPLD  QEEMES

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Recombinant p38α/SAPK2a (T106M) nucleotide sequence:

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1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgatat  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgat
181  ggtgatgta  aattaacaca  gtctatggcc  atcatagctt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcgggtttg
301  gatattagat  acggtgtttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatgttcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tggtgtatga  cgctcttgat
481  gttgttttat  acatggaccc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggttccgc  gtggatccat  gtctcaggag  aggcccacgt  tctaccggca  ggagctgaac
721  aagacaatct  gggaggtgcc  cgagcgttac  cagaacctgt  ctccagtggg  ctctggcgcc
781  tatggctctg  tgtgtgctgc  ttttgacaca  aagacggggg  tacgtgtggc  agtgaagaag
841  ctctccagac  catttcagtc  catcattcat  gcgaaaagaa  cctacagaga  actgcggtta
901  cttaaacata  tgaaacatga  aaatgtgatt  ggtctgttgg  acgtttttac  acctgcaagg
961  tctctggagg  aattcaatga  tgtgtatctg  gtgatgcata  tcatgggggc  agatctgaac
1021  aacattgtga  aatgtcagaa  gcttacagat  gaccatgttc  agttccttat  ctaccaaatt
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1441  gcaagaaact  atattcagtc  tttgactcag  atgccgaaga  tgaactttgc  gaattgattt
1501  attggtgcca  atcccctggc  tgtcgacttg  ctggagaaga  tgcttgattt  ggactcagat
1561  aagagaatta  cagcggccca  agcccttgca  catgcctact  ttgctcagta  ccacgatcct
1621  gatgatgaac  cagtggccga  tccttatgat  cagtcctttg  aaagcagggg  cctccttata

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1681 gatgagtgga aaagcctgac ctatgatgaa gtcatcagct ttgtgccacc accccttgac  
1741 caagaagaga tggagtctg a
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