eurofins | Discovery Services

Certificate of Analysis

p38α/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 p38α/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 p38α/SAPK2a (T106M) 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.

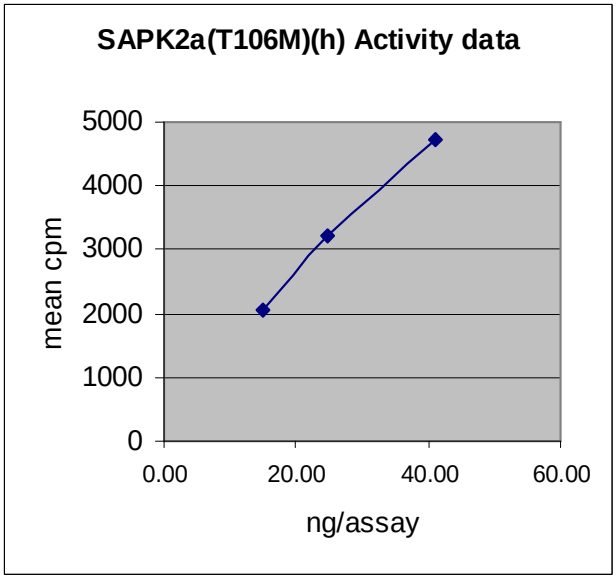
Formulation: 1.879mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid solution at -20°C.

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.

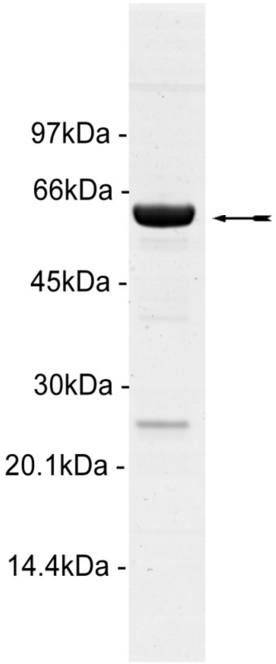
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 p38α/SAPK2a (T106M) with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Representative gel from this lot.
Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active p38α /SAPK2a.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

Certificate of Analysis

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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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.

eurofins | Discovery Services

Certificate of Analysis

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:

```
  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSMSQE RPTFYRQELN
241 KTIWEVPERY QNLSPVGSGA YGSVCAAFDT KTGLRVAVKK LSRPFQSIIH AKRTYRELRL
301 LKHMKHENVI GLLDVFTPAR SLEEFNDVYL VMHLMGADLN NIVKCQKLTD DHVQFLIYQI
361 LRGLKYIHSA DIIHRDLKPS NLAVNEDCEL KILDFGLARH TDDEMTGYVA TRWYRAPEIM
421 LNWMHYNQTV DIWSVGCIMA ELLTGRTLFP GTDHIDQLKL ILRLVGTPGA ELLKKISSES
481 ARNYIQSLTQ MPKMNFANVF IGANPLAVDL LEKMLVLDSD KRITAAQALA HAYFAQYHDP
541 DDEPVADPYD QSFESRDLLI DEWKSLTYDE VISFVPPPLD QEEMES
```

Recombinant p38α/SAPK2a (T106M) nucleotide sequence:

```
   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga 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 aagacggggt tacgtgtggc agtgaagaag
 841 ctctccagac catttcagtc catcattcat gcgaaaagaa cctacagaga actgcggtta
 901 cttaaacata tgaaacatga aaatgtgatt ggtctgttgg acgtttttac acctgcaagg
 961 tctctggagg aattcaatga tgtgtatctg gtgatgcatc tcatgggggc agatctgaac
1021 aacattgtga aatgtcagaa gcttacagat gaccatgttc agttccttat ctaccaaatt
1081 ctccgaggtc taaagtatat acattcagct gacataattc acagggacct aaaacctagt
1141 aatctagctg tgaatgaaga ctgtgagctg aagattctgg attttggact ggctcggcac
1201 acagatgatg aaatgacagg ctacgtggcc actaggtggt acagggctcc tgagatcatg
```

eurofins | Discovery Services

Certificate of Analysis

```
1261 ctgaactgga tgcattacaa ccagacagtt gatatttggt cagtgggatg cataatggcc
1321 gagctgttga ctggaagaac attgtttcct ggtacagacc atattgatca gttgaagctc
1381 attttaagac tcgttggaac cccaggggct gagcttttga agaaaatctc ctcagagtct
1441 gcaagaaact atattcagtc tttgactcag atgccgaaga tgaactttgc gaatgtattt
1501 attggtgcca atcccctggc tgtcgacttg ctggagaaga tgcttgtatt ggactcagat
1561 aagagaatta cagcggccca agcccttgca catgcctact ttgctcagta ccacgatcct
1621 gatgatgaac cagtggccga tccttatgat cagtcctttg aaagcaggga cctccttata
1681 gatgagtgga aaagcctgac ctatgatgaa gtcatcagct ttgtgccacc acccccttgac
1741 caagaagaga tggagtcctg a
```

Reviewed and approved by site quality representative.