eurofins | Discovery Services

Certificate of Analysis

SAPK4, unactive

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-250, 14-250-K, 14-250M
Parent Lot # 2447956

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, full length human SAPK4 expressed in *E.coli* cells. Purified using glutathione sepharose. Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 69kDa.

Specific Activity (Parent lot# 2447956): As provided, this lot demonstrated <1% of maximum activity. Activated by phosphorylation with MKK6 (S599D, T603D) (cat# 14-537).

Formulation: 1.56mg/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.

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

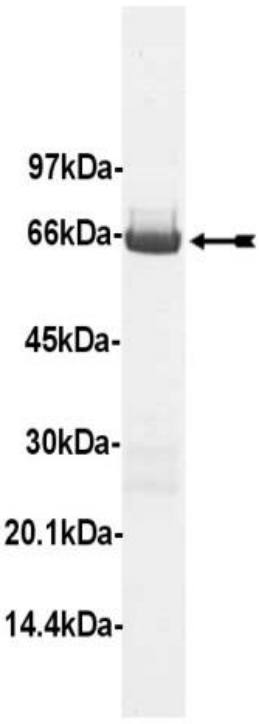
Quality Control Testing

Activation Assay: 4µM SAPK4, unactive was activated using 300nM MKK6 (S599D, T603D), diluted 5-40 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.

DDMKK6	SAPK4, unactive	Mean cpm	Comments
0.6µg	276ng	12834	Kinase activity
None	276ng	48	Background

MS Tryptic Fingerprint: Confirmed identity as SAPK4 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 SAPK4, unactive.



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. **10 x SAPK4 activation Buffer:** 500mM Tris/HCl, pH7.5, 1mM EGTA, 1mM Na_3VO_4, 1% 2-mercaptoethanol.
2. **5 x SAPK4 assay buffer:** 125mM Tris/HCl pH7.5, 0.1mM EGTA.
3. **Enzyme dilution buffer:** 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
4. **Magnesium/ATP Cocktail (5 x stock):** 500µM cold ATP and 50mM magnesium acetate.
5. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM magnesium acetate and 0.25mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)
6. **DD-MKK6, active:** Use at a final assay concentration of 300nM (0.024mg/ml). Prepare a 0.24mg/ml stock. Add 5µl of stock per assay point.
7. **SAPK4, unactive:** Use a final assay concentration of 4µM (0.276mg/ml). Prepare a 1.38mg/ml stock. Add 10µl 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 use 2.5µl per assay point.

Assay Procedure:

Stage One: *Activation of SAPK4 by MKK6 (S599D, T603D)*

1. Add 5 µl of SAPK2a activation buffer to a microcentrifuge tube (final reaction volume 50µl.)
2. Add 5µl **DD-MKK6, active**.
3. Add **10µl SAPK4, unactive**.
4. Add 20µl of dH_2O.
5. Add 10µl of the 5 x cold Magnesium/ATP mixture.
6. Incubate for 60 minutes at 30°C.
7. Stop reaction by diluting 5-40 fold and incubate on ice.

Stage Two: *Phosphorylation of MBP by activated SAPK4*

1. Add 5µl of SAPK4 assay buffer per assay to wells (final reaction volume 25µl).
2. Add 2.5µl of **MBP**.
3. Add **5µl** of **Stage One** reaction mixture.
4. Add 2.5µl of dH_2O.
5. Add 10µl of the 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. Spot 10µl 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 scintillation cocktail.
12. 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.

eurofins | Discovery Services

Certificate of Analysis

eurofins | Discovery Services

Certificate of Analysis

SAPK4 Sequence Information

Protein Human SAPK4

Tags *N*-terminal GST

Native sequence M230 of the recombinant protein is equivalent to M1 of human SAPK4

Accession number EMBL Y10488

Recombinant SAPK4 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 LVPRGSPEFM SLIRKKGFYK
241 QDVNKTAWEL PKTYVSPTHV GSGAYGSVCS AIDKRSGEKV AIKKLSRPFQ SEIFAKRAYR
301 ELLLLKHMQH ENVIGLLDVF TPASSLRNFY DFYLVMPFMQ TDLQKIMGME FSEEKIQYLV
361 YQMLKGLKYI HSAGVVHRDL KPGNLAVNED CELKILDFGL ARHADAEMTG YVVTRWYRAP
421 EVILSWMHYN QTVDIWSVGC IMAEMLTGKT LFKGKDYLDQ LTQILKVTGV PGTEFVQKLN
481 DKAAKSYIQS LPQTPRKDFT QLFPRASPQA ADLLEKMLEL DVDKRLTAAQ ALTHPFFEPF
541 RDPEEETEAQ QPFDDSLEHE KLTVDEWKQH IYKEIVNFSP IARKDSRRRS GMKL
```

Recombinant SAPK4 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 gtggatcccc ggaattcatg agcctcatcc ggaaaaaggg cttctacaag
 721 caggacgtca acaagacagc ctgggagctg cccaagacct acgtgtcccc gacgcacgtc
 781 ggcagcgggg cctatggctc cgtgtgctcg gccatcgaca agcggtcagg ggagaaggtg
 841 gccatcaaga agctgagccg acccctttcag tccgagatct tcgccaagcg cgcctaccgg
 901 gagctgctgc tgctgaagca catgcagcat gagaacgtca ttgggctcct ggatgtcttc
 961 accccagcct cctccctgcg caacttctat gacttctacc tggtgatgcc cttcatgcag
1021 acggatctgc agaagatcat ggggatggag ttcagtgagg agaagatcca gtacctggtg
1081 tatcagatgc tcaaaggcct taagtacatc cactctgctg gggtcgtgca cagggacctg
1141 aagccaggca acctggctgt gaatgaggac tgtgaactga agattctgga ttttgggctg
1201 gcgcgacatg cagacgccga gatgactggc tacgtggtga cccgctggta ccgagccccc
1261 gaggtgatcc tcagctggat gcactacaac cagacagtgg acatctggtc tgtgggctgt
1321 atcatggcag agatgctgac agggaaaact ctgttcaagg ggaaagatta cctggaccag
1381 ctgacccaga tcctgaaagt gaccggggtg cctggcacgg agtttgtgca gaagctgaac
1441 gacaaagcgg ccaaatccta catccagtcc ctgccacaga cccccaggaa ggatttcact
1501 cagctgttcc cacgggccag cccccaggct gcggacctgc tggagaagat gctggagcta
1561 gacgtggaca agcgcctgac ggccgcgcag gccctcaccc atcccttctt tgaacccttc
```

eurofins | Discovery Services

Certificate of Analysis

```
1621 cgggaccctg aggaagagac ggaggcccag cagccgtttg atgattcctt agaacacgag
1681 aaactcacag tggatgaatg gaagcagcac atctacaagg agattgtgaa cttcagcccc
1741 attgcccgga aggactcacg gcgccggagt ggcatgaagc tgtag
```

Reviewed and approved by site quality representative.