

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.

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.

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

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**

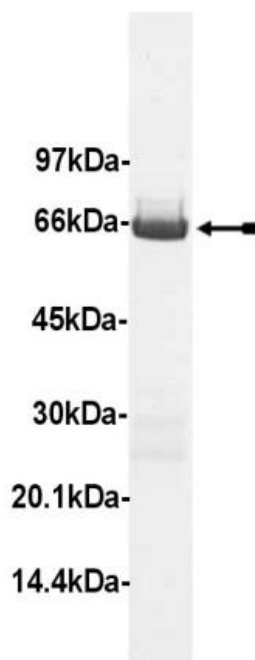
Quality Control Testing

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.



Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **10 x SAPK4 activation Buffer:** 500mM Tris/HCl, pH7.5, 1mM EGTA, 1mM Na₃VO₄, 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 Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA.
4. **Magnesium/ATP Cocktail (5 x stock):** 500μM cold ATP and 50mM magnesium acetate.
5. **[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.)
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, inactive:** 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, inactive**.
4. Add 20μl of dH₂O.
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 dH₂O.
5. Add 10μl of the 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. 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.

Certificate of Analysis

SAPK4 Sequence Information

<u>Protein</u>	Human SAPK4
<u>Tags</u>	N-terminal GST
<u>Native sequence</u>	M230 of the recombinant protein is equivalent to M1 of human SAPK4
<u>Accession number</u>	EMBL Y10488

Recombinant SAPK4 amino acid sequence:

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1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSPEFM SLIRKKGFYK
241 QDVNKTAWEL PKTYVSPTHV GSGAYGSVCS AIDKRSGEKV AIKKLSRPFQ SEIFAKRAYR
301 ELLLLKHMQL ENVIGLLDVF TPASSLRNFY DFYLVMPFMQ TDLQKIMGME FSEEKIQYLV
361 YQMLKGLKYI HSAGVVHRDL KPGNLAVNED CELKILDFGL ARHADAEMTG YVVRWYRAP
421 EVILSWMHYN QTVDIWSVGC IMAEMLTGKT LFKGKDYLQ LTQILKVTGV PGTEFVQKLN
481 DKAASKYIQS LPQTPRKDFT QLFPRASPQA ADLLEKMLEL DVDKRLTAAQ ALTHPFFPEPF
541 RDPEEETEAQ QPFDDSLHEH KLTVDDEWKQH IYKEIVNFSP IARKDSRRRS GMKL

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Recombinant SAPK4 nucleotide sequence:

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1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaaccac tcgacttctt
61 ttggaatatc ttgaagaaaa atatgaagag catttgatat agcgcgatga aggtgataaa
121 tggcgaaaca aaaagtgtga attgggtttg gagtttccca atcttcctta ttatatattgat
181 ggtgatgtta aattaacaca gtctatggcc atcatagctt atatatagctga caagcacaaac
241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcgggtttg
301 gatattagat acggtgtttc gagaattgca tatagtaaaag actttgaaac tctcaaagtt
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1501 cagctgttcc cacgggccag ccccaggct gcggacctgc tggagaagat gctggagcta
1561 gacgtggaca agcgcctgac ggccgcgcag gccctcacc atcccttctt tgaacccttc

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Certificate of Analysis

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