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Certificate of Analysis

SAPK4, active

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-249-K
Lot # D8EN044U

Product Description: *N*-terminal GST tagged, full length human SAPK4 expressed in *E.coli* cells. Purified using glutathione sepharose. Activated using a constitutively active mutant of MKK6 and repurified using glutathione sepharose. Purity 99% by SDS-PAGE and Coomassie blue staining. MW = 68.9kDa.

Specific Activity (lot# D8EN044U): 252U/mg, where one unit of SAPK4 activity is defined as 1nmol phosphate incorporated into 0.33mg/ml MBP per minute at 30°C with a final ATP concentration of 100µM.

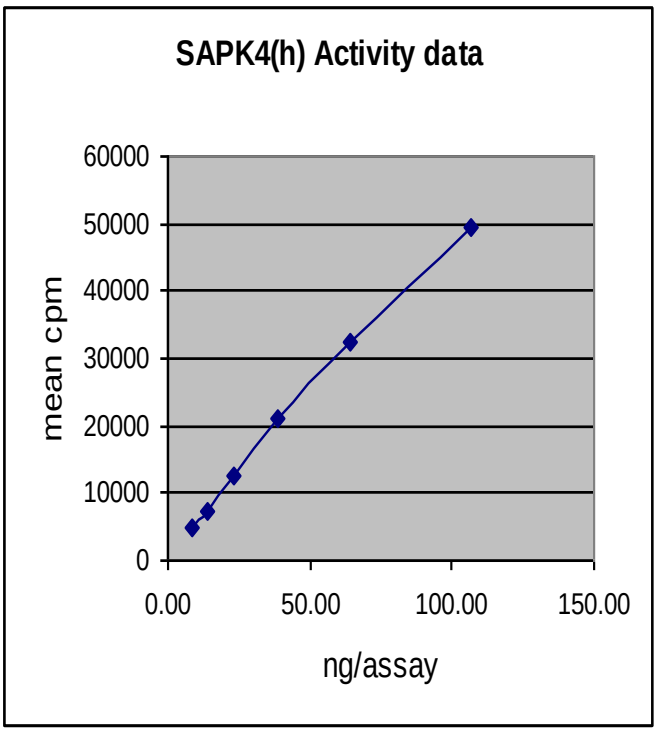
Formulation: 3.127mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 50% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Liquid at -20°C.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

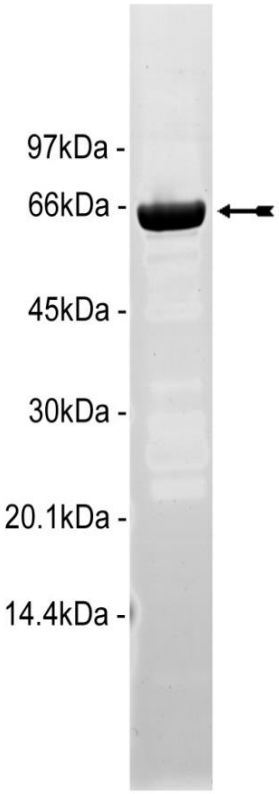
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 8.44–106ng of this lot of enzyme phosphorylated 0.33mg/ml 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 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, 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 concentration of 0.33mg/ml. Prepare a 3.33mg/ml stock. Use 2.5µl of stock per assay point.
3. **SAPK4, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na_3VO_4, 0.1% 2-mercaptoethanol. Use 8.44–106ng 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 5 x reaction buffer to wells.
2. Add 2.5µl of **MBP**.
3. Add **2.5µl (8.44–106ng) SAPK4, 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 twice for 5 minutes with 75mM phosphoric acid.
10. Wash 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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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:

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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 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
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Recombinant SAPK4 nucleotide sequence:

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   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
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 781 ggcagcgggg cctatggctc cgtgtgctcg gccatcgaca agcggtcagg ggagaaggtg
 841 gccatcaaga agctgagccg acccctttcag tccgagatct tcgccaagcg cgcctaccgg
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1081 tatcagatgc tcaaaggcct taagtacatc cactctgctg gggtcgtgca cagggacctg
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1201 gcgcgacatg cagacgccga gatgactggc tacgtggtga cccgctggta ccgagccccc
1261 gaggtgatcc tcagctggat gcactacaac cagacagtgg acatctggtc tgtgggctgt
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1501 cagctgttcc cacgggccag cccccaggct gcggacctgc tggagaagat gctggagcta
1561 gacgtggaca agcgcctgac ggccgcgcag gccctcaccc atcccttctt tgaacccttc
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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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Reviewed and approved by site quality representative.