Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SAPK4 [1 – 365]

Enzyme description:- SAPK4 [1 - 365]

Clone number:- DU 981

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 10 mg/L

Calculated molecular mass:- 68, 628 daltons

Purity:- >85 %

Activation protocol:-
SAPK4 (3.5 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM MgAc, 0.1 mM ATP with 200 nM MBP-MKK6 DD [DU 1662] at 30 °C for 30 min. Following activation, SAPK4 is re-purified by GSH Sepharose chromatography.

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 50 % glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -20 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 0.1 mM sodium vanadate, 10 mM magnesium acetate

Substrate:-
Myelin Basic Protein Final concentration: 0.3 mg/ml

Specific activity range:- 75 – 150 U/mg

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Clone Data Sheet

SAPK4 [1 - 365]

Protein SAPK4 [1 – 365]

Clone number DU 981

Species Human

Accession number Y10488

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL
EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL
DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA
WPLQGWQATFGGGDHPPKSDLVPRGSPEF**MSLIRKKGFYKQDVNKTAWEL
PKTYVSPTHVGSGAYGSVCSAIDKRSGEKVAIKKLSRPFQSEIFAKRAYR
ELLLLKHMQHENVIGLLDVFTPASSLRNFYDFYLVMPFMQTDLQKIMGME
FSEEKIQYLVYQMLKGLKYIHSAGVVHRDLKPGNLAVNEDCELKILDFGL
ARHADAEMTGYVVTRCYRAPEVILSWMHYNQTVDIWSVGCIMAEMLTGKT
LFKGKDYLDQLTQILKVTGVPGTEFVQKLNDKAAKSYIQSLPQTPRKDFT
QLFPRASPQAADLLEKMLELDVDKRLTAAQALTHPFFEPFRDPEEETEAQ
QPFDDSLEHEKLTVDEWKQHIYKEIVNFSPIARKDSRRRSGMKL**

Native sequence Amino acids M1- L365 (end) of human SAPK4.
Residue M230 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

The following amino acid substitution is present:
W – C, where residue W187 of the native sequence is C416 of the fusion protein

Protease cleavage Thrombin (LVPRGS) at residues 221 – 226

Cloning sites *Eco*RI site of pGEX 4T-1

Nucleotide sequence of insert

ATGAGCCTCATCCGGAAAAAGGGCTTCTACAAGCAGGACGTCAACAAGAC
CGCCTGGGAGCTGCCCAAGACCTACGTGTCCCCGACGCACGTCGGCAGCG
GGGCCTATGGCTCCGTGTGCTCGGCCATCGACAAGCGGTCAGGGGAGAAG
GTGGCCATCAAGAAGCTGAGCCGACCCTTTCAGTCCGAGATCTTCGCCAA
GCGCGCCTACCGGGAGCTGCTGCTGCTGAAGCACATGCAGCATGAGAACG
TCATTGGGCTCCTGGATGTCTTCACCCCAGCCTCCTCCCTGCGCAACTTC
TATGACTTCTACCTGGTGATGCCCTTCATGCAGACGGATCTGCAGAAGAT
CATGGGGATGGAGTTCAGTGAGGAGAAGATCCAGTACCTGGTGTATCAGA
TGCTCAAAGGCCTTAAGTACATCCACTCTGCTGGGGTCGTGCACAGGGAC
CTGAAGCCAGGCAACCTGGCTGTGAATGAGGACTGTGAACTGAAGATTCT
GGATTTTGGGCTGGCGCGACATGCAGACGCCGAGATGACTGGCTACGTGG
TGACCCGCTGTTACCGAGCCCCCGAGGTGATCCTCAGCTGGATGCACTAC
AACCAGACAGTGGACATCTGGTCTGTGGGCTGTATCATGGCAGAGATGCT
GACAGGGAAAACTCTGTTCAAGGGGAAAGATTACCTGGACCAGCTGACCC
AGATCCTGAAAGTGACCGGGGTGCCTGGCACGGAGTTTGTGCAGAAGCTG
AACGACAAAGCGGCCAAATCCTACATCCAGTCCCTGCCACAGACCCCCAG
GAAGGATTTCACTCAGCTGTTCCCACGGGCCAGCCCCCAGGCTGCGGACC
TGCTGGAGAAGATGCTGGAGCTAGACGTGGACAAGCGCCTGACGGCCGCG
CAGGCCCTCACCCATCCCTTCTTTGAACCCTTCCGGGACCCTGAGGAAGA
GACGGAGGCCCAGCAGCCGTTTGATGATTCCTTAGAACACGAGAAACTCA
CAGTGGATGAATGGAAGCAGCACATCTACAAGGAGATTGTGAACTTCAGC
CCCATTGCCCGGAAGGACTCACGGCGCCGGAGTGGCATGAAGCTGtag