Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active SAPK2b [1 – 364]

Enzyme description:- SAPK2b [1 – 364]

Clone number:- DU 1791

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 10 mg/L

Calculated molecular mass:- 67, 979 daltons

Purity:- >85 %

Activation protocol:-
SAPK2b (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, SAPK2a 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, 0.1mM EGTA, 0.1mM 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

SAPK2b [1 – 364]

Protein	SAPK2b [1 - 364]
Clone number	DU 1791
Species	Human
Accession number	Y14440
Tags	N-terminal GST
Bacterially-expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPEF**MSGPRAGFYRQ ELNKTVWEVPQRLQGLRPVGSGAYGSVCSAYDARLRQKVAVKKLSRPF QSLIHARRTYRELRLLKHLKHENVIGLLDVFTPATSIEDFSEVYLVTT LMGADLNNIVKCQALSDEHVQFLVYQLLRGLKYIHSAGIIHRDLKPSN VAVNEDCELRILDFGLARQADEEMTGYVATRWYRAPEIMLNWMHYNQT VDIWSVGCIMAELLQGKALFPGSDYIDQLKRIMEVVGTPSPEVLAKIS SEHARTYIQSLPPMPQKDLSSIFRGANPLAIDLLGRMLVLDSDQRVSA AEALAHAYFSQYHDPEDEPEAEPYDESVEAKERTLEEWKELTYQEVLS FKPPEPPKPPGSLEIEQ**
Native sequence	Amino acids M1 – Q364 (end) of human SAPK2b. Residue M230 of the GST-fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	Thrombin (LVPRGS) residues 221 - 226
Cloning sites	*Eco*RI site of pGEX-4T1

Nucleotide sequence of insert

ATGTCGGGCCCTCTCGCCGGCTTCTACCGGCAGGAGCTGAACAAGACC
GTGTGGGAGGTGCCGCAGCGGCTGCAGGGGCTGCGCCCGGTGGGCTCC
GGCGCCTACGGCTCCGTTTGTTCGGCCTACGACGCCCGGCTGCGCCAG
AAGGTGGCGGTGAAGAAGCTGTCGCGCCCCTTCCAGTCGCTGATCCAC
GCGCGCAGAACGTACCGGGAGCTGCGGCTGCTCAAGCACCTGAAGCAC
GAGAACGTCATCGGGCTTCTGGACGTCTTCACGCCGGCCACGTCCATC
GAGGACTTCAGCGAAGTGTACTTGGTGACCACCCTGATGGGCGCCGAC
CTGAACAACATCGTCAAGTGCCAGGCGCTGAGCGACGAGCACGTTCAA
TTCCTGGTTTACCAGCTGCTGCGCGGGCTGAAGTACATCCACTCGGCC
GGGATCATCCACCGGGACCTGAAGCCCAGCAACGTGGCTGTGAACGAG
GACTGTGAGCTCAGGATCCTGGATTTCGGGCTGGCGCGCCAGGCGGAC
GAGGAGATGACCGGCTATGTGGCCACGCGCTGGTACCGGGCACCTGAG
ATCATGCTCAACTGGATGCATTACAACCAAACAGTGGATATCTGGTCC
GTGGGCTGCATCATGGCTGAGCTGCTCCAGGGCAAGGCCCTCTTCCCG
GGAAGCGACTACATTGACCAGCTGAAGCGCATCATGGAAGTGGTGGGC
ACACCCAGCCCTGAGGTTCTGGCAAAAATCTCCTCGGAACACGCCCGG
ACATATATCCAGTCCCTGCCCCCCATGCCCCAGAAGGACCTGAGCAGC
ATCTTCCGTGGAGCCAACCCCCTGGCCATAGACCTCCTTGGAAGGATG
CTGGTGCTGGACAGTGACCAGAGGGTCAGTGCAGCTGAGGCACTGGCC
CACGCCTACTTCAGCCAGTACCACGACCCCGAGGATGAGCCAGAGGCC
GAGCCATATGATGAGAGCGTTGAGGCCAAGGAGCGCACGCTGGAGGAG
TGGAAGGAGCTCACTTACCAGGAAGTCCTCAGCTTCAAGCCCCCAGAG
CCACCGAAGCCACCTGGCAGCCTGGAGATTGAGCAGtga