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Standard Operating Procedure

Preparation of active SAPK2b/p38 β

<u>Enzyme description:-</u>	Active SAPK2b/p38 β
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Expression conditions:-</u>	Induce expression with 250 μ M IPTG once culture reaches OD _{600nm} = 0.4. Express for 16 hours at 26°C.
<u>Tag:-</u>	N-terminal GST
<u>Purification method:-</u>	GSH- agarose.
<u>Expression level:-</u>	10 mg/L
<u>Molecular mass:-</u>	65 kDa by SDS-PAGE
<u>Purity:-</u>	>85%
<u>Contaminants:-</u>	No major contaminating proteins as judged by SDS-PAGE.

Activation protocol:-

Fresh SAPK2b/ p38 β (0.2 mg/ml - 3.5 μ M) is activated in 50mM Tris/HCl pH 7.5, 0.1mM EGTA, 0.1 % β -mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 200 nM active MKK6 DD at 30°C for 30 min. Following activation, the enzyme is separated from the MKK6 DD by GSH agarose chromatography. The active SAPK2b/ p38 β is then dialysed into enzyme storage buffer, and stored at -20°C.

Enzyme storage buffer:-

50 mM Tris/HCl pH 7.5, 50% glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β -mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -20°C

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CLONE DATA SHEET – Human SAPK2b/p38 β

<u>Protein</u>	Human SAPK2b/ p38 β
<u>Accession number</u>	Y14440
<u>Tags</u>	GST
<u>Bacterially-expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRN KKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML KMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCL DAFPKLVCFKKRIEAIPOIDKYLKSSKYIAWPLQGWQATFGGG DHPPKSDLVPRGSPEFMSGPRAGFYRQELNKTVWEVPQRLQ GLRPVGSAYGSVCSAYDARLRQKVAVKKLSRPFQSLIHAR RTYRELRLKHLKHENVIGLLDVFTPATSIEDFSEVYLVTTLM GADLNNIVKCQALSDEHVQFLVYQLLRGLKYIHSAGIIHRD LKPSNVAVNEDCELRIIDFGLARQADEEMTGYVATRWRRA PEIMLNWMHYNQTVDIWSVGCIMAELLOGKALFPGSDYID QLKRIMEVVGTPSPEVLAKISSEHARTYIQSLPPMPQKDLSSI FRGANPLAIDLLGRMLVLDSDQRVSAEALAHAYFSQYHD PEDEPEAEPYDESVEAKERTLEEWKELTYQEVLSFKPPEPPKP PGSLEIEQ
<u>Native sequence</u>	M230 of the GST-fusion protein is equivalent to M1 of human SAPK2 β / p38 β
<u>Protease cleavage site</u>	Thrombin (LVPRGS) residues 221-226 of the fusion protein
<u>Cloning sites</u>	EcoRI site of pGEX-4T1
<u>Nucleotide sequence of SAPK2b/p38β ORF</u>	ATGTCGGGCCCTCTCGCCGGCTTCTACCGGCAGGAGCTGAACAAGACC GTGTGGGAGGTGCCGCAGCGGCTGCAGGGGCTGCGCCCGGTGGGCTCC GGCGCCTACGGCTCCGTTTGTTCGGCCTACGACGCCCGGCTGCGCCAG AAGGTGGCGGTGAAGAAGCTGTCGCGCCCCTTCCAGTCGCTGATCCAC GCGCGCAGAACGTACCGGGAGCTGCGGCTGCTCAAGCACCTGAAGCAC GAGAACGTCATCGGGCTTCTGGACGTCTTACGCCGGCCACGTCCATC GAGGACTTCAGCGAAGTGTACTTGGTGACCACCCTGATGGGCGCCGAC CTGAACAACATCGTCAAGTGCCAGGCGCTGAGCGACGAGCACGTTCAA TTCCTGGTTTACCAGCTGCTGCGCGGGCTGAAGTACATCCACTCGGCC GGGATCATCCACCGGGACCTGAAGCCCAGCAACGTGGCTGTGAACGAG

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GACTGTGAGCTCAGGATCCTGGATTTTCGGGCTGGCGCGCCAGGCGGAC
GAGGAGATGACCGGCTATGTGGCCACGCGCTGGTACCGGGCACCTGAG
ATCATGCTCAACTGGATGCATTACAACCAAACAGTGGATATCTGGTCC
GTGGGCTGCATCATGGCTGAGCTGCTCCAGGGCAAGGCCCTCTTCCCG
GGAAGCGACTACATTGACCAGCTGAAGCGCATCATGGAAGTGGTGGGC
ACACCCAGCCCTGAGGTTCTGGCAAAAATCTCCTCGGAACACGCCCGG
ACATATATCCAGTCCCTGCCCCCATGCCCCAGAAGGACCTGAGCAGC
ATCTTCCGTGGAGCCAACCCCTGGCCATAGACCTCCTTGGAAGGATG
CTGGTGCTGGACAGTGACCAGAGGGTCAGTGCAGCTGAGGCACTGGCC
CACGCCTACTTCAGCCAGTACCACGACCCCGAGGATGAGCCAGAGGCC
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CCACCGAAGCCACCTGGCAGCCTGGAGATTGAGCAGTGA