University of Dundee

Standard Operating Procedure

Preparation of active SAPK4/p38δ

Enzyme description:- Active SAPK4/p38δ

Source:- Recombinant

Expression system:- *E.coli*

Expression conditions:- Induce expression with 200 μM IPTG once culture reaches OD_{600nm} = 0.4. Express for 16 hours at 26°C.

Tag:- N-terminal GST

Purification method:- GSH- agarose

Expression level:- 10 mg/L

Molecular mass:- 71 kDa by SDS-PAGE

Purity:- >85%

Contaminants:- No major contaminating proteins as judged by SDS-PAGE.

Activation protocol:-

Fresh SAPK4/p38δ (0.2 mg/ml - 3.5 μM) 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 SAPK4/p38δ 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 SAPK4/p38δ

Protein	Human SAPK4/p38δ
Accession number	Y10488
Tags	GST amino terminal
Bacterially-expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRN KKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML KMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCL DAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGG DHPPKSDLVPRGSPEF**MSLIRKKGFYKQDVNKTAWELPKTY** **VSPTHVGSGAYGSVCSAIDKRSGEKVAIKKLSRPFQSEIFAK** **RAYRELLLLKHMQHENVIGLLDVFTPASSLRNFYDFYLVMPF** **MQTDLQKIMGMEFSEEKIQYLVYQMLKGLKYIHSAGVVHR** **DLKPGNLAVNEDCELKILDFGLARHADAEMTGYVVTRCYR** **APEVILSWMHYNQTVDIWSVGCIMAEMLTGKTLFKGKDYL** **DQLTQILKVTGVPGTEFVQKLNDKAAKSYIQSLPQTPRKDF** **TQLFPRASPQAADLLEKMLELDVDKRLTAAQALTHPFFEPF** **RDPEEETEAQQPFDDSLEHEKLTVDEWKQHIYKEIVNFSPIA** **RKDSRRRSGMKL**
Native sequence	M230 of the GST-fusion protein is equivalent to M1 of human SAPK4/p38δ.
Protease cleavage site	Thrombin (LVPRGS) at residues 221 –226 of the fusion protein
Cloning sites	EcoRI site of pGEX-4T1

Nucleotide sequence of SAPK4/p38δ ORF

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ATGAGCCTCATCCGGAAAAAGGGCTTCTACAAGCAGGACGTCAACAAG
ACCGCCTGGGAGCTGCCCAAGACCTACGTGTCCCCGACGCACGTCGGC
AGCGGGGCCTATGGCTCCGTGTGCTCGGCCATCGACAAGCGGTCAGGG
GAGAAGGTGGCCATCAAGAAGCTGAGCCGACCCTTTCAGTCCGAGATC
TTCGCCAAGCGCGCCTACCGGGAGCTGCTGCTGCTGAAGCACATGCAG
CATGAGAACGTCATTGGGCTCCTGGATGTCTTCACCCCAGCCTCCTCC
CTGCGCAACTTCTATGACTTCTACCTGGTGATGCCCTTCATGCAGACG
GATCTGCAGAAGATCATGGGGATGGAGTTCAGTGAGGAGAAGATCCAG
TACCTGGTGTATCAGATGCTCAAAGGCCTTAAGTACATCCACTCTGCT
GGGGTCGTGCACAGGGACCTGAAGCCAGGCAACCTGGCTGTGAATGAG
GACTGTGAACTGAAGATTCTGGATTTTGGGCTGGCGCGACATGCAGAC
GCCGAGATGACTGGCTACGTGGTGACCCGCTGTTACCGAGCCCCCGAG
GTGATCCTCAGCTGGATGCACTACAACCAGACAGTGGACATCTGGTCT
GTGGGCTGTATCATGGCAGAGATGCTGACAGGGAAAACTCTGTTCAAG
GGGAAAGATTACCTGGACCAGCTGACCCAGATCCTGAAAGTGACCGGG
GTGCCTGGCACGGAGTTTGTGCAGAAGCTGAACGACAAAGCGGCCAAA
TCCTACATCCAGTCCCTGCCACAGACCCCCAGGAAGGATTTCACTCAG
CTGTTCCCACGGGCCAGCCCCCAGGCTGCGGACCTGCTGGAGAAGATG
CTGGAGCTAGACGTGGACAAGCGCCTGACGGCCGCGCAGGCCCTCACC
CATCCCTTCTTTGAACCCTTCCGGGACCCTGAGGAAGAGACGGAGGCC
CAGCAGCCGTTTGATGATTCCTTAGAACACGAGAAACTCACAGTGGAT
GAATGGAAGCAGCACATCTACAAGGAGATTGTGAACTTCAGCCCCATT
GCCCGGAAGGACTCACGGCGCCGGAGTGGCATGAAGCTGTAG
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