University of Dundee

Standard Operating Procedure

Preparation of active SAPK2α/p38

Enzyme description:- Active SAPK2α/p38

Source:- Recombinant

Expression system:- *E.coli*

Expression conditions:- Induce expression with 100 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:- ~50 mg/L

Molecular mass:- 64 kDa by SDS-PAGE

Purity:- >85%

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

Activation protocol:-

Fresh SAPK2a/ p38 (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 SAPK2a/p38 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 SAPK2α/p38

Protein	Human SAPK2α / p38
Accession number	L35264
Tags	GST
Bacterially-expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRN KKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML KMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCL DAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGG DHPPKSDLVPRGS**MSQERPTFYRQELNKTIWEVPERYQNLSP** **VGSGAYGSVCAAFDTKTGLRVAVKKLSRPFQSIIHAKRTYR** **ELRLLKHMKHENVIGLLDVFTPARSLEEFNDVYLVTHLMGA** **DLNNIVKCQKLTDDHVQFLIYQILRGLKYIHSADIIHRDLKP** **SNLAVNEDCELKILDFGLARHTDDEMTGYVATRWYRAPEI** **MLNWMHYNQTVDIWSVGCIMAELLTGRTLFPGTDHIDQL** **KLILRLVGTPGAELLKKISSESARNYIQSLTQMPKMNFANVF** **IGANPLAVDLLEKMLVLDSDKRITAAQALAHAYFAQYHDP** **DDEPVADPYDQSFESRDLLIDEWKSLTYDEVISFVPPPLDQE** **EMES**
Native sequence	M227 of the GST-fusion protein is equivalent to M1 of human SAPK2α/p38.
Protease cleavage site	Thrombin (LVPRGS) residues 221-226 of the GST fusion protein
Cloning sites	BamHI site of pGEX-4T1

ORF of unmodified protein

ATGTCTCAGGAGAGGCCCACGTTCTACCGGCAGGAGCTGAACAAGACA
ATCTGGGAGGTGCCCGAGCGTTACCAGAACCTGTCTCCAGTGGGCTCT
GGCGCCTATGGCTCTGTGTGTGCTGCTTTTGACACAAAAACGGGGTTA
CGTGTGGCAGTGAAGAAGCTCTCCAGACCATTTCAGTCCATCATTCAT
GCGAAAAGAACCTACAGAGAACTGCGGTTACTTAAACATATGAAACAT
GAAAATGTGATTGGTCTGTTGGACGTTTTTACACCTGCAAGGTCTCTG
GAGGAATTCAATGATGTGTATCTGGTGACCCATCTCATGGGGGCAGAT
CTGAACAACATTGTGAAATGTCAGAAGCTTACAGATGACCATGTTCAG
TTCCTTATCTACCAAATTCTCCGAGGTCTAAAGTATATACATTCAGCT
GACATAATTCACAGGGACCTAAAACCTAGTAATCTAGCTGTGAATGAA
GACTGTGAGCTGAAGATTCTGGATTTTGGACTGGCTCGGCACACAGAT
GATGAAATGACAGGCTACGTGGCCACTAGGTGGTACAGGGCTCCTGAG
ATCATGCTGAACTGGATGCATTACAACCAGACAGTTGATATTTGGTCA
GTGGGATGCATAATGGCCGAGCTGTTGACTGGAAGAACATTGTTTCCT
GGTACAGACCATATTGATCAGTTGAAGCTCATTTTAAGACTCGTTGGA
ACCCCAGGGGCTGAGCTTTTGAAGAAAATCTCCTCAGAGTCTGCAAGA
AACTATATTCAGTCTTTGACTCAGATGCCGAAGATGAACTTTGCGAAT
GTATTTATTGGTGCCAATCCCCTGGCTGTCGACTTGCTGGAGAAGATG
CTTGTATTGGACTCAGATAAGAGAATTACAGCGGCCCAAGCCCTTGCA
CATGCCTACTTTGCTCAGTACCACGATCCTGATGATGAACCAGTGGCC
GATCCTTATGATCAGTCCTTTGAAAGCAGGGACCTCCTTATAGATGAG
TGGAAAAGCCTGACCTATGATGAAGTCATCAGCTTTGTGCCACCACCC
CTTGACCAAGAAGAGATGGAGTCCTGA