University of Dundee

Standard Operating Procedure

Preparation of active SAPK3/p38γ

Enzyme description:- Active SAPK3/p38γ

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 SAPK3/p38γ (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 SAPK3/p38γ is then dialysed into enzyme storage buffer, and stored at –70°C.

Enzyme storage buffer:-

50 mM Tris/HCl pH 7.5, 270 mM sucrose,150 mM NaCl, 0.1 mM EGTA, 0.1 % β-mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF,1 mM Benzamidine.

Storage temperature:- Aliquot, snap freeze and store at -70°C.

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CLONE DATA SHEET – human SAPK3/p38γ

Protein	Human SAPK3/p38γ
Accession number	Y10487
Tags	GST
Bacterially-expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRN KKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML KMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCL DAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGG DHPPKSDLVPRGSPEF**MSSPPPARSGFYRQEVTKTAWEVRAV YRDLQPVGSGAYGAVCSAVDGRTGAKVAIKKLYRPFQSEL FAKRAYRELRLLKHMRHENVIGLLDVFTPDETLDDFTDFYL VMPFMGTDLGKLMKHEKLGEDRIQFLVYQMLKGLRYIHA AGIIHRDLKPGNLAVNEDCELKILDFGLARQADSEMTGYVV TRWYRAPEVILNWMRYTQTVDIWSVGCIMAEMITGKTLFK GSDHLDQLKEIMKVTGTPPAEFVQRLQSDEAKNYMKGLPE LEKKDFASILTNASPLAVNLLEKMLVLDAEQRVTAGEALAH PYFESLHDTEDEPQVQKYDDSFDDVDRTLDEWKRVTYKEV LSFKPPRQLGARVSKETPL**
Native sequence	M230 of the GST-fusion protein is equivalent to M1 of human SAPK3/p38γ.
Protease cleavage site	Thrombin (LVPRGS) residues 221-226 of the fusion protein
Cloning sites	EcoRI site of pGEX-4T1

Nucleotide sequence of SAPK3/p38γ ORF

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ATGAGCTCTCCGCCGCCCGCCCGCAGTGGCTTTTACCGCCAGGAGGTG
ACCAAGACGGCCTGGGAGGTGCGCGCCGTGTACCGGGACCTGCAGCCC
GTGGGCTCGGGCGCCTACGGCGCGGTGTGCTCGGCCGTGGACGGCCGC
ACCGGCGCTAAGGTGGCCATCAAGAAGCTGTATCGGCCCTTCCAGTCC
GAGCTGTTCGCCAAGCGCGCCTACCGCGAGCTGCGCCTGCTCAAGCAC
ATGCGCCACGAGAACGTGATCGGGCTGCTGGACGTATTCACTCCTGAT
GAGACCCTGGATGACTTCACGGACTTTTACCTGGTGATGCCGTTCATG
GGCACCGACCTGGGCAAGCTCATGAAACATGAGAAGCTAGGCGAGGAC
CGGATCCAGTTCCTCGTGTACCAGATGCTGAAGGGGCTGAGGTATATC
CACGCTGCCGGCATCATCCACAGAGACCTGAAGCCCGGCAACCTGGCT
GTGAACGAAGACTGTGAGCTGAAGATCCTGGACTTCGGCCTGGCCAGG
CAGGCAGACAGTGAGATGACTGGTTACGTGGTGACCCGGTGGTACCGG
GCTCCCGAGGTCATCTTGAATTGGATGCGCTACACGCAGACGGTGGAC
ATCTGGTCTGTGGGCTGCATCATGGCGGAGATGATCACAGGCAAGACG
CTGTTCAAGGGCAGCGACCACCTGGACCAGCTGAAGGAGATCATGAAG
GTGACGGGGACGCCTCCGGCTGAGTTTGTGCAGCGGCTGCAGAGCGAT
GAGGCCAAGAACTACATGAAGGGCCTCCCCGAATTGGAGAAGAAGGAT
TTTGCCTCTATCCTGACCAATGCAAGCCCTCTGGCTGTGAACCTCCTG
GAGAAGATGCTGGTGCTGGACGCGGAGCAGCGGGTGACGGCAGGCGAG
GCGCTGGCCCATCCCTACTTCGAGTCCCTGCACGACACGGAAGATGAG
CCCCAGGTCCAGAAGTATGATGACTCCTTTGACGACGTTGACCGCACA
CTGGATGAATGGAAGCGTGTTACTTACAAAGAGGTGCTCAGCTTCAAG
CCTCCCCGGCAGCTGGGGGCCAGGGTCTCCAAGGAGACGCCTCTGTGA
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