Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active p42MAPK [2 – 360]

Enzyme description:- p42MAPK [2 – 360]

Clone number:- DU 650

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 20 mg/L

Calculated molecular mass:- 68, 721 daltons

Purity:- >95 %

Activation protocol:-
p42MAPK (3.5 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 100 nM active GST-MKK1-His(6) [DU 1843] at 30 °C for 30 min. Following activation, the MKK1 is removed by chromatography on Ni^{2+}-NTA agarose. The active p42MAPK is further purified by chromatography on Mono-Q.

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, 0.1 mM EGTA, 10 mM MgAc

Substrate:-
Myelin basic protein Final concentration: 0.3 mg/ml

Specific activity range:- 350 – 700 U/mg

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Clone Data Sheet

p42MAPK [2 - 360]

Protein	p42MAPK [2 – 360]
Clone number	DU 650
Species	Human
Accession number	NM_002745
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEG DKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIA DKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKD FETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPD FMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQID KYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGP LGSPNSRVD**AAAAAAGAGPEMVRGQVFDVGPRYTNLSY** **IGEGAYGMVCSAYDNVNKVRVAIKKISPFEHQTYCQRT** **LREIKILLRFRHENIIGINDIIRAPTIEQMKDVYIVQD** **LMETDLYKLLKTQHLSNDHICYFLYQILRGLKYIHSAN** **VLHRDLKPSNLLLNTTCDLKICDFGLARVADPDHDHTG** **FLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAE** **MLSNRPIFPGKHYLDQLKHILGILGSPSQEDLNCIINL** **KARNYLLSLPHKNKVPWNRLFPNADSKALDLLDKMLTF** **NPHKRIEVEQALAHPYLEQYYDPSDEPIAEAPFKFDME** **LDDLPKEKLKELIFEETARFQPGYRS**
Native sequence	Amino acids A2 – S360 (end) of human p42MAPK. Residue A238 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Sal*1 and *Not*1 of pGEX6P-3

Nucleotide sequence of insert

GCGTCGACGCGGCGGCGGCGGCGGGCGCGGGCCCGGAGATGG
TCCGCGGGCAGGTGTTCGACGTGGGGCCGCGCTACACCAACCTCT
CGTACATCGGCGAGGGCGCCTACGGCATGGTGTGCTCTGCTTATG
ATAATGTCAACAAAGTTCGAGTAGCTATCAAGAAAATCAGCCCCT
TTGAGCACCAGACCTACTGCCAGAGAACCCTGAGGGAGATAAAAA
TCTTACTGCGCTTCAGACATGAGAACATCATTGGAATCAATGACA
TTATTCGAGCACCAACCATCGAGCAAATGAAAGATGTATATATAG
TACAGGACCTCATGGAAACAGATCTTTACAAGCTCTTGAAGACAC
AACACCTCAGCAATGACCATATCTGCTATTTTCTCTACCAGATCC
TCAGAGGGTTAAAATATATCCATTCAGCTAACGTTCTGCACCGTG
ACCTCAAGCCTTCCAACCTGCTGCTCAACACCACCTGTGATCTCA
AGATCTGTGACTTTGGCCTGGCCCGTGTTGCAGATCCAGACCATG
ATCACACAGGGTTCCTGACAGAATATGTGGCCACACGTTGGTACA
GGGCTCCAGAAATTATGTTGAATTCCAAGGGCTACACCAAGTCCA
TTGATATTTGGTCTGTAGGCTGCATTCTGGCAGAAATGCTTTCTA
ACAGGCCCATCTTTCCAGGGAAGCATTATCTTGACCAGCTGAAAC
ACATTTTGGGTATTCTTGGATCCCCATCACAAGAAGACCTGAATT
GTATAATAAATTTAAAAGCTAGGAACTATTTGCTTTCTCTTCCAC
ACAAAAATAAGGTGCCATGGAACAGGCTGTTCCCAAATGCTGACT
CCAAAGCTCTGGACTTATTGGACAAAATGTTGACATTCAACCCAC
ACAAGAGGATTGAAGTAGAACAGGCTCTGGCCCACCCATATCTGG
AGCAGTATTACGACCCGAGTGACGAGCCCATCGCCGAAGCACCAT
TCAAGTTCGACATGGAATTGGATGACTTGCCTAAGGAAAAGCTCA
AAGAACTAATTTTTGAAGAGACTGCTAGATTCCAGCCAGGATACA
GATCTtaagcggccgcgc