Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active p44MAPK [2 – 379]

Enzyme description:- p44MAPK [2 – 379]

Clone number:- DU 1509

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 10 mg/L

Calculated molecular mass:-

Monoisotopic 69, 757.66 daltons
Average Mass 69, 802.46 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.06

Purity:- >90 %

Activation protocol:-

p44MAPK (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 p44MAPK 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

Division of Signal Tranduction Therapy

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:- To be determined

Division of Signal Tranduction Therapy

Clone Data Sheet

p44MAPK [2 - 379]

Protein	p44MAPK [2 – 379]
Clone number	DU 1509
Species	Human
Accession number	BC013992
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKK FELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERA EISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFED RLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFK KRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF QGPLGS**AAAAAQGGGGGEPRRTEGVGPGVPGEVEMVKGQPFDVGP RYTQLQYIGEGAYGMVSSAYDHVRKTRVAIKKISPFEHQTYCQRT LREIQILLRFRHENVIGIRDILRASTLEAMRDVYIVQDLMETDLY KLLKSQQLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLSN TTCDLKICDFGLARIADPEHDHTGFLTEYVATRWYRAPEIMLNSK GYTKSIDIWSVGCILAEMLSNRPIFPGKHYLDQLNHILGILGSPS QEDLNCIINMKARNYLQSLPSKTKVAWAKLFPKSDSKALDLLDRM LTFNPNKRITVEEALAHPYLEQYYDPTDEPVAEEPFTFAMELDDL PKERLKELIFQETARFQPGVLEAP**

Native sequence	Amino acids A2 – P379 (end) of human p44MAPK. Residue A232 of the fusion protein is equivalent to A2 of the native enzyme. The GST tag is located at residues 1 – 220. The following amino acid substitution is present: I – S, where I174 of the native sequence is S404 of the fusion protein
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Sal*1 of pGEX6P-1

Nucleotide sequence of insert

ggatccGCGGCGGCGGCGGCTCAGGGGGGCGGGGGCGGGGAGCCC
CGTAGAACCGAGGGGGTCGGCCCGGGGGTCCCGGGGGAGGTGGAG
ATGGTGAAGGGGCAGCCGTTCGACGTGGGCCCGCGCTACACGCAG
TTGCAGTACATCGGCGAGGGCGCGTACGGCATGGTCAGCTCGGCC
TATGACCACGTGCGCAAGACTCGCGTGGCCATCAAGAAGATCAGC
CCCTTCGAACATCAGACCTACTGCCAGCGCACGCTCCGGGAGATC
CAGATCCTGCTGCGCTTCCGCCATGAGAATGTCATCGGCATCCGA
GACATTCTGCGGGCGTCCACCCTGGAAGCCATGAGAGATGTCTAC
ATTGTGCAGGACCTGATGGAGACTGACCTGTACAAGTTGCTGAAA
AGCCAGCAGCTGAGCAATGACCATATCTGCTACTTCCTCTACCAG
ATCCTGCGGGGCCTCAAGTACATCCACTCCGCCAACGTGCTCCAC
CGAGATCTAAAGCCCTCCAACCTGCTCAGCAACACCACCTGCGAC
CTTAAGATTTGTGATTTCGGCCTGGCCCGGATTGCCGATCCTGAG
CATGACCACACCGGCTTCCTGACGGAGTATGTGGCTACGCGCTGG
TACCGGGCCCCAGAGATCATGCTGAACTCCAAGGGCTATACCAAG
TCCATCGACATCTGGTCTGTGGGCTGCATTCTGGCTGAGATGCTC
TCTAACCGGCCCATCTTCCCTGGCAAGCACTACCTGGATCAGCTC
AACCACATTCTGGGCATCCTGGGCTCCCCATCCCAGGAGGACCTG
AATTGTATCATCAACATGAAGGCCCGAAACTACCTACAGTCTCTG
CCCTCCAAGACCAAGGTGGCTTGGGCCAAGCTTTTCCCCAAGTCA
GACTCCAAAGCCCTTGACCTGCTGGACCGGATGTTAACCTTTAAC
CCCAATAAACGGATCACAGTGGAGGAAGCGCTGGCTCACCCCTAC
CTGGAGCAGTACTATGACCCGACGGATGAGCCAGTGGCCGAGGAG
CCCTTCACCTTCGCCATGGAGCTGGATGACCTACCTAAGGAGCGG
CTGAAGGAGCTCATCTTCCAGGAGACAGCACGCTTCCAGCCCGGA
GTGCTGGAGGCCCCCtaggtcgac