Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MKK4 [34 - 397]

Enzyme description:- MKK4 [34 - 397]

Clone number:- DU 1788

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GST Sepharose

Expression level:- 5-10 mg/L

Calculated molecular mass:- 67, 574 daltons

Purity:- >70 %

Activation protocol:-

GST-MKK4 (4 µM) is activated in 50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc, 0.1mM ATP, with 100 µg/ml His-MEKK1 [DU 1847] at 30 °C for 30 min. Following activation, the GST-MKK4 is re-purified by GSH Sepharose chromatography.

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:-
Two step assay in which MKK4 activates unactive JNK 1, 2 or 3 [DU 700, DU 699 or DU 1511], which then phosphorylates ATF2 [DU 1787].

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 % 2−mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Specific activity range:- 600 – 1200 U/mg

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Clone Data Sheet

MKK4 [34 – 397]

Protein	MKK4 [34 – 397]
Clone number	DU 1788
Species	Mouse
Accession number	U18310
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNPLYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLHMFEDRLCHKTYLN GDHVTHPDFMLYDALAVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRG**SMQGKRKALKLNFAN** **PPVKSTARFTLNPNTTGVQNPHIERLRTHSIESSGKLKISPEQHWDFT** **AEDLKDLGEIGRGAYGSVNKMVHKPSGQIMAVKRIRSTVDEKEQKQLL** **MDLDVVMRSSDCPYIVQFYGALFREGDCWICMELMSTSFDKFYKYVYS** **VLDDVIPEEILGKITLATVKALNHLKENLKIIHRDIKPSNILLDRSGN** **IKLCDFGISGQLVDSIAKTRDAGCRPYMAPERIDPSASRQGYDVRSDV** **WSLGITLYELATGRFPYPKWNSVFDQLTQVVKGDPPQLSNSEEREFSP** **SFINFVNLCLTKDESKRPKYKELLKHPFILMYEERTVEVACYVCKILD** **QMPATPSSPMYVD**
Native sequence	Amino acids S34 – D397 (end) of mouse MKK4. Residue S226 of the fusion protein is equivalent to S34 of the native enzyme The GST tag is located at residues 1 - 220.
Protease cleavage	Thrombin (LVPRGS) at residues 221 - 226
Cloning sites	*Bam*HI sites of pGEX-2T

Nucleotide sequence of insert

GGATCCATGCAGGGTAAGCGCAAAGCACTGAAGTTGAATTTTGCAAAT
CCACCTGTCAAATCGACAGCACGGTTTACCCTGAATCCTAATACTACA
GGAGTCCAGAACCCACACATAGAGAGACTGAGAACACACAGCATTGAG
TCATCAGGAAAACTGAAGATCTCCCCTGAACAACACTGGGATTTCACT
GCAGAGGACTTGAAAGACCTTGGAGAAATTGGACGAGGAGCTTATGGT
TCTGTCAACAAAATGGTCCACAAACCAAGTGGGCAGATAATGGCAGTT
AAAAGAATTCGGTCAACTGTGGATGAAAAAGAACAAAAACAACTTCTC
ATGGATTTGGATGTAGTAATGCGGAGTAGTGATTGCCCATACATTGTT
CAGTTCTATGGTGCACTCTTCAGAGAGGGCGACTGTTGGATCTGTATG
GAGCTCATGTCTACCTCGTTCGATAAGTTTTACAAATATGTATATAGT
GTGTTAGATGACGTTATTCCGGAAGAGATCTTAGGCAAAATCACTTTA
GCAACTGTGAAAGCACTAAACCACTTAAAAGAAAACTTGAAAATTATT
CACAGAGACATCAAACCTTCCAATATTCTTCTGGACAGAAGTGGAAAT
ATAAAGCTCTGTGATTTCGGCATCAGTGGACAGCTTGTGGACTCTATT
GCCAAGACAAGAGATGCTGGGTGTAGGCCGTATATGGCACCTGAAAGA
ATAGACCCAAGTGCATCAAGACAAGGGTATGATGTCCGCTCTGATGTC
TGGAGTTTGGGGATCACATTGTACGAGTTGGCCACAGGCCGATTTCCT
TATCCAAAGTGGAATAGTGTATTTGATCAGCTAACACAAGTGGTGAAA
GGAGACCCTCCGCAGCTGAGTAATTCTGAAGAAAGGGAGTTCTCCCCC
AGTTTCATCAACTTTGTCAACTTGTGCCTTACGAAGGATGAATCCAAA
AGGCCAAAGTATAAAGAGCTTCTGAAACATCCCTTTATTTTGATGTAT
GAAGAACGTACTGTAGAGGTCGCATGCTATGTTTGTAAAATCCTGGAT
CAGATGCCAGCCACTCCCAGCTCGCCCATGTATGTCGACtgaggatcc