Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MAPKAP-K3 [2 - 382]

Enzyme description:- MAPKAP-K3 [2 - 382]

Clone number:- DU 929

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 10 mg/L

Calculated molecular mass:-
Monoisotopic 69, 635.10 daltons
Average Mass 69, 679.96 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.34

Purity:- >80 %

Activation protocol:-

MAPKAP-K3 (4 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 MgAc, 0.1 mM ATP with 2 U/ml GST-SAPK2a [DU 979] at 30 °C for 45 min. Following activation, the enzyme is repurified by chromatography on MonoS (GST-SAPK2a does not bind MonoS).

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

Substrate:-
KKLNRTLSVA Final concentration: 30 μM

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Clone Data Sheet

MAPKAP-K3 [2 - 382]

Protein	MAPKAP-K3 [2 - 382]
Clone number	DU 929
Species	Human
Accession number	NM_004635
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**DGETAEEQG** **GPVPPPVAPGGPGLGGAPGGRREPKKYAVTDDYQLSKQVLGLGVNGKV** **LECFHRRTGQKCALKLLYDSPKARQEVDHHWQASGGPHIVCILDVYEN** **MHHGKRCLLIIMECMEGGELFSRIQERGDQAFTEREAAEIMRDIGTAI** **QFLHSHNIAHRDVKPENLLYTSKEKDAVLKLTDFGFAKETTQNALQTP** **CYTPYYVAPEVLGPEKYDKSCDMWSLGVIMYILLCGFPPFYSNTGQAI** **SPGMKRRIRLGQYGFPNPEWSEVSEDAKQLIRLLLKTDPTERLTITQF** **MNHPWINQSMVVPQTPLHTARVLQEDKDHWDEVKEEMTSALATMRVDY** **DQVKIKDLKTSNNRLLNKRRKKQAGSSSASQGCNNQ**
Native sequence	Amino acids D2 – Q382 (end) of human MAPKAP-K3. Residue D232 of fusion protein is equivalent to D2 of the native enzyme. The GST tag is located at residues 1- 220.
Protease cleavage	PreScission (LEVLFQGP) at residues 221 – 228
Cloning sites	*Bam*H1 and *Eco*R1 of pGEX6P-1

Nucleotide sequence of insert

GGATCCGATGGTGAAACAGCAGAGGAGCAGGGGGGCCCTGTGCCCCCG
CCAGTTGCACCCGGCGGACCCGGCTTGGGCGGTGCTCCGGGGGGGCGG
CGGGAGCCCAAGAAGTACGCAGTGACCGACGACTACCAGTTGTCCAAG
CAGGTGCTGGGCCTGGGTGTGAACGGCAAAGTGCTGGAGTGCTTCCAT
CGGCGCACTGGACAGAAGTGTGCCCTGAAGCTCCTGTATGACAGCCCC
AAGGCCCGGCAGGAGGTAGACCATCACTGGCAGGCTTCTGGCGGCCCC
CATATTGTCTGCATCCTGGATGTGTATGAGAACATGCACCATGGCAAG
CGCTGTCTCCTCATCATCATGGAATGCATGGAAGGTGGTGAGTTGTTC
AGCAGGATTCAGGAGCGTGGCGACCAGGCTTTCACTGAGAGAGAAGCT
GCAGAGATAATGCGGGATATTGGCACTGCCATCCAGTTTCTGCACAGC
CATAACATTGCCCACCGAGATGTCAAGCCTGAAAACCTACTCTACACA
TCTAAGGAGAAAGACGCAGTGCTTAAGCTCACCGATTTTGGCTTTGCT
AAGGAGACCACCCAAAATGCCCTGCAGACACCCTGCTATACTCCCTAT
TATGTGGCCCCTGAGGTCCTGGGTCCAGAGAAGTATGACAAGTCATGT
GACATGTGGTCCCTGGGTGTCATCATGTACATCCTCCTTTGTGGCTTC
CCACCCTTCTACTCCAACACGGGCCAGGCCATCTCCCCGGGGATGAAG
AGGAGGATTCGCCTGGGCCAGTACGGCTTCCCCAATCCTGAGTGGTCA
GAAGTCTCTGAGGATGCCAAGCAGCTGATCCGCCTCCTGTTGAAGACA
GACCCCACAGAGAGGCTGACCATCACTCAGTTCATGAACCACCCCTGG
ATCAACCAATCGATGGTAGTGCCACAGACCCCACTCCACACGGCCCGA
GTGCTGCAGGAGGACAAAGACCACTGGGACGAAGTCAAGGAGGAGATG
ACCAGTGCCTTGGCCACTATGCGGGTAGACTACGACCAGGTGAAGATC
AAGGACCTGAAGACCTCTAACAACCGGCTCCTCAACAAGAGGAGAAAA
AAGCAGGCAGGCAGCTCCTCTGCCTCACAGGGCTGCAACAACCAGtag
gaattc