Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active MAPKAP-K2 [46 - 400]

Enzyme description:- MAPKAP-K2 [46 - 400]

Clone number:- DU 1714

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and C-terminal Myc

Purification method:- GSH Sepharose

Expression level:- 20 mg/L

Calculated molecular mass:- 70, 022 daltons

Purity:- >90 %

Activation protocol:-
MAPKAP-K2 (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 magnesium acetate, 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, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

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:-
KKLNRTLSVA: Final concentration: 30 µM

Specific activity range:- 1000 – 1500 U/mg

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Clone Data Sheet

MAPKAP-K2 [46 - 400]

Protein MAPKAP-K2 [46 - 400]

Clone number DU 1714

Species Human

Accession NM_032960

Tags N-terminal GST and C-terminal MYC (EQKLISEEDL)

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNPLYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLHMFEDRLCHKTYLN
GDHVTHPDFMLYDALAVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSPGISGGGGGILEAT
ME**FHVKSGLQIKKNAIIDDYKVTSQVLGLGINGKVLQIFNKRTQEKFA**
LKMLQDCPKARREVELHWRASQCPHIVRIVDVYENLYAGRKCLLIVME
CLDGGELFSRIQDRGDQAFTEREASEIMKSIGEAIQYLHSINIAHRDV
KPENLLYTSKRPNAILKLTDFGFAKETTSHNSLTTPCYTPYYVAPEVL
GPEKYDKSCDMWSLGVIMYILLCGYPPFYSNHGLAISPGMKTRIRMGQ
YEFPNPEWSEVSEEVKMLIRNLLKTEPTQRMTITEFMNHPWIMQSTKV
PQTPLHTSRVLKEDKERWEDVKEEMTSALATMRVDYEQIKIKKIEDAS
NPLLLKRRKKARALRAAALGHMEQKLISEEDLK

Native sequence Amino acids F46 – H400 (end) of human MAPKAP-K2.
Residue F243 of the fusion protein is equilivalent to F46 of the native enzyme. The GST tag is located at residues 1 – 220 and the MYC tag is located at residues 599 – 608. The following amino acid is present after the MAPKAP-K2 sequence and before the MYC tag, M at residue 598 and the following amino acid is present after the MYC tag, K at residue 609.

The following amino acid substitutions are present:
E – R, where E394 of the native sequence is R591 of the fusion protein
A – G, where A399 of the native sequence is G596 of the fusion protein

Protease cleavage Thrombin (LVPRGS) at residues 221 – 226

Cloning sites

*Bam*H1 and *Eco*R1 sites of pGEX-4T

Nucleotide sequence of insert

TTCCACGTCAAGTCCGGCCTGCAGATCAAGAAGAACGCCATCATCGAT
GACTACAAGGTCACCAGCCAGGTCCTGGGGCTGGGCATCAACGGCAAA
GTTTTGCAGATCTTCAACAAGAGGACCCAGGAGAAATTCGCCCTCAAA
ATGCTTCAGGACTGCCCCAAGGCCCGCAGGGAGGTGGAGCTGCACTGG
CGGGCCTCCCAGTGCCCCGACATCGTACGGATCGTGGATGTGTACGAG
AATCTGTACGCAGGGAGGAAGTGCCTGCTGATTGTCATGGAATGTTTG
GACGGTGGAGAACTCTTTAGCCGAATCCAGGATCGAGGAGACCAGGCA
TTCACAGAAAGAGAAGCATCCGAAATCATGAAGAGCATCGGTGAGGCC
ATCCAGTATCTGCATTCAATCAACATTGCCCATCGGGATGTCAAGCCT
GAGAATCTCTTATACACCTCCAAAAGGCCCAACGCCATCCTGAAACTC
ACTGACTTTGGCTTTGCCAAGGAAACCACCAGCCACAACTCTTTGACC
ACTCCTTGTTATACACCGTACTATGTGGCTCCAGAAGTGCTGGGTCCA
GAGAAGTATGACAAGTCCTGTGACATGTGGTCCCTGGGTGTCATCATG
TACATTCTGCTGTGTGGGTATCCCCCCTTCTACTCCAACCACGGCCTT
GCCATCTCTCCGGGCATGAAGACTCGCATCCGAATGGGCCAGTATGAA
TTTCCCAACCCAGAATGGTCAGAAGTATCAGAGGAAGTGAAGATGCTC
ATTCGGAATCTGCTGAAAACAGAGCCCACCCAGAGAATGACCATCACC
GAGTTTATGAACCACCCTTGGATCATGCAATCAACAAAGGTCCCTCAA
ACCCCACTGCACACCAGCCGGGTCCTGAAGGAGGACAAGGAGCGGTGG
GAGGATGTCAAGGAGGAGATGACCAGTGCCTTGGCCACAATGCGCGTT
GACTACGAGCAGATCAAGATAAAAAAGATTGAAGATGCATCCAACCCT
CTGCTGCTGAAGAGGCGGAAGAAAGCTCGGGCCCTGGAGGCTGCGGCT
CTGGGCCACATGGAGCAGAAGCTGATCAGCGAGGAGGACCTGAAG