University of Dundee

Standard Operating Procedure

Preparation of active human MAPKAP-K2

Enzyme description:- Active human MAPKAP-K2

Source:- Recombinant

Expression system:- *E.coli*

Expression conditions:- Induce expression with 125 μM IPTG once culture reaches OD_{600nm} = 0.4. Express for 16 hours at 26°C.

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

Purification method:- GSH-agarose

Expression level:- ~50 mg/L

Molecular mass:- 66 kDa by SDS-PAGE

Purity:- >90%

Contaminants:- The preparation also contains some minor degradation products.

Activation protocol:-

Fresh MAPKAP-K2 (4 μM–0.25 mg/ml) is activated in 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 0.1 % β-mercaptoethanol, 0.1 mM sodium vanadate, 10 mM magnesium acetate, 0.1 mM ATP with 2 U/ml SAPK2a/p38 at 30°C for 45 min. Following activation, the enzyme is separated from the SAPK2a/p38 by chromatography on MonoS/Hi-Trap SP (GST-SAPK2a/p38 does not bind MonoS). The peak fractions containing active MAPKAP-K2 are pooled and dialysed into storage buffer.

Enzyme storage buffer:-
50mM Tris/HCl pH 7.5, 50% glycerol, 150 mM NaCl, 0.1mM EGTA, 0.1 % β-mercaptoethanol, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF

Storage temperature:- -20°C.

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CLONE DATA SHEET – human MAPKAP-K2

Protein	Human MAPKAP-K2, transcript variant 2 (Δ 1-45)
Accession	NM_032960
Tags	N-Terminal GST and C-Terminal Myc (EQKLISEEDL)
Bacterially-expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRN KKFELGLEFPNPLYYIDGDVKLTQSMAIIRYIADKHNMLGGCP KERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEML HMFEDRLCHKTYLNGDHVTHPDFMLYDALAVVLYMDPMCL DAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGG DHPPKSDLVPRGSPGISGGGGGILEATME**FHVKSGLQIKKNAI IDDYKVTSQVLGLGINGKVLQIFNKRTQEKFALKMLQDCP KARREVELHWRASQCPHIVRIVDVYENLYAGRKCLLIVMEC LDGGELFSRIQDRGDQAFTEREASEIMKSIGEAIQYLHSINIA HRDVKPENLLYTSKRPNAILKLTDFGFAKETTSHNSLTTPCY TPYYVAPEVLGPEKYDKSCDMWSLGVIMYILLCGYPPFYSN HGLAISPGMKTRIRMGQYEFPNPEWSEVSEEVKMLIRNLLK TEPTQRMTITEFMNHPWIMQSTKVPQTPLHTSRVLKEDKER WEDVKEEMTSALATMRVDYEQIKIKKIEDASNPLLLKRRKK ARALRAAALGHMEQKLISEEDLK**
Native sequence	F243 of the fusion protein is equivalent to F46 of human MAPKAP-K2. The MAPKAK-K2 sequence terminates at H597 and is followed by a Myc (EQKLISEEDL) tag before the fusion protein terminates at K609. The fusion protein contains a R591E (R304E native) and G596A (G309A native) substitution as compared to the Genbank entry.
Protease cleavage site	Thrombin (LVPRGS) at residues 221-226 of the fusion protein.
Cloning sites	EcoR*1* and Sal*1* sites of pGEX-KG
Human MAPKAP-K2 transcript varient 2 nucleotide sequence	TTCCACGTCAAGTCCGGCCTGCAGATCAAGAAGAACGCCATCATCGAT GACTACAAGGTCACCAGCCAGGTCCTGGGGCTGGGCATCAACGGCAAA GTTTTGCAGATCTTCAACAAGAGGACCCAGGAGAAATTCGCCCTCAAA ATGCTTCAGGACTGCCCCAAGGCCCGCAGGGAGGTGGAGCTGCACTGG CGGGCCTCCCAGTGCCCCGACATCGTACGGATCGTGGATGTGTACGAG

AATCTGTACGCAGGGAGGAAGTGCCTGCTGATTGTCATGGAATGTTTG
GACGGTGGAGAACTCTTTAGCCGAATCCAGGATCGAGGAGACCAGGCA
TTCACAGAAAGAGAAGCATCCGAAATCATGAAGAGCATCGGTGAGGCC
ATCCAGTATCTGCATTCAATCAACATTGCCCATCGGGATGTCAAGCCT
GAGAATCTCTTATACACCTCCAAAAGGCCCAACGCCATCCTGAAACTC
ACTGACTTTGGCTTTGCCAAGGAAACCACCAGCCACAACTCTTTGACC
ACTCCTTGTTATACACCGTACTATGTGGCTCCAGAAGTGCTGGGTCCA
GAGAAGTATGACAAGTCCTGTGACATGTGGTCCCTGGGTGTCATCATG
TACATTCTGCTGTGTGGGTATCCCCCCTTCTACTCCAACCACGGCCTT
GCCATCTCTCCGGGCATGAAGACTCGCATCCGAATGGGCCAGTATGAA
TTTCCCAACCCAGAATGGTCAGAAGTATCAGAGGAAGTGAAGATGCTC
ATTCGGAATCTGCTGAAAACAGAGCCCACCCAGAGAATGACCATCACC
GAGTTTATGAACCACCCTTGGATCATGCAATCAACAAAGGTCCCTCAA
ACCCCACTGCACACCAGCCGGGTCCTGAAGGAGGACAAGGAGCGGTGG
GAGGATGTCAAGGAGGAGATGACCAGTGCCTTGGCCACAATGCGCGTT
GACTACGAGCAGATCAAGATAAAAAAGATTGAAGATGCATCCAACCCT
CTGCTGCTGAAGAGGCGGAAGAAAGCTCGGGCCCTGGAGGCTGCGGCT
CTGGGCCACATGGAGCAGAAGCTGATCAGCGAGGAGGACCTGAAG