University of Dundee

Standard Operating Procedure

Preparation of active MNK1 [2 – 424]

Enzyme description:- MNK1 [2 - 424]

Clone number:- DU 1385

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and C-terminal His(6)

Purification method:- GSH Sepharose

Expression level:- 3 mg/L

Calculated molecular mass:- 74, 869 daltons

Purity:- 90 %

Activation protocol:-

MNK1 (2.5 μM) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.2 % 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP with 100 μg/ml GST-SAPK2a [DU 979] at 30 °C for 30 mins. Following activation, MNK1 is re-purified by Ni^{2+}-NTA agarose chromatography.

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -20 °C [Long term stability to be determined]

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:-
eIF4E [DU 1129] Final concentration: 0.5 mg/ml

Specific activity range:- To be determined

University of Dundee

Clone Data Sheet - MNK1 [2 - 424]

Protein	MNK1 [2 - 424]
Clone number	DU 1385
Species	Human
Accession number	AB000409 (lacks an inactivating 41 amino acid insertion in the catalytic domain)
Tags	N-terminal GST and C-terminal His(6)
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLE FPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDI RYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDF MLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQ GWQATFGGGDHPPKSDLEVLFQGPLGS**VSSQKLEKPIEMGSSEPLPIADGD RRRKKKRRGRATDSLPGKFEDMYKLTSELLGEGAYAKVQGAVSLQNGKEYA VKIIEKQAGHSRSRVFREVETLYQCQGNKNILELIEFFEDDTRFYLVFEKL QGGSILAHIQKQKHFNEREASRVVRDVAAALDFLHTKGIAHRDLKPENILC ESPEKVSPVKICDFDLGSGMKLNNSCTPITTPELTTPCGSAEYMAPEVVEV FTDQATFYDKRCDLWSLGVVLYIMLSGYPPFVGHCGADCGWDRGEVCRVCQ NKLFESIQEGKYEFPDKDWAHISSEAKDLISKLLVRDAKQRLSAAQVLQHP WVQGQAPEKGLPTPQVLQRNSSTMDLTLFAAEAIALNRQLSQHEENELAEE PEALADGLCSMKLSPPCKSRLARRRALAQAGRGEDRSPPTAL**HHHHHH
Native sequence	Amino acids V2 – L424 (end) of human MNK1. Residue V232 of the fusion protein is equivalent to V2 of the native enzyme. The GST tag is located at residues 1 – 220 and the His(6) tag is located at residues 655 – 660.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Bam*H1 and *Eco*R1 site of pGEX 6P-1

Nucleotide Sequence of insert

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCA
ACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAG
AGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAA
AAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATAT
TGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTT
ATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAG
CGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAG
ATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTC
TCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATG
TTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCA
TGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTG
TTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTA
GTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAA
GTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCT
GGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGAT
CTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCGTATCTTCTCA
AAAGTTGGAAAAACCTATAGAGATGGGCAGTAGCGAACCCCTTC
CCATCGCAGATGGTGACAGGAGGAGGAAGAAGAAGCGGAGGGGC
CGGGCCACTGACTCCTTGCCAGGAAAGTTTGAAGATATGTACAA
GCTGACCTCTGAATTGCTTGGAGAGGGAGCCTATGCCAAAGTTC
AAGGTGCCGTGAGCCTACAGAATGGCAAAGAGTATGCCGTCAAA
ATCATCGAGAAACAAGCAGGGCACAGTCGGAGTAGGGTGTTTCG
AGAGGTGGAGACGCTGTATCAGTGTCAGGGAAACAAGAACATTT
TGGAGCTGATTGAGTTCTTTGAAGATGACACAAGGTTTTACTTG
GTCTTTGAGAAATTGCAAGGAGGTTCCATCTTAGCCCACATCCA
GAAGCAAAAGCACTTCAATGAGCGAGAAGCCAGCCGAGTGGTGC
GGGACGTTGCTGCTGCCCTTGACTTCCTGCATACCAAAGGCATT
GCTCATCGTGATCTGAAACCAGAAAATATATTGTGTGAATCTCC
AGAAAAGGTGTCTCCAGTGAAAATCTGTGACTTTGACTTGGGCA
GTGGGATGAAACTGAACAACTCCTGTACCCCCATAACCACACCA
GAGCTGACCACCCCATGTGGCTCTGCAGAATACATGGCCCCTGA
GGTAGTGGAGGTCTTCACGGACCAGGCCACATTCTACGACAAGC
GCTGTGACCTGTGGAGCCTGGGCGTGGTCCTCTACATCATGCTG
AGTGGCTACCCACCCTTCGTGGGTCACTGCGGGGCCGACTGTGG
CTGGGACCGGGGCGAGGTCTGCAGGGTGTGCCAGAACAAGCTGT
TTGAAAGCATCCAGGAAGGCAAGTATGAGTTTCCTGACAAGGAC
TGGGCACACATCTCCAGTGAAGCCAAAGACCTCATCTCCAAGCT
CCTGGTGCGAGATGCAAAGCAGAGACTTAGCGCCGCCCAAGTTC
TGCAGCACCCATGGGTGCAGGGGCAAGCTCCAGAAAAGGGACTC
CCCACGCCGCAAGTCCTCCAGAGGAACAGCAGCACAATGGACCT
GACGCTCTTCGCAGCTGAGGCCATCGCCCTTAACCGCCAGCTAT
CTCAGCACGAAGAGAACGAACTAGCAGAGGAGCCAGAGGCACTA

GCTGATGGCCTCTGCTCCATGAAGCTTTCCCCTCCCTGCAAGTC
ACGCCTGGCCCGGAGACGGGCCCTGGCCCAGGCAGGCCGTGGTG
AAGACAGGAGCCCGCCCACAGCACTCCATCACCATCACCATCAC
tga