

University of Dundee

Standard Operating Procedure

Preparation of active MNK2 alpha [2 - 465]

<u>Enzyme description:-</u>	MNK2 alpha [2 - 465]
<u>Clone number:-</u>	DU 1214
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal GST and C-terminal His(6)
<u>Purification method:-</u>	GSH Sepharose
<u>Expression level:-</u>	2 mg/L
<u>Calculated molecular mass:-</u>	79, 339 daltons
<u>Purity:-</u>	90 %

Activation protocol:-

MNK2 alpha (2.5 μ M) is activated in 50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM magnesium acetate, 0.1 mM ATP with 100 μ g/ml GST-SAPK2a [DU 979] at 30 °C for 30 mins. Following activation, MNK2 alpha is re-purified by Ni²⁺-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

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:- 25 – 50 U/mg

University of Dundee

Clone Data Sheet - MNK2 alpha [2 - 465]

Protein MNK2 alpha [2 - 465]

Clone number DU 1214

Species Human

Accession number AF237775

Tags N-terminal GST and C-terminal His(6)

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLEGA
VLDIRYGVSR IAYS KDFETLKVDFLSKLP EMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLVFOGPLG**SVQKKPAELQGFHRS**
FKGQNPFELAFSLDQPDHGDSDFGLQCSARPDMPASQPIDIPDAK**KR**GK
KKKRGRATDSFSGRFEDVYQLQEDVLGEGAHARVQTCINLITSQ**EYAVK**
IIEKQPGHIRSRVFREVEMLYQ**CQ**HRNVLELIEFFEEEDRFYLV**FEKM**
RGSILSHIHKRRHFNELEASVVVQDVASALDFLHNKGIAHRDLKPENI
LCEHPNQVSPVKICDFDLGSGIKLNGDCSP**I**STPELLTPCGSAEYMAPE
VVEAFSEEAS**I**YDKRCDLWSLGVILYILLSGYPPFVGRCGSDCGWDRGE
ACPACQ**N**MLFESIQEGKYEPDKDWAHISCAAKDLISKLLVRDAK**Q**RLS
AAQVLQHPWVQGCAPENTLPTPMVLQ**R**NSCAKDLTSFAAEAIAMNR**Q**LA
QHEDLAE**E**EAAGQGPVLRATSRCLQ**L**SPPSQSKLAQR**R**Q**R**ASLSSA
PVVLVGDHAHHHHHH

Native sequence Amino acids V2 – A465 (end) of human MNK2 alpha.
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 696 – 701.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Bam*H1 and *Eco*R1 site of pGEX 6P-1

University of Dundee

Nucleotide
Sequence of insert

ggatccGTGCAGAAGAAACCAGCCGAACTTCAGGGTTTCCACCGTTTCGT
TCAAGGGGCAGAACCCCTTCGAGCTGGCCTTCTCCCTAGACCAGCCCGA
CCACGGAGACTCTGACTTTGGCCTGCAGTGCTCAGCCCGCCCTGACATG
CCCGCCAGCCAGCCATTGACATCCCGGACGCCAAGAAGAGGGGCAAGA
AGAAGAAGCGCGGCCGGGCCACCGACAGCTTCTCGGGCAGGTTTGAAGA
CGTCTACCAGCTGCAGGAAGATGTGCTGGGGGAGGGCGCTCATGCCCGA
GTGCAGACCTGCATCAACCTGATCACCAGCCAGGAGTACGCCGTCAAGA
TCATTGAGAAGCAGCCAGGCCACATTCGGAGCAGGGTTTTTCAGGGAGGT
GGAGATGCTGTACCAGTGCCAGGGACACAGGAACGTCTTAGAGCTGATT
GAGTTCTTCGAGGAGGAGGACCGCTTCTACCTGGTGTGTTGAGAAGATGC
GGGAGGGCTCCATCCTGAGCCACATCCACAAGCGCCGGCAGTTCAACGA
GCTGGAGGCCAGCGTGGTGGTGCAGGACGTGGCCAGCGCCTTGACTTT
CTGCATAACAAAGGCATCGCCACAGGGACCTAAAGCCGGAAAACATCC
TCTGTGAGCACCCCAACCAGGTCTCCCCGTGAAGATCTGTGACTTCGA
CCTGGGCAGCGGCATCAAACCTAACGGGGACTGCTCCCTATCTCCACC
CCGGAGCTGCTCACTCCGTGCGGCTCGGCGGAGTACATGGCCCCGGAGG
TAGTGGAGGCCTTCAGCGAGGAGGCTAGCATCTACGACAAGCGCTGCGA
CCTGTGGAGCCTGGGCGTCATCTTGTATATCTACTCAGCGGCTACCCG
CCCTTCGTGGGCCGCTGTGGCAGCGACTGCGGCTGGGACCGCGGCGAGG
CCTGCCCTGCCTGCCAGAACATGCTGTTTGAGAGCATCCAGGAGGGCAA
GTACGAGTTCCCCGACAAGGACTGGGCCACATCTCCTGCGCTGCCAAA
GACCTCATCTCCAAGCTGCTGGTCCGTGACGCCAAGCAGAGGCTGAGTG
CCGCCAAGTCCTGCAGCACCCCTGGGTTTCAGGGGTGCGCCCCGGAGAA
CACCTTGCCCACTCCCATGGTCTGCAGAGGAACAGCTGTGCCAAAGAC
CTCACGTCCTTCGCGGCTGAGGCCATTGCCATGAACCGGCAGCTGGCCC
AGCACGACGAGGACCTGGCTGAGGAGGAGGCCGCGGGGCAGGGCCAGCC
CGTCTGGTCCGAGCTACCTCACGCTGCCTGCAGCTGTCTCCACCCTCC
CAGTCCAAGCTGGCGCAGCGGCGCAAAGGGCCAGTCTGTCTCGGCC
CAGTGGTCCTGGTGGGAGACCACGCCACCATCACCATCACCATTGAGA
ATTC