MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active TNK2 [1 - 389]

Enzyme description:- TNK2 [1 – 389]

Clone number:- DU 60935

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 71, 334.53 daltons
Average Mass 71, 380.55 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.03

Purity:- >75 %

Activation protocol:- Constitutively Active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 10 mM DTT, 10 mM magnesium acetate, 0. 1mM EGTA

Substrate:-
EAIYAAPFAKKK Final concentration: 300 μM

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Clone Data Sheet

TNK2 [1 – 389]

Protein	TNK2 [1 – 389]
Clone number	DU 60935
Species	Human
Accession number	NM_005781
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**MQPEEGTGW LLELLSEVQLQQYFLRLRDDLNVTRLSHFEYVKNEDLEKIGMGRPGQR RLWEAVKRRKALCKRKSWMSKVFSGKRLEAEFPPHHSQSTFRKTSPAP GGPAGEGPLQSLTCLIGEKDLRLLEKLGDGSFGVVRRGEWDAPSGKTV SVAVKCLKPDVLSQPEAMDDFIREVNAMHSLDHRNLIRLYGVVLTPPM KMVTELAPLGSLLDRLRKHQGHFLLGTLSRYAVQVAEGMGYLESKRFI HRDLAARNLLLATRDLVKIGDFGLMRALPQNDDHYVMQEHRKVPFAWC APESLKTRTFSHASDTWMFGVTLWEMFTYGQEPWIGLNGSQILHKIDK EGERLPRPEDCPQDIYNVMVQCWAHKPEDRPTFVALRDFLLEAQ**
Native sequence	Amino acids M1 – Q389 (end residue is R1038) of human TNK2. Residue M232 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 - *Not*1 sites of pFastBac GST

Nucleotide sequence of insert

ggatccATGCAGCCAGAGGAGGGCACAGGCTGGCTGCTGGAGCTGCTG
TCCGAGGTGCAGCTGCAACAGTACTTCCTGCGGCTCCGAGATGACCTC
AACGTCACCCGCCTGTCCCACTTTGAGTACGTCAAGAATGAGGACCTG
GAGAAGATCGGCATGGGTCGGCCTGGCCAGCGGCGGCTGTGGGAGGCT
GTGAAGAGGAGGAAGGCCTTGTGCAAACGCAAGTCGTGGATGAGTAAG
GTGTTCAGTGGAAAGCGACTGGAGGCTGAGTTCCCACCTCATCACTCT
CAGAGCACCTTCCGGAAGACCTCGCCCGCCCCTGGGGGCCCAGCAGGG
GAGGGGCCCCTGCAGAGCCTCACCTGCCTCATTGGGGAGAAGGACCTG
CGCCTCCTGGAGAAGCTGGGTGATGGTTCCTTTGGCGTGGTGCGCAGG
GGCGAGTGGGACGCGCCCTCAGGGAAGACGGTGAGTGTGGCTGTGAAG
TGCCTGAAGCCCGATGTCCTGAGCCAGCCAGAAGCCATGGACGACTTC
ATCCGGGAGGTCAATGCCATGCACTCGCTCGACCACCGAAACCTCATC
CGCCTCTACGGGGTGGTGCTCACGCCGCCCATGAAGATGGTGACAGAG
CTGGCACCTCTGGGATCGTTGTTGGACCGGCTACGTAAGCACCAGGGC
CACTTCCTCCTGGGGACTCTGAGCCGCTACGCTGTGCAGGTGGCTGAG
GGCATGGGCTACCTGGAGTCCAAGCGCTTTATTCACCGTGACCTGGCT
GCCCGCAATCTGCTGTTGGCTACCCGCGACCTGGTCAAGATCGGGGAC
TTTGGGCTGATGCGAGCACTACCTCAGAATGACGACCATTACGTCATG
CAGGAACATCGCAAGGTGCCCTTCGCCTGGTGTGCCCCCGAGAGCCTG
AAGACACGCACCTTCTCCCATGCCAGCGACACCTGGATGTTCGGGGTG
ACACTGTGGGAAATGTTCACCTACGGCCAGGAGCCCTGGATCGGCCTC
AACGGCAGTCAGATCCTGCATAAGATCGACAAGGAGGGGGAGCGGCTG
CCCCGGCCCGAGGACTGTCCCCAGGACATCTACAACGTCATGGTCCAG
TGCTGGGCTCACAAGCCAGAGGACAGACCCACGTTTGTGGCCCTGCGG
GACTTCCTGCTGGAGGCCCAGtgagcggccgc