MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active PKN1 [1 - 948]

Enzyme description:- PKN1 [1 – 948]

Clone number:- DU 62981

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 132, 077.79 daltons
Average Mass 132, 160.73 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.79

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:-
KKLNRTLSVA Final concentration: 300 uM

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Clone Data Sheet

PKN1 [1 – 948]

Protein PKN1 [1 – 948]

Clone number DU 62981

Species Human

Accession number Q16512-2

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVE**MAE**
ANNPSEQELESEPRSWSLLEQLGLAGADLAAPGVQQQLELERERLRRE
IRKELKLKEGAENLRRATTDLGRSLGPVELLLRGSSRRLDLLHQQLQE
LHAHVVLPDPAATHDGPQSPGAGGPTCSATNLSRVAGLEKQLAIELKV
KQGAENMIQTYSNGSTKDRKLLLTAQQMLQDSKTKIDIIRMQLRRALQ
AGQLENQAAPDDTQGSPDLGAVELRIEELRHHFRVEHAVAEGAKNVLR
LLSAAKAPDRKAVSEAQEKLTESNQKLGLLREALERRLGELPADHPKG
RLLREELAAASSAAFSTRLAGPFPATHYSTLCKPAPLTGTLEVRVVGC
RDLPETIPWNPTPSMGGPGTPDSRPPFLSRPARGLYSRSGSLSGRSSL
KAEAENTSEVSTVLKLDNTVVGQTSWKPCGPNAWDQSFTLELERAREL
ELAVFWRDQRGLCALKFLKLEDFLDNERHEVQLDMEPQGCLVAEVTFR
NPVIERIPRLRRQKKIFSKQQGKAFQRARQMNIDVATWVRLLRRLIPN
ATGTGTFSPGASPGSEARTTGDISVEKLNLGTDSDSSPQKSSRDPPSS
PSSLSSPIQESTAPELPSETQETPGPALCSPLRKSPLTLEDFKFLAVL
GRGHFGKVLLSEFRPSGELFAIKALKKGDIVARDEVESLMCEKRILAA
VTSAGHPFLVNLFGCFQTPEHVCFVMEYSAGGDLMLHIHSDVFSEPRA
IFYSACVVLGLQFLHEHKIVYRDLKLDNLLLDTEGYVKIADFGLCKEG
MGYGDRTSTFCGTPEFLAPEVLTDTSYTRAVDWWGLGVLLYEMLVGES
PFPGDDEEEVFDSIVNDEVRYPHFLSAEAIGIMRRLLRRNPERRLGSS
ERDAEDVKKQPFFRTLGWEALLARRLPPPFVPTLSGRTDVSNFDEEFT
GEAPTLSPPRDARPLTAAEQAAFLDFDFVAGGC

Native sequence Amino acids M1 – C948 (end) of human PKN1.
Residue M238 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 *Xho*1 - *Not*1 into *Sal*1 - *Not*1 sites of pFastBac GST

Nucleotide sequence of insert

gtcgagATGGCGGAGGCCAATAACCCCTCGGAGCAGGAGCTGGAGAGTGAGCC
TCGCAGCTGGTCCCTGCTAGAGCAGCTGGGCCTGGCCGGGGCAGACCTGGCGG
CCCCCGGGGTACAGCAGCAGCTGGAGCTGGAGCGGGAGCGGCTGCGGCGGGAA
ATCCGCAAGGAGCTGAAGCTGAAGGAGGGTGCTGAGAACCTGCGGCGGGCCAC
CACTGACCTGGGCCGCAGCCTGGGCCCCGTAGAGCTGCTGCTGCGGGGCTCCT
CGCGCCGCCTCGACCTGCTGCACCAGCAGCTGCAGGAGCTGCACGCCCACGTG
GTGCTTCCCGACCCGGCGGCCACCCACGATGGCCCCCAGTCCCCTGGTGCGGG
TGGCCCCACCTGCTCGGCCACCAACCTGAGCCGCGTGGCGGGCCTGGAGAAGC
AGTTGGCCATTGAGCTGAAGGTGAAGCAGGGGGCGGAGAACATGATCCAGACC
TACAGCAATGGCAGCACCAAGGACCGGAAGCTGCTGCTGACAGCCCAGCAGAT
GTTGCAGGACAGTAAGACCAAGATTGACATCATCCGCATGCAACTCCGCCGGG
CGCTGCAGGCCGGCCAGCTGGAGAACCAGGCAGCCCCGGATGACACCCAAGGG
AGTCCTGACCTGGGGGCTGTGGAGCTGCGCATCGAAGAGCTGCGGCACCACTT
CCGAGTGGAGCACGCGGTGGCCGAGGGTGCCAAGAACGTACTGCGCCTGCTCA
GCGCTGCCAAGGCCCCGGACCGCAAGGCAGTCAGCGAGGCCCAGGAGAAATTG
ACAGAATCCAACCAGAAGCTGGGGCTGCTGCGGGAGGCTCTGGAGCGGAGACT
TGGGGAGCTGCCCGCCGACCACCCCAAGGGGCGGCTGCTGCGAGAAGAGCTCG
CTGCGGCCTCCTCCGCTGCCTTCAGCACCCGCCTGGCCGGGCCCTTTCCCGCC
ACGCACTACAGCACCCTGTGCAAGCCCGCGCCGCTCACAGGGACCCTGGAGGT
ACGAGTGGTGGGCTGCAGAGACCTCCCAGAGACCATCCCGTGGAACCCTACCC
CCTCAATGGGGGGACCTGGGACCCCAGACAGCCGCCCCCCCTTCCTGAGCCGC
CCAGCCCGGGGCCTTTACAGCCGAAGCGGAAGCCTCAGTGGCCGGAGCAGCCT
CAAAGCAGAAGCCGAGAACACCAGTGAAGTCAGCACTGTGCTTAAGCTGGATA
ACACAGTGGTGGGGCAGACGTCTTGGAAGCCATGTGGCCCCAATGCCTGGGAC
CAGAGCTTCACTCTGGAGCTGGAAAGGGCACGGGAACTGGAGTTGGCTGTGTT
CTGGCGGGACCAGCGGGGCCTGTGTGCCCTCAAATTCCTAAAGTTGGAGGATT
TCTTGGACAATGAGAGGCATGAGGTGCAGCTGGACATGGAACCCCAGGGCTGC
CTGGTGGCTGAGGTCACCTTCCGCAACCCTGTCATTGAGAGGATTCCTCGGCT
CCGACGGCAGAAGAAAATTTTCTCCAAGCAGCAAGGGAAGGCGTTCCAGCGTG
CTAGGCAGATGAACATCGATGTCGCCACGTGGGTGCGGCTGCTCCGGAGGCTC
ATCCCCAATGCCACGGGCACAGGCACCTTTAGCCCTGGGGCTTCTCCAGGATC
CGAGGCCCGGACCACGGGTGACATATCGGTGGAGAAGCTGAACCTCGGCACTG
ACTCGGACAGCTCACCTCAGAAGAGCTCGCGGGATCCTCCTTCCAGCCCATCG
AGCCTGAGCTCCCCCATCCAGGAATCCACTGCTCCCGAGCTGCCTTCGGAGAC
CCAGGAGACCCCAGGCCCCGCCCTGTGCAGCCCTCTGAGGAAGTCACCTCTGA
CCCTCGAAGATTTCAAGTTCCTGGCGGTGCTGGGCCGGGGTCATTTTGGGAAG
GTGCTCCTCTCCGAATTCCGGCCCAGTGGGGAGCTGTTCGCCATCAAGGCTCT
GAAGAAAGGGGACATTGTGGCCCGAGACGAGGTGGAGAGCCTGATGTGTGAGA
AGCGGATATTGGCGGCAGTGACCAGTGCGGGACACCCCTTCCTGGTGAACCTC
TTCGGCTGTTTCCAGACACCGGAGCACGTGTGCTTCGTGATGGAGTACTCGGC
CGGTGGGGACCTGATGCTGCACATCCACAGCGACGTGTTCTCTGAGCCCCGTG
CCATCTTTTATTCCGCCTGCGTGGTGCTGGGCCTACAGTTTCTTCACGAACAC
AAGATCGTCTACAGGGACCTGAAGTTGGACAATTTGCTCCTGGACACCGAGGG
CTACGTCAAGATCGCAGACTTTGGCCTCTGCAAGGAGGGGATGGGCTATGGGG
ACCGGACCAGCACATTCTGTGGGACCCCGGAGTTCCTGGCCCCTGAGGTGCTG
ACGGACACGTCGTACACGCGAGCTGTGGACTGGTGGGGACTGGGTGTGCTGCT
CTACGAGATGCTGGTTGGCGAGTCCCCATTCCCAGGGGATGATGAGGAGGAGG
TCTTCGACAGCATCGTCAACGATGAGGTTCGCTACCCCCACTTCCTGTCGGCC
GAAGCCATCGGCATCATGAGAAGGCTGCTTCGGAGGAACCCAGAGCGGAGGCT
GGGATCTAGCGAGAGAGATGCAGAAGATGTGAAGAAACAGCCCTTCTTCAGGA
CTCTGGGCTGGGAAGCCCTGTTGGCCCGGCGCCTGCCACCGCCCTTTGTGCCC
ACGCTGTCCGGCCGCACCGACGTCAGCAACTTCGACGAGGAGTTCACCGGGGA
GGCCCCCACACTGAGCCCGCCCCGCGACGCGCGGCCCCTCACAGCCGCGGAGC
AGGCAGCCTTCCTGGACTTCGACTTCGTGGCCGGGGGCTGCtaggcggccgc