MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active PKN1 [460 - 942]

Enzyme description:- PKN1 [460 – 942]

Clone number:- DU 68070

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 81, 376.31 daltons
Average Mass 81, 428.17 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.40

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 [460 – 942]

Protein PKN1 [460 – 942]

Clone number DU 68070

Species Human

Accession number Q16512-2

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVEM**DN**
ERHEVQLDMEPQGCLVAEVTFRNPVIERIPRLRRQKKIFSKQQGKAFQ
RARQMNIDVATWVRLLRRLIPNATGTGTFSPGASPGSEARTTGDISVE
KLNLGTDSDSSPQKSSRDPPSSPSSLSSPIQESTAPELPSETQETPGP
ALCSPLRKSPLTLEDFKFLAVLGRGHFGKVLLSEFRPSGELFAIKALK
KGDIVARDEVESLMCEKRILAAVTSAGHPFLVNLFGCFQTPEHVCFVM
EYSAGGDLMLHIHSDVFSEPRAIFYSACVVLGLQFLHEHKIVYRDLKL
DNLLLDTEGYVKIADFGLCKEGMGYGDRTSTFCGTPEFLAPEVLTDTS
YTRAVDWWGLGVLLYEMLVGESPFPGDDEEEVFDSIVNDEVRYPRFLS
AEAIGIMRRLLRRNPERRLGSSERDAEDVKKQPFFRTLGWEALLARRL
PPPFVPTLSGRTDVSNFDEEFTGEAPTLSPPRDARPLTAAEQAAFLDF
D

Native sequence Amino acids D460 – D942 (end residue C948) of human PKN1. Residue D239 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

gacaatgagaggcatgAGGTGCAGCTGGACATGGAACCCCAGGGCTGC
CTGGTGGCTGAGGTCACCTTCCGCAACCCTGTCATTGAGAGGATTCCT
CGGCTCCGACGGCAGAAGAAAATTTTCTCCAAGCAGCAAGGGAAGGCG
TTCCAGCGTGCTAGGCAGATGAACATCGATGTCGCCACGTGGGTGCGG
CTGCTCCGGAGGCTCATCCCCAATGCCACGGGCACAGGCACCTTTAGC
CCTGGGGCTTCTCCAGGATCCGAGGCCCGGACCACGGGTGACATATCG
GTGGAGAAGCTGAACCTCGGCACTGACTCGGACAGCTCACCTCAGAAG
AGCTCGCGGGATCCTCCTTCCAGCCCATCGAGCCTGAGCTCCCCCATC
CAGGAATCCACTGCTCCCGAGCTGCCTTCGGAGACCCAGGAGACCCCA
GGCCCCGCCCTGTGCAGCCCTCTGAGGAAGTCACCTCTGACCCTCGAA
GATTTCAAGTTCCTGGCGGTGCTGGGCCGGGGTCATTTTGGGAAGGTG
CTCCTCTCCGAATTCCGGCCCAGTGGGGAGCTGTTCGCCATCAAGGCT
CTGAAGAAAGGGGACATTGTGGCCCGAGACGAGGTGGAGAGCCTGATG
TGTGAGAAGCGGATATTGGCGGCAGTGACCAGTGCGGGACACCCCTTC
CTGGTGAACCTCTTCGGCTGTTTCCAGACACCGGAGCACGTGTGCTTC
GTGATGGAGTACTCGGCCGGTGGGGACCTGATGCTGCACATCCACAGC
GACGTGTTCTCTGAGCCCCGTGCCATCTTTTATTCCGCCTGCGTGGTG
CTGGGCCTACAGTTTCTTCACGAACACAAGATCGTCTACAGGGACCTG
AAGTTGGACAATTTGCTCCTGGACACCGAGGGCTACGTCAAGATCGCA
GACTTTGGCCTCTGCAAGGAGGGGATGGGCTATGGGGACCGGACCAGC
ACATTCTGTGGGACCCCGGAGTTCCTGGCCCCTGAGGTGCTGACGGAC
ACGTCGTACACGCGAGCTGTGGACTGGTGGGGACTGGGTGTGCTGCTC
TACGAGATGCTGGTTGGCGAGTCCCCATTCCCAGGGGATGATGAGGAG
GAGGTCTTCGACAGCATCGTCAACGACGAGGTTCGCTACCCCCGCTTC
CTGTCGGCCGAAGCCATCGGCATCATGAGAAGGCTGCTTCGGAGGAAC
CCAGAGCGGAGGCTGGGATCTAGCGAGAGAGATGCAGAAGATGTGAAG
AAACAGCCCTTCTTCAGGACTCTGGGCTGGGAAGCCCTGTTGGCCCGG
CGCCTGCCACCGCCCTTTGTGCCCACGCTGTCCGGCCGCACCGACGTC
AGCAACTTCGACGAGGAGTTCACCGGGGAGGCCCCCACACTGAGCCCG
CCCCGCGACGCGCGGCCCCTTACAGCCGCGGAGCAGGCAGCCTTCCTG
GACTTCGACtgagcggccgc