MRC PPU REAGENTS

Standard Operating Procedure

Preparation of active PYK2 [2 – 1009]

Enzyme description:- PYK2 [2 - 1009]

Clone number:- DU 1019

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Calculated molecular mass:-
Monoisotopic 119, 382.69 daltons
Average Mass 119, 459.41 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.84

Purity:- 85 %

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, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 5 mM $MnCl_2$

Substrate:-
Poly Glu Tyr (4:1) Final concentration: 0.1 mg/ml

MRC PPU REAGENTS

Clone Data Sheet

PYK2 [2 - 1009]

Protein	PYK2 [2 - 1009]
Clone number	DU 1109
Species	Human
Accession number	NM_173176.2
Tags	N-terminal His(6)
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMDPEF**SGVSEPLSRVKLGTLRRPE** **GPAEPMVVVPVDVEKEDVRILKVCFYSNSFNPGKNFKLVKCTVQTEIRE** **IITSILLSGRIGPNIRLAECYGLRLKHMKSDEIHWLHPQMTVGEVQDKY** **ECLHVEAEWRYDLQIRYLPEDFMESLKEDRTTLLYFYQQLRNDYMQRYA** **SKVSEGMALQLGCLELRRFFKDMPHNALDKKSNFELLEKEVGLDLFFPK** **QMQENLKPKQFRKMIQQTFQQYASLREEECVMKFFNTLAGFANIDQETY** **RCELIQGWNITVDLVIGPKGIRQLTSQDAKPTCLAEFKQIRSIRCLPLE** **EGQAVLQLGIEGAPQALSIKTSSLAEAENMADLIDGYCRLQGEHQGSLI** **IHPRKDGEKRNSLPQIPMLNLEARRSHLSESCSIESDIYAEIPDETLRR** **PGGPQYGIAREDVVLNRILGEGFFGEVYEGVYTNHKGEKINVAVKTCKK** **DCTLDNKEKFMSEAVIMKNLDHPHIVKLIGIIEEEPTWIIMELYPYGEL** **GHYLERNKNSLKVLTLVLYSLQICKAMAYLESINCVHRDIAVRNILVAS** **PECVKLGDFGLSRYIEDEDYYKASVTRLPIKWMSPESINFRRFTTASDV** **WMFAVCMWEILSFGKQPFFWLENKDVIGVLEKGDRLPKPDLCPPVLYTL** **MTRCWDYDPSDRPRFTELVCSLSDVYQMEKDIAMEQERNARYRTPKILE** **PTAFQEPPPKPSRPKYRPPPQTNLLAPKLQFQVPEGLCASSPTLTSPME** **YPSPVNSLHTPPLHRHNVFKRHSMREEDFIQPSSREEAQQLWEAEKVKM** **RQILDKQQKQMVEDYQWLRQEEKSLDPMVYMNDKSPLTPEKEVGYLEFT** **GPPQKPPRLGAQSIQPTANLDRTDDLVYLNVMELVRAVLELKNELCQLP** **PEGYVVVVKNVGLTLRKLIGSVDDLLPSLPSSSRTEIEGTQKLLNKDLA** **ELINKMRLAQQNAVTSLSEECKRQMLTASHTLAVDAKNLLDAVDQAKVL** **ANLAHPPAE**
Native sequence	Amino acids S2 – E1009 (end) of human PYK2. Residue S31 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQ) residues 18 - 24
Cloning sites	*Eco*R1 and *Xba*1 sites of pFastBac.Hta

Nucleotide Sequence Of Insert

```
gaattcTCTGGGGTGTCCGAGCCCCTGAGTCGAGTAAAGTTGGGCACGTT
ACGCCGGCCTGAAGGCCCTGCAGAGCCCATGGTGGTGGTACCAGTAGATG
TGGAAAAGGAGGACGTGCGTATCCTCAAGGTCTGCTTCTATAGCAACAGC
TTCAATCCTGGGAAAAACTTCAAACTGGTCAAATGCACTGTCCAGACGGA
GATCCGGGAGATCATCACCTCCATCCTGCTGAGCGGGCGGATCGGGCCCA
ACATCCGGTTGGCTGAGTGCTATGGGCTGAGGCTGAAGCACATGAAGTCC
GATGAGATCCACTGGCTGCACCCACAGATGACAGTGGGTGAGGTGCAGGA
CAAGTATGAGTGTCTGCACGTGGAAGCCGAGTGGAGGTATGACCTTCAAA
TCCGCTACTTGCCAGAAGACTTCATGGAGAGCCTGAAGGAGGACAGGACC
ACGCTGCTCTATTTTTACCAACAGCTCCGGAACGACTACATGCAGCGCTA
CGCCAGCAAGGTCAGCGAGGGCATGGCCCTGCAGCTGGGCTGCCTGGAGC
TCAGGCGGTTCTTCAAGGATATGCCCCACAATGCACTTGACAAGAAGTCC
AACTTCGAGCTCCTAGAAAAGGAAGTGGGGCTGGACTTGTTTTTCCCAAA
GCAGATGCAGGAGAACTTAAAGCCCAAACAGTTCCGGAAGATGATCCAGC
AGACCTTCCAGCAGTACGCCTCGCTCAGGGAGGAGGAGTGCGTCATGAAG
TTCTTCAACACTCTCGCCGGCTTCGCCAACATCGACCAGGAGACCTACCG
CTGTGAACTCATTCAAGGATGGAACATTACTGTGGACCTGGTCATTGGCC
CTAAAGGGATCCGCCAGCTGACTAGTCAGGACGCAAAGCCCACCTGCCTG
GCCGAGTTCAAGCAGATCAGGTCCATCAGGTGCCTCCCGCTGGAGGAGGG
CCAGGCAGTACTTCAGCTGGGCATTGAAGGTGCCCCCCAGGCCTTGTCCA
TCAAAACCTCATCCCTAGCAGAGGCTGAGAACATGGCTGACCTCATAGAC
GGCTACTGCCGGCTGCAGGGTGAGCACCAAGGCTCTCTCATCATCCATCC
TAGGAAAGATGGTGAGAAGCGGAACAGCCTGCCCCAGATCCCCATGCTAA
ACCTGGAGGCCCGGCGGTCCCACCTCTCAGAGAGCTGCAGCATAGAGTCA
GACATCTACGCAGAGATTCCCGACGAAACCCTGCGAAGGCCCGGAGGTCC
ACAGTATGGCATTGCCCGTGAAGATGTGGTCCTGAATCGTATTCTTGGGG
AAGGCTTTTTTGGGGAGGTCTATGAAGGTGTCTACACAAATCATAAAGGG
GAGAAAATCAATGTAGCTGTCAAGACCTGCAAGAAAGACTGCACTCTGGA
CAACAAGGAGAAGTTCATGAGCGAGGCAGTGATCATGAAGAACCTCGACC
ACCCGCACATCGTGAAGCTGATCGGCATCATTGAAGAGGAGCCCACCTGG
ATCATCATGGAATTGTATCCCTATGGGGAGCTGGGCCACTACCTGGAGCG
GAACAAGAACTCCCTGAAGGTGCTCACCCTCGTGCTGTACTCACTGCAGA
TATGCAAAGCCATGGCCTACCTGGAGAGCATCAACTGCGTGCACAGGGAC
ATTGCTGTCCGGAACATCCTGGTGGCCTCCCCTGAGTGTGTGAAGCTGGG
GGACTTTGGTCTTTCCCGGTACATTGAGGACGAGGACTATTACAAAGCCT
CTGTGACTCGTCTCCCCATCAAATGGATGTCCCCAGAGTCCATTAACTTC
CGACGCTTCACGACAGCCAGTGACGTCTGGATGTTCGCCGTGTGCATGTG
GGAGATCCTGAGCTTTGGGAAGCAGCCCTTCTTCTGGCTGGAGAACAAGG
ATGTCATCGGGGTGCTGGAGAAAGGAGACCGGCTGCCCAAGCCTGATCTC
TGTCCACCGGTCCTTTATACCCTCATGACCCGCTGCTGGGACTACGACCC
CAGTGACCGGCCCCGCTTCACCGAGCTGGTGTGCAGCCTCAGTGACGTTT
ATCAGATGGAGAAGGACATTGCCATGGAGCAAGAGAGGAATGCTCGCTAC
CGAACCCCCAAAATCTTGGAGCCCACAGCCTTCCAGGAACCCCCACCCAA
GCCGAGCCGACCTAAGTACAGACCCCCTCCGCAAACCAACCTCCTGGCTC
CAAAGCTGCAGTTCCAGGTTCCTGAGGGTCTGTGTGCCAGCTCTCCTACG
CTCACCAGCCCTATGGAGTATCCATCTCCCGTTAACTCACTGCACACCCC
ACCTCTCCACCGGCACAATGTCTTCAAACGCCACAGCATGCGGGAGGAGG
ACTTCATCCAACCCAGCAGCCGAGAAGAGGCCCAGCAGCTGTGGGAGGCT
GAAAAGGTCAAAATGCGGCAAATCCTGGACAAACAGCAGAAGCAGATGGT
GGAGGACTACCAGTGGCTCAGGCAGGAGGAGAAGTCCCTGGACCCCATGG
TTTATATGAATGATAAGTCCCCATTGACGCCAGAGAAGGAGGTCGGCTAC
CTGGAGTTCACAGGGCCCCCACAGAAGCCCCCGAGGCTGGGCGCACAGTC
CATCCAGCCCACAGCTAACCTGGACCGGACCGATGACCTGGTGTACCTCA
ATGTCATGGAGCTGGTGCGGGCCGTGCTGGAGCTCAAGAATGAGCTCTGT
```

CAGCTGCCCCCCGAGGGCTACGTGGTGGTGGTGAAGAATGTGGGGCTGAC
CCTGCGGAAGCTCATCGGGAGCGTGGATGATCTCCTGCCTTCCTTGCCGT
CATCTTCACGGACAGAGATCGAGGGCACCCAGAAACTGCTCAACAAAGAC
CTGGCAGAGCTCATCAACAAGATGCGGCTGGCGCAGCAGAACGCCGTGAC
CTCCCTGAGTGAGGAGTGCAAGAGGCAGATGCTGACGGCTTCACACACCC
TGGCTGTGGACGCCAAGAACCTGCTCGACGCTGTGGACCAGGCCAAGGTT
CTGGCCAATCTGGCCCACCCACCTGCAGAGtgatctaga