MRC PPU REAGENTS

Standard Operating Procedure

Preparation of MINDY1 [1 - 469]

Enzyme description:- MINDY1 [1 – 469]

Clone number:- DU 59325

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal MBP

Purification method:- Amylose resin

Calculated molecular mass:-

Monoisotopic 95, 984.03 daltons
Average Mass 96, 044.08 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 4.81

Purity:- >80 %

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

MRC PPU REAGENTS

Clone Data Sheet

MINDY1 [1 – 469]

Protein MINDY1 [1 – 469]

Clone number DU 59325

Species Human

Accession number Q8N5J2.2

Tags N-terminal MBP

Bacterially expressed protein

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF
PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWD
AVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKG
KSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAG
LTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSK
VNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTD
EGLEAVNKDKPLGAVALKSYEEELVKDPRIAATMENAQKGEIMPNIPQ
MSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGD
DDDKVPEFLEVLFQGPLGS**MEYHQPEDPAPGKAGTAEAVIPENHEVLA
GPDEHPQDTDARDADGEARELREPADQALLPSQCGDNLESPLPEASSAP
PGPTLGTLPEVETIRACSMPQELPQSPRTRQPEPDFYCVKWIPWKGEQ
TPIITQSTNGPCPLLAIMNILFLQWKVKLPPQKEVITSDELMAHLGNC
LLSIKPQEKSEGLQLNFQQNVDDAMTVLPKLATGLDVNVRFTGVSDFE
YTPECSVFDLLGIPLYHGWLVDPQSPEAVRAVGKLSYNQLVERIITCK
HSSDTNLVTEGLIAEQFLETTAAQLTYHGLCELTAAAKEGELSVFFRN
NHFSTMTKHKSHLYLLVTDQGFLQEEQVVWESLHNVDGDSCFCDSDFH
LSHSLGKGPGAEGGSGSPEKQLQVDQDYLIALSLQQQQPRGPLGLTDL
ELAQQLQQEEYQQQQAAQPVRMRTRVLSLQGRGATSGRPAGERRQRPK
HESDCILL**

Native sequence Amino acids M1 – L469 (end) of human MINDY1.
Residue M404 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 – 392.

Protease cleavage PreScission (LEVLFQGP) residues 393 – 400

Cloning sites *Blg*II / *Not*1 into *Bam*H1 / *Not*1 sites of pMEX3C

Nucleotide Sequence Of Insert

agatctATGGAATACCATCAGCCTGAGGATCCAGCCCCTGGTAAGGCCGGGACTGCAGAAGCAGT
CATCCCTGAAAACCATGAGGTTCTGGCAGGCCCAGATGAGCACCCTCAGGACACAGATGCAAGAG
ATGCTGATGGGGAGGCTAGAGAACGGGAGCCAGCAGACCAAGCTTTGCTGCCTAGCCAGTGTGGG
GACAACCTTGAGTCCCCTCTGCCTGAAGCTAGCTCAGCTCCACCGGGGCCAACCCTTGGGACACT
GCCTGAAGTAGAGACAATAAGGGCATGCTCCATGCCCCAGGAGCTTCCTCAGTCCCCCAGGACCC
GACAGCCTGAGCCAGATTTCTACTGTGTCAAGTGGATCCCTTGGAAAGGAGAACAGACACCCATC
ATCACCCAGAGCACTAACGGCCCTTGCCCTCTCCTTGCCATCATGAACATCCTCTTTCTTCAGTG
GAAGGTGAAGCTCCCCCCGCAGAAGGAAGTGATCACATCGGATGAGCTCATGGCCCATCTTGGAA
ACTGCCTCCTGTCCATCAAGCCCCAGGAGAAGTCAGAGGGACTTCAGCTTAATTTTCAGCAGAAT
GTGGATGATGCAATGACAGTGCTGCCTAAACTGGCCACAGGTCTGGATGTCAATGTGCGATTCAC
AGGCGTCTCTGATTTTGAGTATACACCCGAGTGCAGTGTCTTTGACCTGCTAGGCATACCTCTGT
ACCATGGCTGGCTTGTTGATCCACAGAGTCCTGAGGCTGTGCGTGCAGTTGGGAAACTGAGTTAC
AACCAGCTGGTGGAGAGGATCATCACCTGCAAACACTCCAGTGACACCAACCTCGTGACAGAAGG
CCTGATTGCAGAGCAGTTCCTGGAGACCACCGCGGCCCAGCTGACCTACCACGGACTGTGTGAGC
TGACAGCAGCTGCTAAGGAGGGTGAACTTAGCGTCTTTTTCCGAAACAACCACTTTAGCACCATG
ACTAAGCATAAGAGTCACTTATACCTACTGGTCACTGACCAGGGCTTTCTACAGGAGGAGCAAGT
CGTATGGGAGAGCCTGCACAATGTGGATGGAGACAGCTGCTTTTGTGACTCTGACTTTCACCTGA
GTCATTCCCTGGGCAAGGGGCCTGGAGCAGAAGGTGGGAGTGGCTCCCCAGAAAAGCAGCTGCAG
GTAGACCAGGACTACCTGATTGCTCTGTCCCTGCAGCAGCAACAGCCACGAGGCCCGCTGGGGCT
TACCGACTTGGAGCTGGCCCAGCAGCTTCAGCAAGAGGAGTATCAACAGCAGCAGGCAGCGCAGC
CAGTGCGGATGCGGACGCGGGTCCTGTCACTGCAGGGGAGAGGAGCCACATCTGGACGCCCAGCC
GGGGAGCGTCGGCAGAGGCCGAAGCACGAGTCAGACTGCATTCTGCTGtaggcggccgc