

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of MINDY3 [1 - 445]

Enzyme description:- MINDY3 [1 – 445]

Clone number:- DU 47870

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 76, 499.18 daltons

Average Mass 76, 548.74 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.93

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

MINDY3 [1 – 445]

Protein MINDY3 [1 – 445]

Clone number DU 47870

Species Human

Accession number Q9H8M7-1

Tags N-terminal GST

**Bacterially
expressed protein**

MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMSELTKELM
ELVWGTKSSPGLSDTIFCRWTQGFVFSESEGSALQFEGGPCAVIAPV
QAFLLKKLLFSSEKSSWRDCSEEEQKELLCHTLCDILESACCDHSGSY
CLVSWLRGKTTEETASISGSPAESSQVEHSSALAVEELGFERFHALI
QKRSFRSLPELKDAVLDQYSMWGNKFGVLLFLYSVLLTKGIENIKNEI
EDASEPLIDPVYGHGSQSLINLLLLTGHAVSNVWDGDRECSGMKLLGIH
EQAAVGFLTLMEALRYCKVGSYLKSPKFPWIWVGETHLTVFFAKDMA
LVAPEAPSEQARRVFQTYDPEDNGFIPDSLLEDVMKALDLVSDPEYIN
LMKNKLDPEGLGIILLGPFLQEFFPDQGSSGPESFTVYHYNGLKQSNY
NEKVMYVEGTAVVMGFEDPMLQTDTPIKRCLQTKWPYIELLWTTDRS
PSLN

Native sequence Amino acids M1 – N445 (end) of human MINDY3.
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 (LEVLFGQP) residues 221 – 228

Cloning sites *Bam*H1 and *Not*I sites of pGEX6P-1

MRC PPU REAGENTS

Nucleotide Sequence Of Insert

ggatccATGTCCGAACTGACTAAAGAGCTGATGGAGCTGGTGTGGGGCACCAAGAGCAGCCCCGG
TCTCTCGGACACCATTTTCTGCCGCTGGACGCAAGGGTTTGTGTTTAGTGAATCAGAGGGATCTG
CATTAGAACAGTTTGAAGGTGGCCCCTGTGCTGTTATTGCACCTGTTCAGGCATTTCTTTTGAAG
AAGCTCCTGTTTTCTTCGGAGAAGTCTTCTTGGCGGGATTGTTTCAGAGGAAGAGCAGAAGGAACT
CCTTTGTCATACCTTGTGTGATATTTTAGAAAGTGCTTGTGTGACCACTCTGGATCATACTGCT
TGGTTTCATGGTTAAGAGGAAAAGACAACCTGAGGAACTGCTAGTATTTCTGGGAGTCCTGCAGAG
TCTAGTTGCCAAGTGGAAACATTCTTCTGCCCTTGGCTGTCTGAAGAGCTTGGCTTTGAGCGATTTC
TGCATTAATTCAAAAAAGATCGTTTCAGAAGTTTACCAGAATTAAAAGATGCTGTCTTGGACCAGT
ATTCAATGTGGGGAAATAAATTTGGAGTATTGCTTTTTCTGTATTCTGTATTACTGACAAAGGGC
ATTGAAAACATAAAAAACGAAATTGAAGATGCAAGTGAACCCTTGATAGATCCTGTATATGGACA
TGGCAGCCAAAGTTTAATTAATCTCCTGCTGACGGGACATGCTGTTTCTAATGTATGGGATGGTG
ATAGAGAGTGCTCAGGAATGAAACTTCTTGGTATACATGAACAAGCAGCAGTAGGATTTTTAACA
CTAATGGAAGCTTTAAGATACTGTAAGGTTGGTTCTTACTTGAAATCTCCAAAATTCCTATTTG
GATTGTTGGCAGTGAGACTCACCTCACCGTATTTTTTGGCAAGGATATGGCTTTAGTTGCCCTG
AAGCTCCTTCAGAACAAGCCAGAAGAGTTTTTCAAACCTACGACCCAGAAGATAATGGATTCATA
CCCGATTCACTTCTGGAAGATGTGATGAAAGCATTGGACCTTGTTTCAGATCCTGAATATATAAA
TCTCATGAAGAATAAATTAGATCCAGAAGGATTAGGAATCATATTATTGGGCCCATTTCTTCAAG
AATTTTTTCTCTGATCAGGGCTCCAGTGGTCCAGAATCTTTTACTGTCTACCACTACAATGGATTG
AAGCAGTCAAATTATAATGAAAAGGTCATGTACGTAGAAGGGACTGCAGTTGTGATGGGTTTTGA
AGATCCCATGCTACAGACAGATGACACTCCTATTAAACGCTGTCTGCAAACCAAATGGCCATACA
TTGAGTTACTCTGGACCACAGATCGCTCTCCTTCACTAAATtaagcggccgc