

MRC PPU REAGENTS

Standard Operating Procedure

Preparation of MINDY2 [1 - 621]

Enzyme description:- MINDY2 [1 – 621]

Clone number:- DU 63404

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 93, 871.08 daltons

Average Mass 93, 930.38 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.65

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

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Clone Data Sheet

MINDY2 [1 – 621]

Protein MINDY2 [1 – 621]

Clone number DU 63404

Species Human

Accession number Q8NBR6.3

Tags N-terminal GST

Bacterially
expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAIEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKY
LKSSKYIAWPLQGWQATFGGGDHPKSDLEVLFGQPLGSMESSPESLQ
**PLEHGVAAGPASGTGSSQEGLOETRLAAGDGPVWAAETSGGNLGA
AARRSLPDSASPAGSPEVPGPCSSSAGLDLKDSGLESPAAAEAPLRGQ
YKVTASPETA VAGVGH
ELGTAGDAGARPDLAGTCQAE
LTAAGSEEPSS
AGGLSSSCSDPSPPGESPSLDSLESF
SNLHSFPSSCEF
NSEE
GAENRV
PEEEEGAAVLP
GAVPLCKEEEGEETAQVLAASKER
FPGQSVYHIKWIQ
WKEENTPIITQ
NENGPCLLAILNVLLAWKVKLPPME
IITAEQLME
YLGDYMLDAKPK
EISEIQRLN
YEQNMSDAMAILHKLQ
TGLDVNVRFTG
VRVFEYTPECIV
FDLLDIPLYHG
WLVDPOIDDIVKAVG
NCSYNQLVEK
IISCKQSDNSE
LVSEGFVAEQFL
NNTATQ
LTYHGLCELTSTV
QEGELC
VFFRNNHFST
MTKYKQQLYLL
VTDQGF
LTEEKVVWESL
HNVDGDGNFC
DSEFHLRPP
SDPETVYKQ
QDQIDQDYL
MALSLOQEQ
QSQEINWEQ
IP
EGISDLELAK
KLQEEEDRR
ASQYYQEQE
QAAAAAAAAA
ASTQAQ
QGQPAQ
ASPSSGRQSG
NSERKRKEP
REKDK
EKEKEK
NSCVIL**

Native sequence Amino acids M1 – L621 (end) of human MINDY2.
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 (LEVLFQGP) residues 221 – 228

Cloning sites *Bgl*II / *Bgl*II into *Bam*H1 sites of pGEX6P-1

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Nucleotide Sequence Of Insert

agatctATGGAGAGCAGCCCCGAGAGCCTGCAGCCGCTAGAACACGGGGTGGCGGCCGGGCCAGC
GTCAGGGACAGGTTCTTCGCAGGAAGGGCTACAGGAGACCAGGCTCGCCGCTGGTGATGGTCCTG
GGGTATGGGCGGCGGAGACCAGCGGCGGGAATGGGCTGGGGGCGGCGGCCGCCAGGAGGAGCCTC
CCGACTCGGCTTCTCCCGCGGGCTCTCCTGAGGTTCCCGGACCCTGCAGCTCCTCCGCGGGTTT
GGAATTGAAGGACAGTGGTTTGGAGAGTCCTGCTGCCGCCGAGGCGCCTCTGAGAGGGCAGTACA
AGGTGACCGCCTCCCCGGAGACAGCCGTGGCCGGAGTGGGTCATGAGTTGGGTACCGCCGGAGAC
GCGGGAGCCCCCGGATCTCGCCGGCACCTGCCAAGCAGAAGTACCAGCCGCCGGCTCCGAAGA
GCCAGCAGCGCCGGCGGCCTCAGCAGCAGTTGCAGCGACCCGAGCCCTCCTGGGGAATCTCCGA
GCCTGGACTCTCTGGAGTCGTTCTCTAACCTGCATTCTTTTCCAGTAGCTGCGAGTTCAATAGT
GAGGAGGGAGCGGAGAACAGGGTCCCTGAGGAGGAGGAGGGCGCGGCGGTGTTGCCCGGGCTGT
TCCTCTGTGCAAGGAGGAGGAGGGGGAGGAGACCGCTCAGGTGCTGGCGGCCCTCAAGGAACGCT
TCCCGGGACAATCTGTGTATCACATCAAGTGGATCCAGTGGAAGGAAGAGAACACACCCATCATC
ACCCAGAATGAGAACGGACCCTGCCCTTGCTGGCCATCCTCAATGTTTTGCTCCTGGCCTGGAA
GGTAAACTTCCACCGATGATGAAATCATAACTGCTGAGCAGCTGATGGAATATTTAGGAGATT
ACATGCTTGATGCAAAGCCAAAAGAAAATTCAGAAATCAACGTTTAAATTATGAACAGAATATG
AGTGATGCCATGGCAATTTTGCACAAACTACAGACAGGCCTGGATGTAAATGTAAGATTTCACTGG
TGTTTCGAGTGTTTGAATATACACCAGAATGCATAGTATTTGATCTTCTTGATATTCCTTTTGTACC
ATGGGTGGTTAGTAGACCCTCAGATTGATGACATTGTAAAAGCTGTTGGTAACTGCAGCTACAAC
CAACTAGTGGAGAAGATCATCTCTTGTAACAGTCAGACAATAGTGAGCTGGTTAGTGAAGGCTT
TGTAGCTGAGCAGTTTCTAAATAACACAGCCACTCAACTGACATAACCATGGATTATGTGAACTAA
CTTCAACGGTTCAGGAAGGAGAACTTTGTGTGTTCTTTCGGAATAATCATTTTAGCACCATGACC
AAATACAAGGGTCAACTGTATTTGTTGGTAACGGACCAGGGGTTTCTTACTGAAGAGAAAGTTGT
TTGGGAAAGCCTACACAACGTAGATGGTGATGAAATTTCTGTGACTCAGAATTTATCTTCGAC
CTCCTTCAGATCCTGAAACTGTATACAAAGGACAACAAGATCAGATAGATCAGGATTATCTTATG
GCATTATCTCTACAACAAGAACAGCAGAGCCAAGAGATCAATTGGGAACAAATCCCGGAAGGAAT
CAGTGATTTGGAAGTAGCAAAGAAACTCCAAGAGGAAGAGGACAGACGGGCTTCTCAATACTATC
AGGAACAGGAACAAGCAGCAGCTGCTGCTGCTGCTGCTTCTACACAGGCTCAGCAGGGCCAGCCA
GCACAAGCCTCTCCATCAAGTGGAAGACAATCTGGGAATAGTGAACGTAAACGGAAGGAACCACG
AGAAAAAGATAAAGAAAAAGAAAAGGAAAAAATAGCTGTGTTATTTTgtaagatct