MRC PPU REAGENTS

Standard Operating Procedure

Preparation of MINDY2 [241 - 504]

Enzyme description:-	MINDY2 [241 – 504]
Clone number:-	DU 55390
Source:-	Recombinant
Expression system:-	*E.coli*
Tag:-	N-terminal GST
Purification method:-	GSH Sepharose

Calculated molecular mass:-

Monoisotopic	58, 144.20 daltons
Average Mass	58, 182.02 daltons

[cysteines reduced, methionines have not been oxidised

Theoretical pI:-	4.97
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 [241 – 504]

Protein	MINDY2 [241 – 504]
Clone number	DU 55390
Species	Human
Accession number	Q8NBR6.3
Tags	N-terminal GST
Bacterially expressed protein	MAPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSLEVLFQGPLGSPEF M**SVYHIKWIQWKEENTPIITQNENGPCPLLAILNVLLLAWKVKLPPMM** **EIITAEQLMEYLGDYMLDAKPKEISEIQRLNYEQNMSDAMAILHKLQT** **GLDVNVRFTGVRVFEYTPECIVFDLLDIPLYHGWLVDPQIDDIVKAVG** **NCSYNQLVEKIISCKQSDNSELVSEGFVAEQFLNNTATQLTYHGLCEL** **TSTVQEGELCVFFRNNHFSTMTKYKGQLYLLVTDQGFLTEEKVVWESL** **HNVDGDGNFCDSEFHLRPPSDPETV**
Native sequence	Amino acids S241 – V504 (L621 end residue) of human MINDY2. Residue S242 of the fusion protein is equivalent to S241 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 – 228
Cloning sites	*Eco*R1 and *Not*1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

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gaattcatgTCTGTGTATCACATCAAGTGGATCCAGTGGAAGGAAGAGAACACACCCATCATCAC
CCAGAATGAGAACGGACCCTGCCCCTTGCTGGCCATCCTCAATGTTTTGCTCCTGGCCTGGAAGG
TGAAACTTCCACCGATGATGGAAATCATAACTGCTGAGCAGCTGATGGAATATTTAGGAGATTAC
ATGCTTGATGCAAAGCCAAAAGAAATTTCAGAAATTCAACGTTTAAATTATGAACAGAATATGAG
TGATGCCATGGCAATTTTGCACAAACTACAGACAGGCCTGGATGTAAATGTAAGATTCACTGGTG
TTCGAGTGTTTGAATATACACCAGAATGCATAGTATTTGATCTTCTTGATATTCCTTTGTACCAT
GGGTGGTTAGTAGACCCTCAGATTGATGACATTGTAAAAGCTGTTGGTAACTGCAGCTACAACCA
ACTAGTGGAGAAGATCATCTCTTGTAAACAGTCAGACAATAGTGAGCTGGTTAGTGAAGGCTTTG
TAGCTGAGCAGTTTCTAAATAACACAGCCACTCAACTGACATACCATGGATTATGTGAACTAACT
TCAACGGTTCAGGAAGGAGAACTTTGTGTGTTCTTTCGGAATAATCATTTTAGCACCATGACCAA
ATACAAGGGTCAACTGTATTTGTTGGTAACGGACCAGGGGTTTCTTACTGAAGAGAAAGTTGTTT
GGGAAAGCCTACACAACGTAGATGGTGATGGAAATTTCTGTGACTCAGAATTTCATCTTCGACCT
CCTTCAGATCCTGAAACTGTAtaagcggccgc
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