MCC Protein/Clone Information Sheet WMgMDH

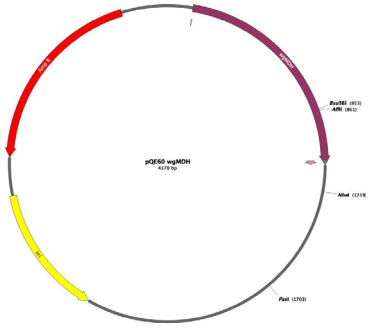
Protein Name: Glyoxysomal Malate Dehydrogenase (WMg MDH)
Organism: Watermelon (*Citrullus lantus*) **Plasmid Name**: pQE60 wgMDH

Clone/Plasmid History: Originally cloned by into pQE60 vector with the His affinity tag on the C terminus of MDH. This is the mature watermelon glyoxysomal MDH (WMgMDH) without presequence. This construct does NOT have the transit vector and instead has an added Met as the start codon after removal of transit peptide sequence. WMgMDH, was prepared by PCR using the Ncol- and Bglll-site and cloned into the same vector. WMgMDH is cloned between the restriction sites Ncol and BgllI; the Ncol-site also provided the start codon. The necessary restriction sites at the 5'-end and 3'- end of the cDNA sequence were added by PCR. Either during cloning or in subsequent manipulations, the NcoI site (C/CATGG) was mutated to CTATGG, destroying the NcoI cut site.

NCBI / Gene Accession Number: https://www.ncbi.nlm.nih.gov/nuccore/M33148 (shown with transit peptide)

SnapGene Plasmid Map: *Downloadable file will include*:

- Resistance, Promotor (for bacterial or mammalian), Sequencing primers, RBS and Kozak sequence, History of cloning, Annotated start and stop of protein, Highlighted tags or TEV/Thrombin sites



NCBI Protein Sequence Accession:
Precursor form: https://www.ncbi.nlm.nih.gov/protein/1SEV_A (shown with precursor transit peptide)
Mature form: https://www.ncbi.nlm.nih.gov/protein/1SMK_A (Mature form lacks transit peptide

UniProt Protein Page: https://www.uniprot.org/uniprotkb/P19446/entry

Crystal Structures: RCSB PDB Page: https://www.rcsb.org/structure/1SEV (transit peptide not seen in crystal structure) and https://www.rcsb.org/structure/1SMK (mature form)

Key Publications:

Gietl C, Seidel C, Svendsen I. Plant glyoxysomal but not mitochondrial malate dehydrogenase can fold without chaperone assistance. Biochim Biophys Acta. 1996 May 20;1274(1-2):48-58. doi: 10.1016/0005-2728(96)00009-6. PMID: 8645694.

Cox B, Chit MM, Weaver T, Gietl C, Bailey J, Bell E, Banaszak L. Organelle and translocatable forms of glyoxysomal malate dehydrogenase. The effect of the N-terminal presequence. FEBS J. 2005 Feb;272(3):643-54. doi: 10.1111/j.1742-4658.2004.04475.x. PMID: 15670147.

Available Mutations: Over 80 prepared. See MCC website for list.

Protein Notes: Transit peptide (aa 1-36) required for entry into glyoxasomes and is cleaved as mature format. WMgMDH is a 320 amino acid protein as a functional homodimer. Mature WMgMDH monomer mw = 34.6 kDa. Biologically Active as a dimer.

pI/ ε_{280} , extinction coefficient (280 nm: calculated using ProtParam.) of protein (WT and/or specific mutant):

Watermelon Glyoxysomal MDH::

pI = 8.22/ ε_{280} , = 0.258mL.mg^{-1}.cm^{-1} extinction coefficient (280 nm: calculated using ProtParam.) of protein: 0.475 mL.mg^{-1}.cm^{-1} (Literature reported value)

Key amino acids / functions studied include: Shown below with comparisons to other isoforms to help avoid confusion

Residue	Flexible Loop	Aspartate	Arginine	Arginine	Aspartate	Arginine	Histidine
Watermelon-g MDH 1sev/1smk Equivalent	117-140	D77	R124	R130	D193	R196	H220
hCytosolic 7rm9.pdb or 7rm9repaired. pdb	85-108	D42	R92	R98	D159	R162	H187
hCytoConstruct. pdb		D61	R111	R117	D178	R1181	H226
hMitochondrial 2DFD.pdb	79-102	D39	R86	R92	D155	R158	H182
hMitoConstruct. pdb		D34	R81	R87	D150	R153	H177
Plasmodium falciparum: 5NFR.pdb	74-97	D32	R81	R87	D147	R150	H174
Ignicoccus Islandicus 6qss,pdb	77-100	D37	R86	R92	D151	R154	H178
Function	Closes over active site on substrate binding	Governs specificity for NAD(H)	Malate/Oxaloacetate/Citrate Binding	Malate/Oxaloacetate/Citrate Binding	Alters Basicity of Catalytic Histidine	Malate/Oxaloacetate/Citrate Binding	Catalytic Base

Recommended Protocols: Enzyme Activity Assay / Protein Expression / Protein Purification

Clone FAQ and Important Points: High protein expression at 37°F 1mm IPTG for 3-4 hour induction. ~0.25-0.8 mg per ml of culture. pQE60 (Qiagen) is a low copy plasmid and will not give high yields of DNA preps. Amp Resistant. Do not freeze thaw purified protein – stability test of proteins in glycerol needed. Stable at 4°C for several days in elution buffer with minimal loss of activity. Stable at 4°C for 1-4 weeks dialyzed against (10 mM KPi,150 mM NaCl, 0.1 mM EDTA, pH 8.0). Long term storage -20 to -80°C (10 mM K-phosphate, 0.1 mM EDTA, 20% glycerol, pH 8.0). Concentrations approaching 1-1.25 mg/ml will precipitate over a short time. Dilute immediately after purification and before dyalysis to 1 mg/ml or less. See MDH Stability Datasheet for more information. Expected Km 146µM for NADH and 76 µM for OAA, (additional info from key publications – see above). Purification easily performed in column or batch format.

See Snap Gene File for details.

Amino Acid Coding Sequence:

MAKGGAPGFKVAILGAAGGIGQPLAMLMKMNPLVSVLHLYDVVNAPGVTADISHMDTGAVVRGFLGQQQLEAALTGMDLI
IVPAGVPRKPGMTRDDLFKINAGIVKTLCEGIAKCCPRAIVNLISNPVNSTVPIAAEVFKKAGTYDPKRLLGVTMLDVVR
ANTFVAEVLGLDPRDVDVPVVGGHAGVTILPLLSQVKPPSSFTQEEISYLTDRIQNGGTEVVEAKAGAGSATLSMAYAAV
KFADACLRGLRGDAGVIECAFVSSQVTELPFFASKVRLGRNGIEEVYSLGPLNEYERIGLEKAKKELAGSIEKGVSFIRS
RSHHHHHH*

Coding Plasmid Sequence:

ATGGCTAAAGGCGGAGCTCCCGGGTTCAAAGTCGCAATACTTGGCGCTGCCGGTGGCATTGGCCAGCCCCTTGCGATGTT
GATGAAGATGAATCCTCTGGTTTCTGTTCTACATCTATATGATGTAGTCAATGCCCCTGGTGTCACCGCTGATATTAGCC
ACATGGACACGGGTGCTGTGGTGCGTGGATTCTTGGGGCAGCAGCAGCTGGAGGCTGCGCTTACTGGCATGGATCTTATT
ATAGTCCCTGCAGGTGTTCCTCGAAAACCAGGAATGACGAGGGATGATCTGTTCAAAATAAACGCAGGAATTGTCAAGAC
TCTGTGTGAAGGGATTGCAAAGTGTTGTCCAAGAGCCATTGTCAACCTGATCAGTAATCCTGTGAACTCCACCGTGCCCA
TCGCAGCTGAAGTTTTCAAGAAGGCTGGAACTTATGATCCAAAGCGACTTCTGGGAGTTACAATGCTCGACGTAGTCAGA
GCCAATACCTTTGTGGCAGAAGTATTGGGTCTTGATCCTCGGGATGTTGATGTTCCAGTTGTTGGCGGTCATGCTGGTGT
AACCATTTTGCCCCTTCTATCTCAGGTGAAGCCTCCAAGTTCTTTCACACAAGAAGAGATTAGTTACCTGACTGATAGGA
TTCAAAATGGTGGAACAGAAGTTGTCGAGGCCAAAGCAGGAGCTGGTTCAGCAACTCTCTCAATGGCTTATGCTGCCGTT
AAGTTTGCAGATGCATGCCTCAGGGGCTTAAGAGGAGATGCTGGTGTCATTGAATGCGCGTTTGTGTCTTCTCAGGTGAC
TGAACTTCCATTCTTTGCATCAAAAGTACGACTTGGTCGCAATGGTATCGAAGAAGTATACTCCCTTGGCCCGCTAAATG
AGTATGAGAGGATTGGATTGGAGAAAGCGAAGAAAGAGTTGGCAGGAAGCATTGAGAAGGGAGTTTCCTTCATCAGAAGC
AGATCTCATCACCATCACCATCACTAA