MCC Protein/Clone Information Sheet
wgMDH Active Site Loop Mutants

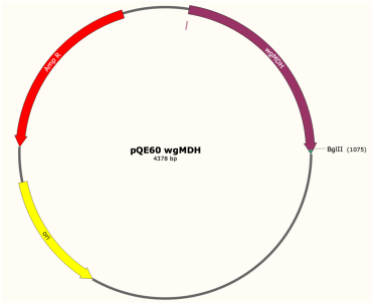
Protein Name: Glyoxysomal Malate Dehydrogenase (wg MDH)
Organism: Watermelon (*Citrullus lantus*) **Plasmid Name**: pQE60 wgMDH

Clone/Plasmid History: Originally cloned by Gietl C *et. al.* (1996) BBA 1274, 48-58 into pQE60 vector with the His affinity tag on the C terminus of MDH. This is the mature watermelon glyoxyl MDH (wgMDH) without presequence. This construct does NOT have the transit peptide (mqpipdvnqr iarisahlhp pksqmeessa lrrancr) and instead has an added Met as the start codon after removal of transit peptide sequence. wgMDH, was prepared by PCR using the NcoI- and BglII-site and cloned into the same vector. wgMDH is cloned between the restriction sites NcoI and BglII; the NcoI-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. Biochemica et Biophysica Acta 1274 (1996) 48-58. And FEBS Journal 272 (2005) 643-654.

NCBI / Gene Accession Number: M33148.1 (shown with transit peptide)

Plasmid Map: *Downloadable file will include*:

- Amp resistance in pQE60 construct (use 100 µg/ml), Promotor (for bacterial or mammalian), Sequencing primers, RBS and Kozak sequence, History of cloning, Annotated start and stop of protein all located on snapgene file



NCBI Protein Sequence Accession: P19446.1 (shown with precursor transit peptide)

UniProt Protein Page: MDHG_CITLA

RCSB PDB Page: 1SEV (with transit peptide) 1SMK (mature form)

Key Publications: Plant glyoxysomal but not mitochondrial malate dehydrogenase can fold without chaperone assistance, Gietl et al.
Organelle and translocatable forms of glyoxysomal malate dehydrogenase: The effect of the N-terminal presequence , Cox et al.

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. wgMDH is a 320 amino acid protein as a functional homodimer. Mature wgMDH monomer mw = 34.5 kDa.

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 dialysis 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.

WILD TYPE - Amino Acid Coding Sequence:

MAKGGAPGFKVAILGAAGGIGQPLAMLMKMNPLVSVLHLYDVVNAPGVTADISHMDTGAVVRGFLGQQQLEAALTGMDLIIVPAGVPRKPG
MTRDDLFKINAGIVKTLCEGIAKCCPRAIVNLISNPVNSTVPIAAEVFKKAGTYDPKRLLGVTMLDVVRANTFVAEVLGLDPRDVDVPVVGGHAGV
TILPLLSQVKPPSSFTQEEISYLTDRIQNGGTEVVEAKAGAGSATLSMAYAAVKFADACLRGLRGDAGVIECAFVSSQVTELPFFASKVRLGRNGIEE
VYSLGPLNEYERIGLEKAKKELAGSIEKGVSFIRSRSHHHHHH*

Coding Region for MDH Plasmid Sequence:

ATGGCTAAAGGCGGAGCTCCCGGGTTCAAAGTCGCAATACTTGGCGCTGCCGGTGGCATTGGCCAGCCCCTTGCGATGTTGATGAAGATG
AATCCTCTGGTTTCTGTTCTACATCTATATGATGTAGTCAATGCCCCTGGTGTCACCGCTGATATTAGCCACATGGACACGGGTGCTGTGGTG
CGTGGATTCTTGGGGCAGCAGCAGCTGGAGGCTGCGCTTACTGGCATGGATCTTATTATAGTCCCTGCAGGTGTTCCTCGAAAACCAGGAA
TGACGAGGGATGATCTGTTCAAAATAAACGCAGGAATTGTCAAGACTCTGTGTGAAGGGATTGCAAAGTGTTGTCCAAGAGCCATTGTCAA
CCTGATCAGTAATCCTGTGAACTCCACCGTGCCCATCGCAGCTGAAGTTTTCAAGAAGGCTGGAACTTATGATCCAAAGCGACTTCTGGGAG
TTACAATGCTCGACGTAGTCAGAGCCAATACCTTTGTGGCAGAAGTATTGGGTCTTGATCCTCGGGATGTTGATGTTCCAGTTGTTGGCGGT
CATGCTGGTGTAACCATTTTGCCCCTTCTATCTCAGGTGAAGCCTCCAAGTTCTTTCACACAAGAAGAGATTAGTTACCTGACTGATAGGATT
CAAAATGGTGGAACAGAAGTTGTCGAGGCCAAAGCAGGAGCTGGTTCAGCAACTCTCTCAATGGCTTATGCTGCCGTTAAGTTTGCAGATG
CATGCCTCAGGGGCTTAAGAGGAGATGCTGGTGTCATTGAATGCGCGTTTGTGTCTTCTCAGGTGACTGAACTTCCATTCTTTGCATCAAAA
GTACGACTTGGTCGCAATGGTATCGAAGAAGTATACTCCCTTGGCCCGCTAAATGAGTATGAGAGGATTGGATTGGAGAAAGCGAAGAAA
GAGTTGGCAGGAAGCATTGAGAAGGGAGTTTCCTTCATCAGAAGCAGATCTCATCACCATCACCATCACTAA

pQE60 G121A wgMDH
pQE60 G121F wgMDH
pQE60 G127A wgMDH
pQE60 G127F wgMDH
pQE60 P119W wgMDH
pQE60 P123A wgMDH
pQE60 P123F wgMDH
pQE60 P126G wgMDH
pQE60 P126R wgMDH
pQE60 P126W wgMDH
pQE60 R210A wgMDH