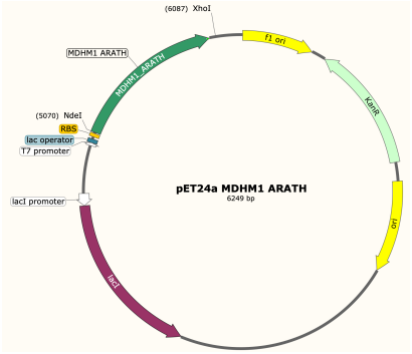
MCC Protein/Clone Information Sheet MDHM1_ARATH

Protein Name: *Arabidopsis thaliana* mitochondrial Malate Dehydrogenase (MDHM1_ARATH)
Organism: *Arabidopsis thaliana* **Plasmid Name**: pET24a MDHM1_ARATH Alternative name: mitochondrial Arabidopsis MDH, amMDH2

Clone/Plasmid History: Mitochondrial MDH in plants is isoform 2. Gene At5g58330. Amino acids 53-443 of the MDH2 gene was synthesized after codon-optimization of Q9ZPO6 (MDHM1_ARATH) version 1, 1999, for expression in BL21 (DE3) and cloned into pET24a vector using a Nde1/EcoR1 digested pET24a The TEV recognition site was also added between the His tag and MDH. The unaltered pET24 does not include a cleavage site. The first 52 amino acids were not included in this construct as these are the mitochondrial transit sequence. The initial M is in place. Thus the initial few resides are "MCSVSQNSQ…". The protein will also include the His tag. The His tag is on the C terminus of MDHM1_ARATH. The N terminus remains unaltered. *Because the gene is synthesized and codon optimize, the nucleotide sequence will not match the accession number*. Please refer to the linked snapgene file or FASTA formatted file linked below for the DNA sequence of the coding region for MDHM1_ARATH.

NCBI / Gene Accession Number: *Because the MDH gene was synthesized and codon optimized as described above its, nucleotide sequence differs from that published in Gene Bank. The complete sequence of the MDHM1_ARATH gene including the targeting sequence can be found at the accession number*. Please refer to the associated snapgene file or FASTA formatted file linked below for the DNA sequence of the coding region NM_100321.3



Plasmid Map: SnapGene file of this construct is available to members of the MCC. Features annotated on the file include the kanamycin resistance gene, bacterial promotors, the ribosome binding site (RBS), the Kozak sequence, sequencing primers, start and stop codons, the His-tag, the TEV sequence and cleavage site, and the cloning history.

NCBI Protein Sequence Accession: The MDHM1_ARATH protein sequence as expressed in plant (with the mitochondrial targeting sequence included) can be found at NP_171936.1

UniProt Knowledge Base Accession: Q9ZPO6 (MDHM1_ARATH)

RCSB PDB Page: 5NUE (peroxide treated), 5NUF (apo)

Key Publications: Mitochondrial malate dehydrogenase lowers leaf respiration and alters photorespiration and plant growth in Arabidopsis, Loss of Mitochondrial Malate Dehydrogenase Activity Alters Seed Metabolism Impairing Seed Maturation and Post-Germination Growth in Arabidopsis, Peroxisomal malate dehydrogenase is not essential for photorespiration in Arabidopsis but its absence causes an increase in the stoichiometry of photorespiratory CO2 release

Protein Notes: Cytoplastic *Arabidopsis thaliana* (NADP MDH). Gene At5g58330 (this clone has been optimized for bacterial expression and will not match the plant gene sequence). In C4 plants, NADP-MDH activity acts to convert oxaloacetate to malate in chloroplasts of mesophyll cells for transport to the bundle sheath cells (Probable). Plays an essential role in the regulation of catalase activity and the accumulation of a hydrogen peroxide-dependent signal by transmitting the redox state of the chloroplast to other cell compartments. The cytosolic isoform is controlled by redox conditions including oxidation by peroxides. This reduces the Km for OAA and NADH by nearly half of the reduced MDH. Similar effects were observed with peroxisomal but not mitochondrial MDH. Mitochondrial MDH plays an important role in OAA->malate production to support mitochondrial respiration and CO_2 assimilation. MDHM1_ARATH is a homodimer and has a predicted mw = 35.57 kDa.

Clone FAQ and Important Points: Weak to moderate protein expression at 37°C 1mm IPTG for 4-6 hour induction. Stronger expression at 20°C (room temp) for 14-24 hrs. pET24a (Novagen) is a low copy plasmid (~40) and will not give high yields of DNA preps. Kan Resistant. The activity in standard enzyme reaction conditions (100 µM NADH/200µM OAA) is moderate to strong and may exhibit OAA feedback inhibition above 200 µM Do not freeze thaw purified protein. Purification easily performed in column or batch format. Stable at 4°C for 1-4 weeks dialyzed against (10 mM K phosphate, 0.1 mM EDTA, pH 8.0). Long term storage has not been studied. Recommended conditions to be tested -20 to -80°C (10-20% Glycerol, 50 mM NaCl, 10 mM K phosphate, pH

8.0). Minimum dialysis and storage buffer suggested, but not tested, (10 mM K phosphate, 0.1 mM EDTA, 20% glycerol, pH 8.0). Inclusion of 0.2 - 1 mM β-ME may be added at user's discretion.

ATGTCCTCTGGTTCTGTACCGGAACGTAAAGTAGCTATTCTGGGTGCTGCTGGTGGCATCGGTCAACCACTGGCTCTGCTGATGAAACTGAACCCACTGGTATCCA
GCCTGTCTCTGTACGACATCGCAAACACTCCAGGTGTTGCAGCTGACGTTGGTCACATCAACACTCGCTCCGAAGTTGTAGGCTACATGGGTGACGACAACCTGG
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AACGGTGTGGAAGAAGTGCTGGACCTGGGTCCACTGAGCGACTTCGAGAAAGAAGGCCTGGAAGCGCTGAAACCGGAACTGAAATCTAGCATCGAGAAAGGT
GTGAAGTTCGCAAACCAGCATCATCACCACCATCAC

~~M~~SSGSVPERKVAILGAAGGIGQPLALLMKLNPLVSSLSLYDIANTPGVAADVGHINTRSEVVGYMGDDNLAKALEGADLVIIPAGVPRKPGMTRDDLFNINAGIVKNLCT
AIAKYCPHALINMISNPVNSTVPIAAEIFKKAGMYDEKKLFGVTTLDVVRARTFYAGKANVPVAEVNVPVIGGHAGVTILPLFSQATPQANLSSDILTALTKRTQDGGTEV
VEAKAGKGSATLSMAYAGALFADACLKGLNGVPDVIECSYVQSTITELPFFASKVRLGKNGVEEVLDLGPLSDFEKEGLEALKPELKSSIEKGVKFANQ**HHHHHH**