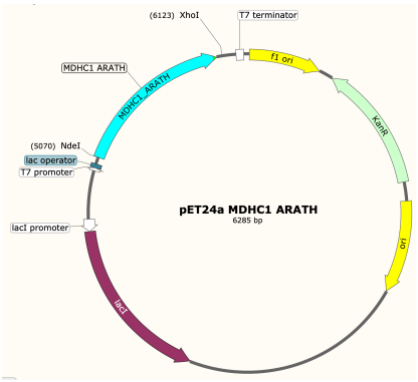
MCC Protein/Clone Information Sheet MDHC1_ARATH

Protein Name: *Arabidopsis thaliana* cytoplasmic Malate Dehydrogenase (MDHC1_ARATH)
Organism: *Arabidopsis thaliana* **Plasmid Name**: pET24a MDHC1_ARATH Alternative Names: Arabidopsis cytosolic MDH, acMDH1

Clone/Plasmid History: Cytosolic MDH in plants is isoform 1. MDH1 gene was synthesized after codon-optimization for expression in BL21 (DE3) using UniProt record P93819 (MDHC1_ARATH) version 2, 1998. The synthesized gene was cloned into pET24a vector using a Nde1/EcoR1 digested pET24a. The unaltered pET24a plasmid does not include a cleavage site. The clone includes a His tag C terminus of MDHC1_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 associated snapgene file or FASTA formatted file linked below for the DNA sequence of the coding region for MDHC1_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 yeast MDHC1_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: A 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 MDHC1_ARATH protein sequence as expressed can be found NP_171936.1

UniProt Knowledge Base Accession: P93819 (MDHC1_ARATH)

RCSB PDB Accession: 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

Available Mutations: None at this time, will become available upon publication.

Protein Notes: 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. Disulfide formation protects from overoxidation and does so by interacting with cellular thioredoxins. 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 CO2 assimilation. Peroxisomal plays a secondary role in recycling carbon and nitrogen in photorespiration. MDHC1_ARATH is a 329 amino acid (plus the 6X His tag on the C terminus). MDHC1_ARATH is a homodimer and has a predicted mw = 35.57 kDa. Km (OAA) 143 µM (NADH) 72 µM.

Key amino acids / functions studied include: Nucleotide binding 12-18, 130-132

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.

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