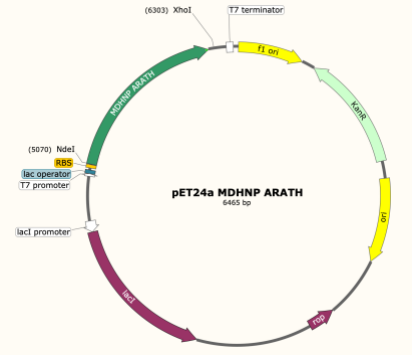
MCC Protein/Clone Information Sheet MDHNP_ARATH

Protein Name: *Arabidopsis thaliana* chloroplastic Malate Dehydrogenase (MDHND_ARATH)
Organism: *Arabidopsis thaliana* **Plasmid Name**: pET24a MDHNP_ARATH Alt names: Arabidopsis chloroplast MDH, acMDH2

Clone/Plasmid History: Chloroplastic MDH in plants belongs in the MDH type 2 family. This gene/protein is the splice isoform 1 of MDHNP. The second splice variant of MDHNP is missing an amino acid in position 59 (including the terminator peptide in counting). Amino acids 53-443 of the MDHNP gene missing the transit peptide but adding an Met in position 1, was synthesized after codon-optimization of Q8H1E2 (MDHNP_ARATH) version 1, 2003, 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 normal pET24 does not include a cleavage site. The first 52 amino acids were not included in this construct as these are the chloroplastic transit sequence. The initial M is in place. Thus the initial few resides are "*M*CSVSQNSQAP". The protein will also include the His tag. Both thrombin cleavage sequence and the His tag are C terminus of MDHNP_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 MDHNP_ARATH.



NCBI / Gene Accession Number: NM_125218.4

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 MDHNP_ARATH protein sequence as expressed in plant (with the targeting sequence included) can be found at NP_568875.2

UniProt Knowledge Base Accession: Q8H1E2 (MDHNP_ARATH)

RCSB PDB Page: QH1E2 (predicted SWISS-MODEL from Chloroplast MDH from flaveria bidentis)

Key Publications: Putative role of the malate valve enzyme NADP-malate dehydrogenase in H2O2 signaling in Arabidopsis.

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

Protein Notes: Chloroplastic *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. Arabadopsis MDHNP is required for reversible regulation of peroxide producing enzymes as light intensity changes. The changes may be due to Cys redox changes. MDHNP_ARATH is a 329 amino acid (plus the thrombin site and 6X His tag on the C terminus). MDHNP_ARATH is a homodimer and has a predicted mw = 48.3 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.

CATATGTGTTCCGTTTCCCAGAACAGCCAAGCTCCAGTTGCAGTTCAAGAGAACGGTCTGGTTAAGACCAAGAAAGAGTGCTATGGCGTGTTCTGCCTGACCTAC
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GGTAGATACCATGCTGCCAGGTGAAGTGCATCATCACCACCATCACTAA

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