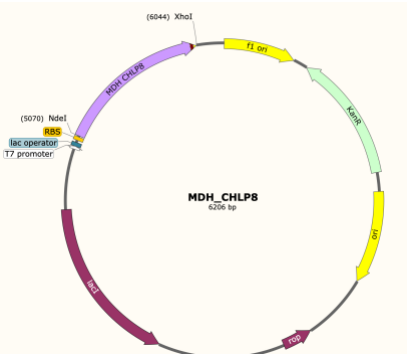
MCC Protein/Clone Information Sheet MDH_CHLP8

Protein Name: *Chlorobium vibrioforme* Malate Dehydrogenase (MDH_CHLP8)
Organism *Chlorobium vibrioforme isolated from bacterial strain NCIB 8327* **Plasmid Name**: pET24a MDH_CHLP8

Clone/Plasmid History: MDH gene was synthesized after codon-optimization MDH_CHLP8 (version 1, 2008) for expression in BL21 (DE3) and cloned into pET24a(+) vector using a NdeI/XhoI digested pET24a. The TEV recognition site was also added between the His tag and MDH. Both the TEV and the His tag are C terminus of MDH_CHLP8. 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 MDH_CHLP8.

NCBI / Gene Accession Number: NC_011027.1 *Because the yeast MDH gene was synthesized and codon optimized as described above its, nucleotide sequence differs from that published in Gene Bank.* There is no current single gene record, only the entire genome sequence. If interested in the genomic sequence, a search could be performed using a similar MDH of this database.

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: WP_012502825.1

UniProt Knowledge Base Accession: B3QPY9 (MDH_CHLP8)

RCSB PDB Page: AlphaFold (AF-B3QPY9-F1) CHLP8 (actual model does not exist. This file is a predicted model using another chlorophil bacterium with 97.7% sequence homology as template)

Key Publications: Malate dehydrogenase from Chlorobium vibrioforme, Chlorobium tepidum, and Heliobacterium gestii: purification, characterization, and investigation of dinucleotide binding by dehydrogenases…, and Malate dehydrogenase from the mesophile Chlorobium vibrioforme and from the mild thermophile Chlorobium tepidum…

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

Protein Notes: *Chlorobaculum parvum (isolated from bacterial strain NCIB 8327): Chlorobaculum parvum*, formerly *Chlorobium vibrioforme* subsp. *thiosulfatophilum*, is a green sulfur photosynthetic bacterium which contains bacteriochlorophyl d and the caratenoid chlorobactene. Reported both as a heterotetramer (dimer of dimers) and as a homodimer. Very little is published and is partially of interest due to divergent homology with other MDH isoforms as this is more similar to LDH than other MDH isoforms with "glycine motifs". A hybrid of this and a thermohillic MDH, this enzyme shows an increased heat stability significantly compared to MDH from other strains. The specific aa involved in the stability were not determined. MDH_CHLP8 is a 310 amino acid (plus the TEV site and 6X His tag on the C terminus). MDH_CHLP8 is a homodimer and has a monomer predicted mw = 33.27 kDa. Km (OAA) 59µM

Key amino acids / functions studied include: Nucleotide binding 7-12 and 117-119

Clone FAQ and Important Points: Expression of this construct is moderate to low at 20°C with an overnight expression. 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 low but measurable. May need to alter conditions to measure activity.
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.

ATGAAGATCACTGTTATCGGTGCTGGTAACGTGGGTGCAACTACTGCATTCCGTATCGCAGACAAGAAACTGGCTCGCGAACTGGTGCTGCTGG
ACGTGGTGGAGGGTATTCCGCAGGGCAAAGGTCTGGATATGTATGAAACTGGTCCGGTTGGCCTGTTCGATACCAAGATCACTGGCAGCAACG
ACTACGCAGACACTGCAGATTCTGACATTGTTATCATTACTGCTGGCCTGCCACGCAAACCAGGTATGACCCGTGAAGACCTGCTGATGAAGAAC
GCTGGTATCGTTAAGGAAGTAACCGATAACATCATGAAACACTCCAAGAATCCGATCATCATCGTAGTATCTAACCCACTGGACATCATGACCCA
TGTTGCTTGGGTTCGTTCCGGTCTGCCGAAAGAACGCGTTATCGGTATGGCTGGTGTGCTGGATGCAGCACGTTTCCGTAGCTTCATCGCTATGG
AGCTGGGTGTTAGCATGCAGGACATCAACGCGTGTGTGCTGGGTGGTCATGGTGACGCTATGGTGCCAGTTGTGAAGTATACCACTGTTGCTGG
TATCCCGATCTCCGATCTGCTGCCAGCTGAAACCATCGACAAACTGGTTGAACGTACCCGTAACGGTGGTGCAGAGATCGTTGAACATCTGAAGC
AGGGTTCTGCGTTCTACGCTCCAGCATCCTCTGTTGTTGAGATGGTAGAATCTATTGTGCTGGATCGTAAACGCGTACTGCCGTGTGCCGTTGGT
CTGGAAGGTCAGTACGGTATCGACAAGACCTTTGTGGGTGTTCCAGTGAAACTGGGTCGCAATGGCGTAGAACAAATCTACGAAATCAACCTGG
ATCAGGCTGACCTGGATCTGCTGCAGAAGTCTGCGAAGATCGTGGATGAGAACTGTAAGATGCTGGAGAGCACCATCGGTGAGAACCTGTACT
TCCAAGGTCATCATCACCACCATCAC

~~M~~4KITVIGAGNVGATTAFRIADKKLARELVLLDVVEGIPQGKGLDMYETGPVGLFDTKITGSNDYADTADSDIVIITAGLPRKPGMTREDLLMKNAGIVKE
VTDNIMKHSKNPIIIVVSNPLDIMTHVAWVRSGLPKERVIGMAGVLDAARFRSFIAMELGVSMQDINACVLGGHGDAMVPVVKYTTVAGIPISDLLPA
ETIDKLVERTRNGGAEIVEHLKQGSAFYAPASSVVEMVESIVLDRKRVLPCAVGLEGQYGIDKTFVGVPVKLGRNGVEQIYEINLDQADLDLLQKSAKIV
DENCKMLESTIGENLYFQGHHHHHH