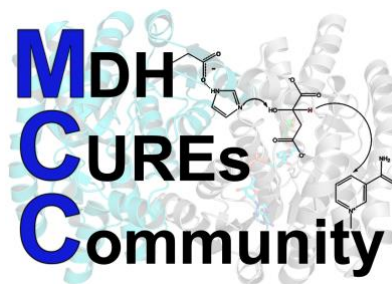


MCC Protein/Clone Information Sheet MDHM_YEAST



Protein Name: *Saccharomyces cerevisiae* mitochondrial Malate Dehydrogenase (MDHM_YEAST)

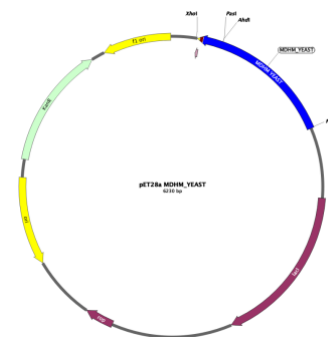
Organism: *S. cerevisiae*, Yeast Strain S288c ATCC 204508, Baker's Yeast.

Plasmid Name: pET28a MDHM_YEAST *Alternative Names:* Yeast MDH1, ymMDH

Clone/Plasmid History: *NOTE – MDH nomenclature in yeast is different from human. Yeast MDH1 is mitochondrial while human MDH1 is cytoplasmic.* The yeast gene name is Mdh1p. This MDH gene (aa 18-334) was synthesized after codon-optimization using Uniprot: P17505 (MDHM_YEAST) version 2, 1994 amino acid sequence but lacking the first 17 amino acids constituting the mitochondrial transit sequence. The synthesized gene was cloned into a pET28 vector using a NcoI/XhoI digested pET28a. *The N-terminal mitochondrial transit peptide (amino acids 1-17; MLSRVAKRAFSSVAVNP) is normally removed in vivo after MDH is synthesized in the cytosol and was not included in this construct. An additional residue (ATG: M) was added before the first coding amino acid to ensure proper start.* The initial few amino acids of this clone are "MYKVTVL..." representing the mature mitochondrial version of MDH. *Because the gene is synthesized and codon optimized, the nucleotide sequence will not match the nucleotide accession number.* A TEV recognition site was also added C terminal of MDH followed by a His Tag. Both the TEV and the His tag are C terminus of MDHM_YEAST.

NCBI / Gene Accession Number : *Because the yeast 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 MDHM_YEAST 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_001179651.1](https://www.ncbi.nlm.nih.gov/nuccore/NM_001179651.1)

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 promoters, 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 MDHM_YEAST protein sequence as expressed in yeast (with the mitochondrial targeting sequence included) can be found at [NP_012838.1](https://www.ncbi.nlm.nih.gov/protein/NP_012838.1)

UniProt Knowledge Base Accession: [P17505 \(MDHM_YEAST\)](https://www.uniprot.org/uniprot/P17505)

RCSB PDB Accession: The structure includes transit peptide bound to processing protease can be found here: [1HR9](https://www.rcsb.org/structure/1HR9) The predicted model using the same protein deleting MPP processing protease [P17505](https://www.rcsb.org/structure/P17505)

Key Publications: [Yeast mitochondrial dehydrogenases are associated in a supramolecular complex, The malate-aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restricted-mediated life span extension in yeast, and Transcriptional response according to strength of calorie restriction in S. cerevisiae.](#)

Protein Notes: *Saccharomyces cerevisiae* is a unicellular fungus. It is commonly known as baker's, brewer's or budding yeast. Mitochondrial MDH in yeast is MDH1. Expression highly changes based on metabolite starting point and its involvement in the asp-malate shuttle in yeast extends replicative lifespan and may play a role in calorie-restricted life extension. Evidence also points to mito yeast MDH is in a large complex with many (most?) other dehydrogenases. This protein [interacts](#) with 117 other yeast mitochondrial proteins. MDHM_YEAST is a 334 amino acid (plus the TEV site and 6X His tag on the C terminus). Predicted mw = 35.65 kDa.

Key amino acids / functions studied include: 24-30 and 135-137 nucleotide binding. S177 and T199 both phosphorylated.

Clone FAQ and Important Points: This is a modest to weak expressor at 37°C 4-6 hrs or 20°C with an overnight incubation. Expression conditions have not been optimized. pET28a (Novagen) is a low copy plasmid (~40) and will not give high yields of DNA preps. The activity in standard enzyme reaction conditions (100 µM NADH/200µM OAA has not been tested. Do not freeze thaw purified protein. Purification easily performed in column or batch format. Stable at 4°C for 6-8 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). Inclusion of 0.2 - 1 mM β-ME may be added at user's discretion. DO NOT FREEZE UNTIL DIALYSIS AND AFTER INCLUSION OF GLYCEROL.

CODING REGION

ATGTACAAGGTTACTGTACTGGGTGCAGGTGGTGGCATTGGTCAACCGCTGTCCCTGCTGCTGAAGCTGAACCACAAAGTTACCGACCTGCGTCTGT
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EKGVNFVASKENLYFQGHHHHHH