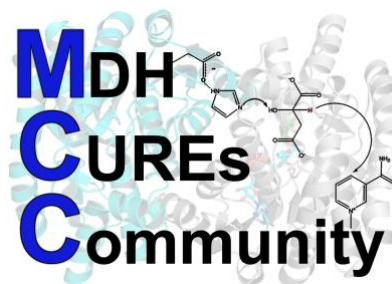


MCC Protein/Clone Information Sheet MDHP_YEAST



Protein Name: *Saccharomyces cerevisiae* peroxisomal Malate Dehydrogenase

(MDHP_YEAST) **Organism:** Yeast Strain S288c ATCC 204508 Baker's Yeast. **Plasmid Name:** pET28a MDHP_YEAST

Alternative Names: Yeast MDH3, ypMDH

Clone/Plasmid History: NOTE – numbering system in yeast is different from human. Yeast MDH2 is cytosolic while human MDH2 is mitochondrial. This clone is peroxisomal Yeast MDH3. The yeast gene name is Mdh3p. The last 3 C-terminal residues (SKL) are the peroxisomal transit signal and were left in the final version. This MDH gene was synthesized after codon-optimization for expression in BL21(DE3) using the sequence from P32419 (MDHP_YEAST) version 3, 2007. The synthesized gene was cloned into pET28 vector using a NcoI/XhoI digested pET28a. The TEV recognition site was also added between the His tag and MDH. Both the TEV and the His tag are C terminus of MDHP_YEAST. The N terminus remains unaltered. Because the gene is synthesized and codon optimized, the nucleotide sequence will not match the nucleotide accession number.

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 MDHP_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 001180137.1](https://www.ncbi.nlm.nih.gov/nuccore/001180137.1)

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 MDHP_YEAST protein sequence as expressed in yeast (with the peroxisomal targeting sequence included) can be found [NP_010205.1](https://www.ncbi.nlm.nih.gov/protein/010205.1)

UniProt Knowledge Base Accession: [P32419 \(MDHP_YEAST\)](https://www.uniprot.org/uniprot/P32419)

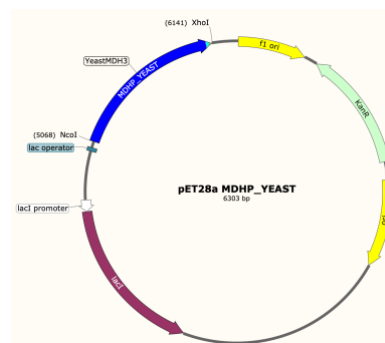
RCSB PDB Page: [5Z13](https://www.rcsb.org/structure/5Z13) (Apo), [5Z12](https://www.rcsb.org/structure/5Z12) (with NAD+), [5Z14](https://www.rcsb.org/structure/5Z14) (with OAA & NAD)

Key Publications: [Structure of glyoxysomal malate dehydrogenase \(MDH3\) from *S. cerevisiae*](#), [Isolation and characterization of the yeast gene encoding the MDH3 isozyme of malate dehydrogenase](#), [Metabolic effects of altering redundant targeting signals for yeast mitochondrial malate dehydrogenase.](#)

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

Protein Notes: *Saccharomyces cerevisiae* is a unicellular fungus. It is commonly known as baker's, brewer's or budding yeast. Both peroxisomes and glyoxysomes are microbodies of yeast sharing many of the same proteins. Peroxisomes are DNA free organelles involved with hydrogen peroxidases involved in fatty acid oxidation and degradation. Glyoxysomes in yeast are a specialized peroxisome containing the glyoxylate cycle. Peroxisomal (glyoxysomal) MDH in yeast is MDH3. The last 3 C-terminal amino acids are the import sequence. MDH3 has lower OAA affinity than MDH1 or MDH2 and the three structures help to identify the active site conformation changes. Readthrough to the C terminus targets MDH3 to peroxisomes but incomplete transcription leaves a soluble/cytosolic active fragment. This MDH is predicted and experimentally found to [interacts](#) with 163 other yeast proteins. MDHP_YEAST is a 343 amino acid (plus the TEV site and 6X His tag on the C terminus). This is a homodimer with a monomer predicted mw = 37.19 kDa. Km (OAA) 300 μ M (NADH (50 μ M)).

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. Kan Resistant. The activity in standard enzyme reaction conditions (100 μ M NADH/200 μ M OAA has not been tested. 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 but recommended conditions for freezing -20 to -80°C (10% 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.



ATGGTGAAAGTTGCTATTCTGGGTGCTTCTGGTGGTGTGGTCAACCACTGTCTCTGCTGCTGAAACTGTCTCCGTACGTTTCTGAG
CTGGCTCTGTACGACATTCGTGCTGCTGAGGGTATCGGCAAGGATCTGAGCCACATCAACACCAATTCCTCCTGTGTAGGTTATGAT
AAGGACAGCATCGAGAACACCCTGTCCAACGCACAAGTTGTAAGAGCCTGGTGACCGCTGTAGGCAAGTTCGCACCGAACGCTCGTATTCTGGTT
ATGATCTGTTCAAGATGAACGCTGGCATCGTTAAGAGCCTGGTGACCGCTGTAGGCAAGTTCGCACCGAACGCTCGTATTCTGGTT
ATCTCCAATCCGGTGAACAGCCTGGTGCCAATCGCAGTGGAACCCCTGAAGAAGATGGGCAAGTTCAAGCCAGGCAACGTTATGG
GTGTAACCAACCTGGATCTGGTGCCTGCAGAGACTTTCCTGGTGGACTACCTGATGCTGAAGAATCCGAAGATTGGCCAGGAACAG
GACAAGACCACTATGCACCGCAAAGTGACCGTGATTGGTGGTCATAGCGGTGAAACCATCATCCCGATCATCACTGACAAATCTCT
GGTGTTCAGCTGGACAAACAATACGAGCACTTCATCCACCGTGTTCAGTTCGGTGGCGATGAAATCGTGAAAGCGAAACAGGGT
GCTGGTTCGCTACTCTGAGCATGGCGTTCGCTGGTGGCAAGTTCGCTGAAGAAGTACTGCGTTCCTTCCACAATGAGAAACCGGA
AACTGAATCTCTGAGCGCATTTCGTTTACCTGCCAGGCCTGAAGAACGGTAAGAAAGCACAGCAGCTGGTTGGCGATAACTCTATCG
AATACTTTAGCCTGCCGATCGTTCTGCGCAATGGCTCTGTAGTTAGCATTGACACCAGCGTGTGGAGAAGCTGTCTCCACGCGAA
GAACAGCTGGTTAACACCGCAGTTAAGGAACTGCGTAAGAACATTGAGAAAGGCAAATCTTTCATTCTGGACAGCTCCAAACTGG
AGAACCTGTACTTCCAAGGTCATCATCACCACCATCAC

MVKVAILGASGGVQPLSLLKLSPLYVSELALYDIRAAEGIGKDLSHINTNSSCVGYDKSIENTLSNAQVVLIPAGVPRKPGLTRDDLK
MNAGIVKSLVAVGKFAPNARILVISNPVNSLVPIAVETLKKMGKFKPGNVMGVTNLDLVRAETFLVDYMLKPNKIGQEQDKTMMHR
KVTVIGGHSGETIPIITDKSLVFQDKQYEHFIHRVQFGGDEIVKAKQGAGSATLSMAFAGAKFAEEVLRFSHNEKPETESLSAFVYLPGL
KNGKKAQQLVGDNSIEYFSLPIVLRNGSVVSIDTSVLEKLSPREEQLVNTAVKELRKNIEKGSFILDS**SKLENLYFQGH**HHHHH