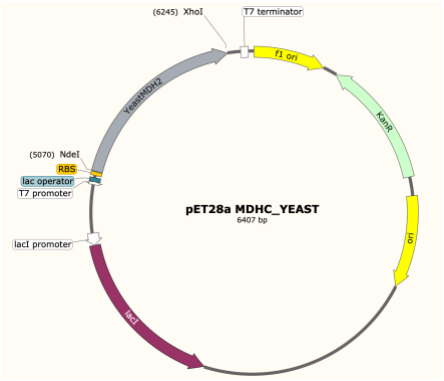
MCC Protein/Clone Information Sheet MDHC_YEAST

Protein Name: *Saccharomyces cerevisiae* cytosolic Malate Dehydrogenase (MDHC_YEAST)
Organism: Yeast Strain S288c ATCC 204508 Baker's Yeast. **Plasmid Name**: pET28a MDHC_YEAST Alternative name Cytoplasmic Yeast MDH2

Clone/Plasmid History: *NOTE – numbering system in yeast is different from human. Yeast MDH2is cytosolic while human MDH2 is mitochondrial.* The yeast gene name is Mdh2p. This MDH gene was synthesized after codon-optimization P22133 (MDHC_YEAST) version 2, 2007, for expression in BL21 (DE3) and cloned into pET28 vector using a NcoI/XhoI digested pET28a. The TEV recognition site was also added between the His tag and MDH. 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 MDHC_YEAST 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 MDHC_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 MDHC_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_001183380.1

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 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 MDHC_YEAST protein sequence as expressed in yeast can be found at: NP_014515.2

UniProt Knowledge Base Accession: P22133 (MDHC_YEAST)

RCSB PDB Page: P22133 (structure based on STML: yeast peroxisomal MDH3 with NAD+) AlphaFold Structure

Key Publications: Isolation, nucleotide sequence analysis, and disruption of the MDH2 gene from Saccharomyces cerevisiae: evidence for three isozymes of yeast MDH, Glucose-induced degradation of the MDH2 isozyme of malate dehydrogenase in yeast.

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. Cytosolic MDH in yeast is MDH2. The first 12 amino acids are targeted for degradation via direction of phosphorylation when yeast is grown in high glucose conditions. The lead 12 residues may not alter kinetics significantly (not well tested). MDH2 null yeast can grow depending on nutrition and thus might be a tool to measure interaction/regulation of cytolic MDH in eukaryotes. Interacts with 1168 other yeast proteins. MDHC_YEAST is a 377 amino acid (plus the TEV site and 6X His tag on the C terminus). Predicted mw = 40.73 kDa.

Key amino acids / functions studied include: 20-26 and 144-146 nucleotide binding. T6 is 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. Kan Resistant. 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 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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