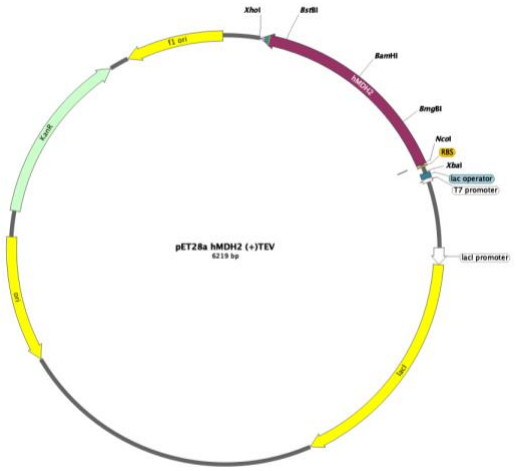
MCC Protein/Clone Information Sheet hMDH2

Protein Name: Mitochondrial Human Malate Dehydrogenase 2 (hMDH2) transcript variant 1
Organism: Homo sapiens (human) MDH2 **Plasmid Name**: pET28a hMDH2. Alternative names: hMDH2, hMDH2V1, and Uniprot MDHM_HUMAN.

Clone/Plasmid History: This MDH gene (aa 25-215) was synthesized after codon-optimization of human MDH2 transcript variant 1 without the first 24 amino acids (mito targeting sequence) using Uniprot: MDMH_HUMAN P46092 version 2, accessed May 2017. The sequence was generated lacking the first 24 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 1-24;* MLSALARPASAALRRSFSTSAQNN*) 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 "MAKVAVL…" representing the mature mitochondrial version of MDH. The clone was created June 13, 2017. 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_HUMAN. *Because the gene is synthesized and codon optimize, the nucleotide sequence will not match the nucleotide accession number*. Please refer to the associated snapgene file or FASTA formatted file for the DNA sequence of the coding region for hMDH2.

NCBI / Gene Accession Number: CR536548.1 *Because the the human mitochondrial 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 human mitochondrial MDH gene including the targeting sequence can be found at the accession number*.

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 hMDH2 protein sequence as expressed in yeast (with the mitochondrial targeting sequence included) can be found CAG38785.1 (hMDH2 with the targeting sequence, not included in this construct)

UniProt Knowledge Base Accession: P40926 (MDHM_HUMAN)

RCSB PDB Page: 4WLN, apo MDH, 4WLE (with citrate), 4WLF (with malate), 4WLO (with NADH and OAA), 2DFD (with malate and NAD)

Key Publications: Aggregation states of mitochondrial malate dehydrogenase.
Mutations in MDH2, Encoding a Krebs Cycle Enzyme, Cause Early-Onset Severe Encephalopathy ;
SIRT3-dependent GOT2 acetylation status affects the malate–aspartate NADH shuttle activity and pancreatic tumor growth,
MDH-CS multienzyme complex dynamics is affected by TCA cycle flux in living yeast

Protein Notes: This is variant 1 of the hMDH 2(mitochondrial) isoform. The version included in this construct does NOT include the mitochondrial targeting sequence (mlsalvrpvsaalrrsfstsaqnn). A Met has been added to the mature hMDH2V1 found in the mitochondria without the targeting sequence. Isoform 2 does not include 41 internal amino acids not present on MD21 isoform 1. hMDH2V1 is a 361 amino acid (with the additional glycine in the N term to maintain reading frame and both a TEV protease recognition site and a 6X His tag placed on the C terminus of hMDH2). Human MDH2V1 has a predicted mw = 34.8 kDa. hMDH2 is reported to be a dimer.

Clone FAQ and Important Points: Strong protein expression at 37°C 1mm IPTG for 3-4 hour induction. Stronger expression at 20°C (room temp) for 14-24 hrs. ~0.5 mg or more per ml of culture. Stronger expression at 20°C (room temp) for 14-24 hrs. pET28a (Novagen) is a low copy plasmid (~40) and will not give high yields of DNA preps. Kan Resistant. Do not freeze thaw purified protein. Purification easily performed in column or batch format. Concentrations approaching 1-1.25 mg/ml will precipitate over a short

time. Dilute immediately after purification and before dyalysis to 1 mg/ml or less. See MDH Stability Datasheet for more information. Stable at 4° for 6-8 weeks dialyzed against (10 mM K phosphate, 0.1 mM EDTA, 20% glycerol, pH 8.0). Long term storage -20 to -80°C (10% Glycerol, 50 mM NaCl, 1 mM β-ME in 10 mM K phosphate, pH 8.0). See MDH Stability Datasheet for more information.

See Snap Gene File for details.

Amino Acid Coding Sequence:

~~M~~AKVAVLGASGGIGQPLSLLLKNSPLVSRLTLYDIAHTPGVAADLSHIETKAAVKGYLGPEQLPDCLKGCDVVVIPAGVP
RKPGMTRDDLFNTNATIVATLTAACAQHCPEAMICVIANPVNSTIPITAEVFKKHGVYNPNKIFGVTTLDIVRANTFVAE
LKGLDPARVNVPVIGGHAGKTIIPLISQCTPKVDFPQDQLTALTGRIQEAGTEVVKAKAGAGSATLSMAYAGARFVFSLV
DAMNGKEGVVECSFVKSQETECTYFSTPLLLGKKGIEKNLGIGKVSSFEEKMISDAIPELKASIKKGEDFVKTLKENLYF
QGHHHHHH

Coding Plasmid Sequence:

TTAGTGATGGTGGTGATGATGACCCTGGAAGTACAGGTTCTCTTTCAGGGTCTTCACGAAATCTTCACCCTTCTTGATGC
TCGCTTTCAGTTCCGGAATTGCGTCGGAGATCATCTTCTCTTCGAAAGAAGAAACTTTACCGATGCCCAGGTTCTTCTCG
ATGCCTTTCTTGCCCAGCAGCAGTGGAGTGCTGAAGTAGGTGCATTCGGTTTCTTGAGACTTCACGAAGGAACATTCCAC
TACACCTTCCTTGCCGTTCATTGCGTCAACCAGAGAGAATACGAAACGAGCACCAGCGTAGGCCATGCTCAGAGTCGCAG
AGCCAGCACCTGCTTTCGCTTTCACCACTTCAGTGCCTGCCTCCTGGATACGACCAGTCAGAGCGGTCAGCTGGTCCTGT
GGGAAGTCTACTTTCGGAGTGCACTGGGAAATCAGCGGAATGATGGTCTTGCCAGCGTGACCACCGATTACCGGAACGTT
TACGCGAGCTGGATCCAGACCTTTCAGTTCAGCCACAAAGGTGTTTGCACGCACGATATCCAGGGTGGTAACACCGAAGA
TCTTGTTCGGGTTGTAAACACCGTGTTTCTTGAATACCTCGGCAGTAATCGGGATGGTGCTGTTAACCGGATTAGCAATC
ACACAGATCATAGCTTCCGGACAGTGCTGAGCGCAAGCAGCGGTCAGGGTCGCAACGATAGTCGCATTAGTATTGAACAG
GTCATCACGGGTCATACCTGGTTTACGTGGAACACCTGCTGGGATAACTACCACGTCACAACCTTTCAGACAATCTGGCA
GCTGTTCTGGACCCAGGTAGCCTTTCACCGCAGCTTTAGTCTCAATGTGGGACAGGTCAGCAGCAACGCCTGGAGTATGT
GCGATATCGTACAGGGTCAGACGGCTAACCAGTGGAGAGTTCTTCAGCAGCAGGGACAGCGGTTGACCAATACCACCAGA
TGCACCCAGAACAGCTACTTTAGCCAT