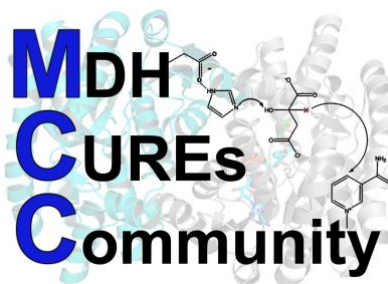


# MCC Protein/Clone Information Sheet hMDH1v3



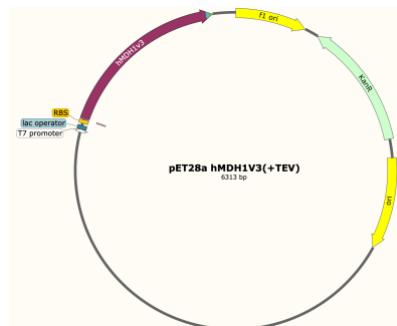
**Protein Name:** Cytosolic Human Malate Dehydrogenase isoform 1 transcript variant 3 (hMDH1v3)

**Organism:** Homo sapiens (human) MDH1 **Plasmid Name:** pET28a hMDH1V3 +TEV. Alternative names: hMDH1v3 and hMDH1V3(+TEV). The Uniprot ID is P40925 MDHC\_HUMAN.

**Clone/Plasmid History:** This is a modification of the hMDH1v3 Human Malate dehydrogenase 1 gene. The original clone was PCR amplified from human brain cDNA pool (purchased from Biochain Inc.). cDNA was re-amplified with added restriction sites (NcoI at 5' and XhoI at 3') and sub-cloned into pET28a expression vector. Glycine added after MET for restriction enzyme and maintained expression frame with the Kozak sequence. A TEV recognition site was added by Gibson cloning, C terminal of MDH followed by a His Tag. Both the TEV and the His tag are C terminus of hMDH1V3. *Because the gene is synthesized and codon optimize, the nucleotide sequence will not match the nucleotide accession number.*

**NCBI / Gene Accession Number:** [NM\\_00119911.1](#) This record states variant 2 but is not considered variant 3. Use this for the human gene sequence, but may not match the sequence for this clone. Please refer to the associated snapgene file or FASTA formatted file linked below for the DNA sequence of the coding region

**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 hMDH1V3C protein sequence as expressed in human tissue can be found at [NP\\_001186040.1](#) This sequence is recorded on NCBI as MDH variant 2. It is NOT variant 2. Using the Uniprot designation hMDH1V3 is the third splice variant of the cytosolic MDH isoform.

**UniProt Knowledge Base Accession:** [MDHC\\_HUMAN P40925](#) The Uniprot page includes information for all three transcripts. This MDH transcript variant is NOT considered the canonical isoform. See MDH variant alignment map for details.

**RCSB PDB Accession:** [7RM9](#) (human MDH), [5MDH](#) (porcine cytosolic MDH with NAD and OAA analogue), [MDHC\\_HUMAN](#) (AlphaFold predicted model using 5MDH with NAD and MAK (OAA analogue)).

**Key Publications:** [Molecular Cloning and Mapping of a Human cDNA for Cytosolic Malate Dehydrogenase \(MDH1\)](#), [Structural basis of substrate specificity in malate dehydrogenases: crystal structure of a ternary complex of porcine cytoplasmic malate dehydrogenase, alpha-ketomalonnate and tetrahydroNAD](#)

**Protein Notes:** This human cytosolic MDH isoform 1 variant 3 (hMDH1v3). Isoform 3 has an additional 89 amino acids on the N terminus that is not present on the canonical MDH1 variant 2. The additional amino acids are due to different 5' terminal exon resulting in a translation variation initiation from an alternative start codon compared to variant 1. Isoform 3 is identical to isoform 2 with an additional M not cleaved on the N terminus. hMDH1v3(+TEV) is a 368 amino acid (with an additional glycine in the N term to maintain reading frame and the 6X His tag on the C terminus). Human MDH1 is a homodimer. The monomer with TEV and His tag, has a predicted mw = 40.61 kDa.

**Key amino acids / functions studied include:** OAA sites by similarity to MDH2 92, 98, 131, and 162. NAD binding 105 and 112 using variant 1 for numbering. See alignment document for more details.

**Clone FAQ and Important Points:** Reasonable protein expression at 37°C 1mM IPTG for 3-4 hour induction. ~0.2-0.5 mg 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. Stable at 4°C for 1-4 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.

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