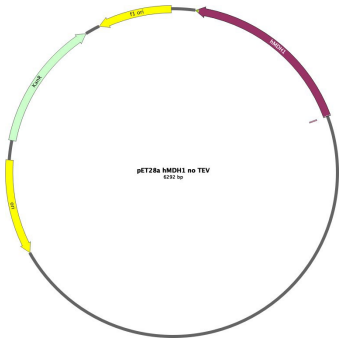
human Cytosolic Protein/Clone Information Sheet hMDH1v3 (- TEV)

Protein Name: Cytosolic Human Malate Dehydrogenase isoform 1 transcript variant 3 (hMDH1v3)
Organism: Homo sapiens (human) MDH1 **Plasmid Name**: pET28a hMDH1 no TEV aka hMDH1v3

Clone/Plasmid History: Human Malate dehydrogenase 1 gene 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. There is no thrombin or TEV sites between the His tag and MDH. This was initially identified as variant 2 but the NCBI nomenclature has changed. The current nomenclature (and correct) is splice variant 3 of human MDH1.

NCBI / Gene Accession: https://www.ncbi.nlm.nih.gov/gene/

Downloadable SnapGene Plasmid Map: Resistance, Promotor (for bacterial or mammalian), Sequencing primers, RBS and Kozak sequence, History of cloning, Annotated start and stop of protein, Highlighted tags or TEV/Thrombin sites.



NCBI Protein Sequence Accession:

Isoform 1: https://www.ncbi.nlm.nih.gov/protein/NP_005908.1

Isoform 2: https://www.ncbi.nlm.nih.gov/protein/NP_001186040.1

Isoform 3: https://www.ncbi.nlm.nih.gov/protein/NP_001186041.1

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Isoform_1      ------------------MSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLLD     42
Isoform_3      ------------------------------------------------------------     0
Isoform_2      MRRCSYFPKDVTVFDKDDKSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLLD     60

Isoform_1      ITPMMGVLDGVLMELQDCALPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMERKDLL     102
Isoform_3      ----------------------------------------------MPRREGMERKDLL     13
Isoform_2      ITPMMGVLDGVLMELQDCALPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMERKDLL     120
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Isoform_1      KANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASKSAPSIPKENFSCLTRLDHNR     162
Isoform_3      KANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASKSAPSIPKENFSCLTRLDHNR     73
Isoform_2      KANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASKSAPSIPKENFSCLTRLDHNR     180
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Isoform_1      AKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVYEALKDDSWLKGE     222
Isoform_3      AKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVYEALKDDSWLKGE     133
Isoform_2      AKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVYEALKDDSWLKGE     240
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Isoform_1      FVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGVPD     282
Isoform_3      FVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGVPD     193
Isoform_2      FVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGVPD     300
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Isoform_1      DLLYSFPVVIKNKTWKFVEGLPINDFSREKMDLTAKELTEEKESAFEFLSSA     334
Isoform_3      DLLYSFPVVIKNKTWKFVEGLPINDFSREKMDLTAKELTEEKESAFEFLSSA     245
Isoform_2      DLLYSFPVVIKNKTWKFVEGLPINDFSREKMDLTAKELTEEKESAFEFLSSA     352
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UniProt Protein Page:

Isoform 1: https://www.uniprot.org/uniprotkb/P40925/entry

Isoform 2:

Isoform 3: https://www.uniprot.org/uniprotkb/A0A5K1VW95/entry

MDHC_HUMAN P40925 The uniprot page includes information for all three transcripts. This MDH transcript variant is NOT considered the canonical isoform. The hMDH1v3 clone is for splice variant 3 which has an extended N term compared to the canonical splice variant 1. See MDH variant alignment map for details.

RCSB PDB Page: 7RM9.pdb

Key Publications:

"Structural Characterization of the Human Cytosolic Malate Dehydrogenase I."**McCue, W.M.**, **Finzel, B.C.** (2022) ACS Omega **7**: 207-214, DOI: 10.1021/acsomega.1c04385

Molecular Cloning and Mapping of a Human cDNA for Cytosolic Malate Dehydrogenase (MDH1), T Tanaka [1], J Inazawa, Y Nakamura, Genomics, 1996 Feb 15;32(1):128-30. doi: 10.1006/geno.1996.0087

Structural basis of substrate specificity in malate dehydrogenases: crystal structure of a ternary complex of porcine cytoplasmic malate dehydrogenase, alpha-ketomalonate and tetrahydoNAD" A D Chapman [1], A Cortés, T R Dafforn, A R Clarke, R L Brady, J Mol Biol, 1999 Jan 15;285(2):703-12. doi: 10.1006/jmbi.1998.2357

Birktoft JJ, Rhodes G, Banaszak LJ. Refined crystal structure of cytoplasmic malate dehydrogenase at 2.5-A resolution. Biochemistry. 1989 Jul 11;28(14):6065-81. doi: 10.1021/bi00440a051. PMID: 2775751.

Available Mutations: None at this time, will become available upon publication. A version of hMDH1v3 with TEV inserted between the His tag and MDH is available as will hMDH1v1 and v2 with TEV.

Protein Notes: This human cytosolic MDH isoform 1 variant 3 (hMDH1v3). Isoform 3 has an additional 89 amino acids on the N terminus 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. hMDH1 no TEV is a 353 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 (no tev) is a homodimer and the monomer has a predicted mw = 39.75 kDa.. Biologically active as a dimer.

Human Cytosolic MDH construct:
pI = 7.14/ ε_{280} , = 0.853mL.mg^{-1}.cm^{-1} extinction coefficient (280 nm: calculated using ProtParam.)

Key amino acids / functions studied include:

Residue	Flexible Loop	Aspartate	Arginine	Arginine	Aspartate	Arginine	Histidine
Watermelon-g MDH 1sev/1smk Equivalent	117-140	D77	R124	R130	D193	R196	H220
hCytosolic 7rm9.pdb or 7rm9repaired.pdb	85-108	D42	R92	R98	D159	R162	H187
hCytoConstruct.pdb		D61	R111	R117	D178	R1181	H226
hMitochondrial 2DFD.pdb	79-102	D39	R86	R92	D155	R158	H182
hMitoConstruct.pdb		D34	R81	R87	D150	R153	H177
Plasmodium falciparum: 5NFR.pdb	74-97	D32	R81	R87	D147	R150	H174
Ignicoccus Islandicus 6qss,pdb	77-100	D37	R86	R92	D151	R154	H178
Function	Closes over active site on substrate binding	Governs specificity for NAD(H)	Malate/Oxaloacetate/Citrate Binding	Malate/Oxaloacetate/Citrate Binding	Alters Basicity of Catalytic Histidine	Malate/Oxaloacetate/Citrate Binding	Catalytic Base

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 }-ME in 10 mM K phosphate, pH 8.0). See MDH Stability Datasheet for more information

>hCyto Construct Amino Acid Coding Sequence

MGRRCSYFPKDVTVFDKDDKSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLLDITPMMGVLDGVLMELQDCA
LPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMERKDLLKANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTAS
KSAPSIPKENFSCLTRLDHNRAKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVYEALKDDSWLKG
EFVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGVPDDLLYSFPVVIKNKTWKFVE
GLPINDFSREKMDLTAKELTEEKESAFEFLSSALEHHHHHH

>pdb|7RM9|B Chain B, Malate dehydrogenase, cytoplasmic

MSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLLDITPMMGVLDGVLMELQDCALPLLKDVIA
TDKEDVAFKDLDVAILVGSMPRREGMERKDLLKANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTA
SKSAPSIPKENFSCLTRLDHNRAKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVY
EALKDDSWLKGEFVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGV
PDDLLYSFPVVIKNKTWKFVEGLPINDFSREKMDLTAKELTEEKESAFEFLSSA

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hCyto          MGRRCSYFPKDVTVFDKDDKSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLL    60
pdb|7RM9|B     ------------------MSEPIRVLVTGAAGQIAYSLLYSIGNGSVFGKDQPIILVLL    41
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hCyto          DITPMMGVLDGVLMELQDCALPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMERKDL    120
pdb|7RM9|B     DITPMMGVLDGVLMELQDCALPLLKDVIATDKEDVAFKDLDVAILVGSMPRREGMERKDL    101
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hCyto          LKANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASKSAPSIPKENFSCLTRLDHN    180
pdb|7RM9|B     LKANVKIFKSQGAALDKYAKKSVKVIVVGNPANTNCLTASKSAPSIPKENFSCLTRLDHN    161
               ************************************************************

hCyto          RAKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVYEALKDDSWLKG    240
pdb|7RM9|B     RAKAQIALKLGVTANDVKNVIIWGNHSSTQYPDVNHAKVKLQGKEVGVYEALKDDSWLKG    221
               ************************************************************

hCyto          EFVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGVP    300
pdb|7RM9|B     EFVTTVQQRGAAVIKARKLSSAMSAAKAICDHVRDIWFGTPEGEFVSMGVISDGNSYGVP    281
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hCyto          DDLLYSFPVVIKNKTWKFVEGLPINDFSREKMDLTAKELTEEKESAFEFLSSALEHHHHH    360
pdb|7RM9|B     DDLLYSFPVVIKNKTWKFVEGLPINDFSREKMDLTAKELTEEKESAFEFLSSA-------    334
               ************************************************************

hCyto          H       361
pdb|7RM9|B     -       334
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Coding Plasmid Sequence:

tcagtggtggtggtggtggtgctcgagggcagaggaaagaaattcaaaagcactttctttttcttctgtcagttcctttg
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