Plasmodium falciparum MDH Clone-Protein Information Sheet MDH_PFALCI

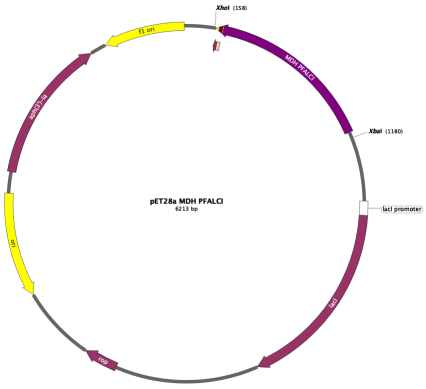
Protein Name: Malate Dehydrogenase (MDH_PFALCI)

Organism: *Plasmodium falciparum* (isolate 3D7) **Plasmid Name**: pET28a MDH_PFALCI Alternative Names: MDH PLAF7 & PfMDH

Clone/Plasmid History: *PFALCI is not a Uniprot number. The Uniprot name is "C6KT25_PLAF7".* MDH gene was synthesized after codon-optimization for expression in BL21 (DE3) using the Uniprot record C6KT25_PLAF7 accessed August 2018. 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 MDH_PFALCI. The N terminus remains unaltered. *Because the gene is synthesized and codon optimize, the nucleotide sequence will not match the nucleotide accession number*. 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 MDH_PFALCI.

NCBI / Gene Accession Number: https://www.ncbi.nlm.nih.gov/gene/3885804. *Because the 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 gene 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.

Downloadable SnapGene 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 MDH_PFALCI protein sequence as expressed in *Plasmodium falciparum* can be found at https://www.ncbi.nlm.nih.gov/protein/AAQ23154.1

UniProt Knowledge Base Accession:
https://www.uniprot.org/uniprotkb/C6KT25/entry#sequences
Is the entry for the Plasmodium falciparum Isolate 3D7, named as a Lactate Dehydrogenase but has the same amino acid sequence as the Malate Dehydrogenase.

RCSB PDB Accession: 5NFR.pdb: https://www.rcsb.org/structure/5NFR

Key Publications:

Tripathi AK, Desai PV, Pradhan A, Khan SI, Avery MA, Walker LA, Tekwani BL. An alpha-proteobacterial type malate dehydrogenase may complement LDH function in Plasmodium falciparum. Cloning and biochemical characterization of the enzyme. Eur J Biochem. 2004 Sep;271(17):3488-502. doi: 10.1111/j.1432-1033.2004.04281.x. PMID: 15317584.

Lunev S, Butzloff S, Romero AR, Linzke M, Batista FA, Meissner KA, Müller IB, Adawy A, Wrenger C, Groves MR. Oligomeric interfaces as a tool in drug discovery: Specific interference with activity of malate dehydrogenase of Plasmodium falciparum in vitro. PLoS One. 2018 Apr 25;13(4):e0195011. doi: 10.1371/journal.pone.0195011. PMID: 29694407; PMCID: PMC5919072.

Natalie Botros, Ellis Bell, & Jessica Bell, **The existence of a Cryptic Allosteric Site on Plasmodium falciparum Malate Dehydrogenase.**, First published: 18 April 2020. **https://doi.org/10.1096/fasebj.2020.34.s1.05326**

Natalie Botros, Ellis Bell, & Jessica Bell., **Potential Drug Design for Plasmodium falciparum Malate Dehydrogenase Targeting the Cryptic Allosteric Site.**: 14 May 2021
https://doi.org/10.1096/fasebj.2021.35.S1.02936

Daniel Armendariz, Diego Hernandez, Megan Keene, Jessica Bell, & Ellis Bell., Exploring the role of the dimer interface in *Plasmodium falciparum* malate dehydrogenase: The impact of Q11I, I15Q, L19N and L22N mutations on quaternary structure and enzymatic properties.. *Journal of Biological Chemistry*Vol. 299Issue 3SupplementPublished in issue: 2023. https://doi.org/10.1016/j.jbc.2023.103634

Diego Hernandez, Jessica Bell, & Ellis Bell., Using enzyme kinetics and computational docking studies to understand substrate and inhibitor interactions with human mitochondrial, human cytosolic, watermelon glyoxysomal and Plasmodium falciparum malate dehydrogenases., https://doi.org/10.1016/j.jbc.2023.103638

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

Protein Notes: *Plasmodium falciparum (isolate 3D7)* is a unicellular protozoan parasite of humans, and the deadliest species of Plasmodium that cause malaria in humans. This MDH is a tetramer. Mutations in the oligomeric site of this MDH that decrease dimers resulted in a loss of oligomerization and activity, while mutants that stabilize the oligomerization increased thermal stability and activity (see reference). Unlike some MDH isoforms, OAA inhibition is not reported, but could be seen under different assay conditions. The current Uniprot records label as lacate dehydrogenase, a pBlast of this protein shows it is MDH. pH optimum for OAA -> malate is 7.0 while in the reverse direction pH optimum is 10.0. Km (OAA) 0.03 mM (NADH) 0.036 mM (malate) 1.35 mM (NAD 0.152 mM). This clone includes a TEV site between the His tag and the coding protein on the C terminus of MDH_PFALCI. The Plasmodium falciparum Malate Dehydrogenase is biologically active as a tetramer.

MW(subunit/biological)/pI/ ε_{280} , extinction coefficient (280 nm: calculated using ProtParam.) of protein (WT and/or specific mutant):

Plasmodium falciparum: **MWt**: 35,715/142,860, **pI**(theoretical): 6.89 ε_{280} 0.375 $mL.mg^{-1}.cm^{-1}$

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

A crystal structure of the tetramer is found in the Protein Data Base (https://www.rcsb.org/structure/5NFR) which has 4 tetramers in the asymmetric unit but several of the tetramers have chains with breaks in one or more of the four chains. We have generated a version of the

tetramer without chain breaks suitable for computational studies(5nfr-tetramer.pdb) and have generated a Landmarks pse file showing critical structural and functional features (*5nfr-Landmarks.pse*)

Clone FAQ and Important Points: Modest protein expression at 37°C 1mm IPTG for 3-4 hour induction 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. This is a high yield – range from 35-90 mg of purified protein per 1000 ml. Dilute after purification to ~0.9 mg/ml or aggregation/precipitation will occur overnight. Stable at 4°C for 4 weeks dialyzed against (10 mM K phosphate, 0.1 mM EDTA, pH 8.0). Long term storage in glycerol > 6 weeks. Recommended -20 to -80°C (10-20% Glycerol, 50 mM NaCl, 10 mM K phosphate, pH 8.0).

Construct Amino Acid Coding Sequence:

~~M~~TKIALIGSGQIGAIVGELCLLENLGDLILYDVVPGIPQGKALDLKHFSTILGVNRNILGTNQIEDIKDADIIVITAGVQRKEGMTREDLIGVNGKIMKSVAE
SVKLHCSKAFVICVSNPLDIMVNVFHKFSNLPHEKICGMAGILDTSRYCSLIADKLKVSAEDVNAVILGGHGDLMVPLQRYTSVNGVPLSEFVKKNMISQ
NEIQEIIQKTRNMGAEIIKLAKASAAFAPAAAITKMIKSYLYNENNLFTCAVYLNGHYNCSNLFVGSTAKINNKGAHPVEFPLTKEEQDLYTESIASVQSNT
QKAFDLIK**ENLYFQGHHHHHH**

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Pfalci-MDH      MTKIALIGSGQIGAIVGELCLLENLGDLILYDVVPGIPQGKALDLKHFSTILGVNRNILG    60
pdb|5NFR|P      MTKIALIGSGQIGAIVGELCLLENLGDLILYDVVPGIPQGKALDLKHFSTILGVNRNILG    60
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Pfalci-MDH      TNQIEDIKDADIIVITAGVQRKEGMTREDLIGVNGKIMKSVAESVKLHCSKAFVICVSNP    120
pdb|5NFR|P      TNQIEDIKDADIIVITAGVQRKEGMTREDLIGVNGKIMKSVAESVKLHCSKAFVICVSNP    120
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Pfalci-MDH      LDIMVNVFHKFSNLPHEKICGMAGILDTSRYCSLIADKLKVSAEDVNAVILGGHGDLMVP    180
pdb|5NFR|P      LDIMVNVFHKFSNLPHEKICGMAGILDTSRYCSLIADKLKVSAEDVNAVILGGHGDLMVP    180
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Pfalci-MDH      LQRYTSVNGVPLSEFVKKNMISQNEIQEIIQKTRNMGAEIIKLAKASAAFAPAAAITKMI    240
pdb|5NFR|P      LQRYTSVNGVPLSEFVKKNMISQNEIQEIIQKTRNMGAEIIKLAKASAAFAPAAAITKMI    240
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Pfalci-MDH      KSYLYNENNLFTCAVYLNGHYNCSNLFVGSTAKINNKGAHPVEFPLTKEEQDLYTESIAS    300
pdb|5NFR|P      KSYLYNENNLFTCAVYLNGHYNCSNLFVGSTAKINNKGAHPVEFPLTKEEQDLYTESIAS    300
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Pfalci-MDH      VQSNTQKAFDLIKENLYFQGHHHHHH      326
pdb|5NFR|P      VQSNTQKAFDLIKGHHHHHH------      320
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Coding Region for MDH Plasmid Sequence:

atgaccaagatcgctctgatcggttctggccaaatcggtgcaattgtaggcgaactgtgtctgctggagaacctgggtgacctgatcctgtacgacgtagttccaggtattccgcaaggtaaag
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ccaagaaatcatccagaagactcgtaacatgggtgcggagatcatcaaactggcgaaagcctctgctgctttcgcaccagcagcagctatcaccaagatgatcaagtcctacctgtacaacg
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