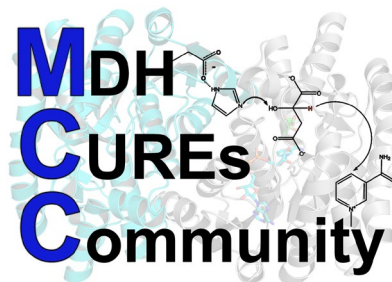


# Ignicoccus Islandicus MDH Protein/Clone Information Sheet MDH\_9CREN



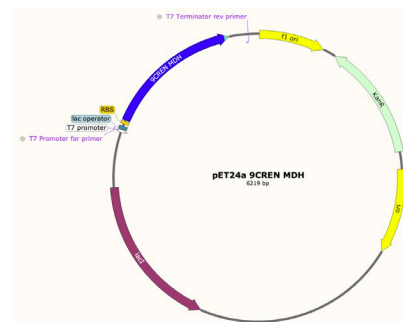
**Protein Name:** *Ignicoccus islandicus* Malate Dehydrogenase (A0A0U3FQH7\_9CREN)

**Organism:** *Ignicoccus islandicus* DSM 13165 **Plasmid Name:** pET24a MDH\_9CREN **Alternate name:** MDH\_9CREN

**Clone/Plasmid History:** MDH gene was synthesized after codon-optimization A0A0U3FQH7\_9CREN version 1 2016, for expression in BL21 (DE3) and cloned into pET24a vector using a Nde1/EcoR1 digested pET24a. The affinity His tag was inserted C-terminal to the MDH gene. Unlike other clones in the MCC, there is NO TEV SITE in this construct. The N terminus remains unaltered. Because the gene is synthesized and codon optimized, the nucleotide sequence will not match the accession number. Please refer to the associated snapgene file or FASTA formatted file shown below for the DNA sequence of the coding region for MDH\_9CREN.

**NCBI / Gene Accession Number:** Because the MDH gene was synthesized and codon optimized as described above its, nucleotide sequence shown below differs from that published in Gene Bank. NZ\_CP006867

**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, and the cloning history.



**NCBI Protein Sequence Accession:** The MDH\_9CREN protein sequence as expressed can be found [WP\\_075049760](https://www.ncbi.nlm.nih.gov/protein/WP_075049760)

**UniProt Knowledge Base Accession** [A0A0U3FQH7](https://www.uniprot.org/uniprot/A0A0U3FQH7) ([A0A0U3FQH7\\_9CREN](https://www.uniprot.org/uniprot/A0A0U3FQH7))

**RCSB PDB Accession:** [6QSS](https://www.rcsb.org/structure/6QSS) co-crystallized with 10 mM Tb-Xo4

## Key Publications:

Roche J, Girard E, Mas C, Madern D. The archaeal LDH-like malate dehydrogenase from *Ignicoccus islandicus* displays dual substrate recognition, hidden allostery and a non-canonical tetrameric oligomeric organization. *J Struct Biol.* 2019 Oct 1;208(1):7-17. doi: 10.1016/j.jsb.2019.07.006. Epub 2019 Jul 10. PMID: 31301348.

The Multifaceted Subunit Interface of Malate Dehydrogenase, [Megan Keene](https://doi.org/10.1096/fasebj.2022.36.S1.0R323), [Isabella Hanson](https://doi.org/10.1096/fasebj.2022.36.S1.0R323), [Daniel Armendariz](https://doi.org/10.1096/fasebj.2022.36.S1.0R323), [Natalie Botros](https://doi.org/10.1096/fasebj.2022.36.S1.0R323), [Hannah Blythe](https://doi.org/10.1096/fasebj.2022.36.S1.0R323), [Jessica Bell](https://doi.org/10.1096/fasebj.2022.36.S1.0R323), [Ellis Bell](https://doi.org/10.1096/fasebj.2022.36.S1.0R323)  
<https://doi.org/10.1096/fasebj.2022.36.S1.0R323>

Using computational and biophysical approaches to explore knock in and knock out mutations of *Ignicoccus islandicus* and watermelon glyoxysomal malate dehydrogenases

Megan Keene, Jessica Bell, Ellis Bell <https://doi.org/10.1016/j.jbc.2023.103635>

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

**Protein Notes:** Originally isolated from *Ignicoccus islandicus* DSM 13165, a genus of Archaea living in a hydrothermal vent growing in ~90°C. **The** Ignicoccus MDH is a canonical NAD (H) dependent enzyme and does not use NADP(H) with the classic Rossmann fold beta 2 asp blocking NADP(H) use. It is a tetramer with LDH like activity. May be a divergent homolog from MDH and LDH with different allosteric properties. The MDH protein is thermostable and will bind and react with both OAA (with substrate inhibition observed above 0.3mM Oxaloacetate and pyruvate (where some sigmoidicity with varied Pyruvate is reported (similar to allosteric LDHs,. MDH\_9CREN is a 310 amino acid (plus the TEV site and 6X His tag on the C terminus). MDH\_9CREN is a homotetramer and has a predicted polypeptide chain mw = 34.38 kDa. And a tetramer molecular weight of 137,500Da. It is biologically active as a tetramer

#### **Ignicoccus Islandicus MDH:**

$pI = 7.96 / \epsilon_{280} = 0.623 \text{ mL} \cdot \text{mg}^{-1} \cdot \text{cm}^{-1}$  extinction coefficient (280 nm: calculated using ProtParam.)

A crystal structure of the tetramer is found in the Protein Data Base (<https://www.rcsb.org/structure/6QSS>) but has chains with breaks in three of the four chains. We have generated a version without chain breaks suitable for computational studies (6qss\_no\_breaks.pdb) and have generated a Landmarks pse file showing critical structural and functional features ([6qss-Landmarks.pse](#))

#### **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:** Weak to moderate protein expression at 37°C 1mM IPTG for 4-6 hour induction. 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. 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  $\beta$ -ME may be added at user's discretion.

>6qss Amino Acid Coding Sequence of available clone:

MARIPYKVAVIGTGRVGATFAYTMAVVPGIARMTLVDVVPGLAKGVMEDIKHAAAVFRRSITVEA  
FEDVSKVENADAIVITAGKPRKADMSRRDLANVNAQIIRDIGDKLRDRNPGALYVVVTNPVDVMT  
MVLDDVIGSKGTVIGTGTSLDTFRFRAAVSELLNVPIVAVDGYVVGEHGEEAFVAWSTVTIKGIHI  
DQYIKERNINISREQIEKYVKDVAASIIASQGATIWGPAATFQEIVVSHLANESKIIPISLPQNIIEGVGR  
VAVSVPTIISGRLKPLVQLLNEEQERLKRAAKAIRNVYESILTHHHHHH

>pdb|6QSS|D Chain D, Malate dehydrogenase

MARIPYKVAVIGTGRVGATFAYTMAVVPGIARMTLVDVVPGLAKGVMEDIKHAAAVFRRSITVEAFEDVS  
KVENADAIVITAGKPRKADMSRRDLANVNAQIIRDIGDKLRDRNPGALYVVVTNPVDVMTMVLDDVIGSK  
GTVIGTGTSLDTFRFRAAVSELLNVPIVAVDGYVVGEHGEEAFVAWSTVTIKGIHIDQYIKERNINISRE  
QIEKYVKDVAASIIASQGATIWGPAATFQEIVVSHLANESKIIPISLPQNIIEGVGRVAVSVPTIISGRLK  
PLVQLLNEEQERLKRAAKAIRNVYESILT

6qss	MARIPYKVAVIGTGRVGFATFAYTMAVVPGIARMTLVDVVPGLAKGVMEDIKHAAAVFRRS	60
pdb 6QSS D	MARIPYKVAVIGTGRVGFATFAYTMAVVPGIARMTLVDVVPGLAKGVMEDIKHAAAVFRRS	60
*****		
6qss	ITVEAFEDVSKVENADAIVITAGKPRKADMSRRDLANVNAQIIRDIGDKLRDRNPGALYV	120
pdb 6QSS D	ITVEAFEDVSKVENADAIVITAGKPRKADMSRRDLANVNAQIIRDIGDKLRDRNPGALYV	120
*****		
6qss	WVTNPVDVMTMVLDDVIGSKGTVIIGTGTSLDTRFRRAAVSELLNVPIIVAVDGYVVGEGHE	180
pdb 6QSS D	WVTNPVDVMTMVLDDVIGSKGTVIIGTGTSLDTRFRRAAVSELLNVPIIVAVDGYVVGEGHE	180
*****		
6qss	EAFVAWSTVTIKGIHIDQYIKERNINISREQIEKYVKDVAASIIASQGATIWGPAATFQE	240
pdb 6QSS D	EAFVAWSTVTIKGIHIDQYIKERNINISREQIEKYVKDVAASIIASQGATIWGPAATFQE	240
*****		
6qss	IVVSHLANESKIIPIISLPQNIIEGVRVAVSVPTIISGRLKPLVQLLNEEEQERLKRAAKA	300
pdb 6QSS D	IVVSHLANESKIIPIISLPQNIIEGVRVAVSVPTIISGRLKPLVQLLNEEEQERLKRAAKA	300
*****		
6qss	IRNVYESILTHHHHHH	316
pdb 6QSS D	IRNVYESILT-----	310
*****		

Coding Sequence:

ATGGCACGTATTCCGTACAAGGTAGCTGTAATCGGTACTGGTCGTGTTGGTGCAACTTTCGC  
GTACACTATGGCAGTTGTTCCAGGTATTGCTCGTATGACCCTGGTTGATGTTGTTCCAGGCC  
TGCGCAAAGGCGTAATGGAAGATATCAAGCACGCTGCTGCTGTATTCCGTCGCTCTATCACC  
GTAGAAGCGTTTGAAGACGTATCTAAAGTTGAGAATGCAGACGCGATTGTTATCACCGCAGG  
TAAACCACGTAAAGCAGACATGTCTCGTCGTGATCTGGCAAATGTGAACGCACAGATCATCCG  
TGACATTGGCGATAAGCTGCGTGATCGTAACCCAGGTGCTCTGTACGTTGTTGTTACTAACC  
CAGTTGACGTAATGACTATGGTGCTGGATGATGTTATCGGTTCCAAGGGTACTGTTATCGG  
CACTGGTACTAGCCTGGACACCTTTCGCTTTCGTGCTGCGGTATCTGAACTGCTGAATGTT  
CGATCGTTGCCGTGGACGGCTATGTGGTTGGTGAACACGGTGAAGAGGCTTTCGTGGCGTG  
GAGCACTGTTACTATCAAAGGTATCCACATTGACCAGTACATCAAGGAGCGTAACATCAACAT  
CAGCCGTGAACAGATCGAGAAGTACGTGAAGGACGTTGCAGCTTCCATCATCGCTTCTCAGG  
GTGCCACCATTTGGGGTCCGGCAGCAACTTTCAGGAAATCGTTGTGTCTCACCTGGCAAAC  
GAGTCCAAGATCATCCCGATCTCCCTGCCACAGAACATTGAAGGCGTTGGTCGTGTGGCTGT  
TTCTGTTCCGACCATCATCTCTGGTCGTCTGAAACCGCTGGTGCAACTGCTGAACGAAGAAG  
AACAGGAGCGTCTGAAGCGTGCAGCGAAAGCCATCCGCAATGTTTACGAAAGCATTCTGACC  
CATCATCACCACCATCAC