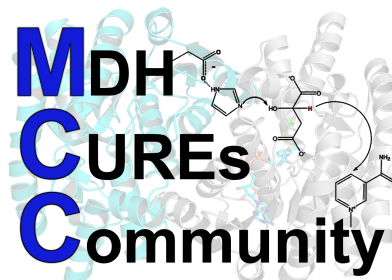


MCC Protein/Clone Information Sheet MDH_9CREN



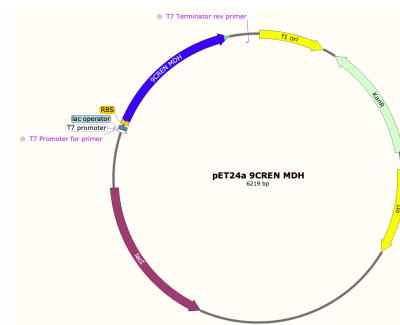
Protein Name: *Ignicoccus islandicus* Malate Dehydrogenase (A0A0U3FQH7_9CREN)

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 differs from that published in Gene Bank. [NZ_CP006867](https://www.ncbi.nlm.nih.gov/nuccore/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 \(A0A0U3FQH7_9CREN\)](https://www.uniprot.org/uniprot/A0A0U3FQH7)

RCSB PDB Accession: [6QSS](https://www.rcsb.org/structure/6QSS) co-crystallized with 10 mM Tb-Xo4, [AF-A0A0U3FQH7-F1](https://www.rcsb.org/structure/AF-A0A0U3FQH7-F1) (AlphaFold)

Key Publications: [The archaeal LDH-like malate dehydrogenase from *Ignicoccus islandicus* displays dual substrate recognition, hidden allostery and a non-canonical tetrameric oligomeric organization](#)

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 more 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 = 33.55 kDa. And a tetramer molecular weight of 134,200Da

Key amino acids / functions studied include: TBA

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 β-ME may be added at user's discretion.

Amino Acid Coding Sequence:

MARIPYKVAVIGTGRVGFAYTMAVVPGIARMTLVDPVPLAKGVMEIKHAAVFRRSITVEAFEDVSKVENADAIVITAGKPRKADMSRRDLAN
VNAQIIRDIGDKLRDRNPGALYVVVTPVDVMTMVLDDVIGSKGTVIGTGTSLDTRFRAAVSELLNPIVAVDGYVVEHGEAFVAVSTVTIKGIHI
DQYIKERNINISREQIEKYVKDVAASIIASQGATIWGPAATFQEIVVSHLANESKIIPISLPQNIIEGVGRVAVSVPTIISGRKPLVQLLNEEEEQERLKRAAKAI
RNVYESILTHHHHHH

Coding Sequence:

ATGGCACGTATCCGTACAAGGTAGCTGTAATCGGTACTGGTCGTGTTGGTGCAACTTTCGCGTACACTATGGCAGTTGTTCCAGGTATTGCTCG
TATGACCCTGGTTGATGTTGTTCCAGGCCTGGCGAAAGGCGTAATGGAAGATATCAAGCACGCTGCTGCTGATTCCGTCGCTCTATCACCGTAG
AAGCGTTTGAAGACGTATCTAAAGTTGAGAATGCAGACGCGATTGTTATCACCGCAGGTAAACCACGTAAAGCAGACATGTCTCGTCGTGATCT
GGCAAATGTGAACGCACAGATCATCCGTGACATTGGCGATAAGCTGCGTGATCGTAACCCAGGTGCTCTGTACGTTGTTGTTACTAACCCAGTTG
ACGTAATGACTATGGTGCTGGATGATGTTATCGGTTCCAAGGGTACTGTTATCGGCACTGGTACTAGCCTGGACACCTTTCGCTTTCGTGCTGCG
GTATCTGAACTGCTGAATGTTCCGATCGTTGCCGTGGACGGCTATGTGGTTGGTGAACACGGTGAAGAGGCTTTCGTGGCGTGGAGCACTGTTA
CTATCAAAGGTATCCACATTGACCAGTACATCAAGGAGCGTAACATCAACATCAGCCGTGAACAGATCGAGAAGTACGTGAAGGACGTTGCAGC
TTCCATCATCGCTTCTCAGGGTGCCACCATTTGGGGTCCGGCAGCAACTTTCAGGAAATCGTTGTGTCTCACCTGGCAAACGAGTCCAAGATCA
TCCCATCTCCCTGCCACAGAACATTGAAGGCGTTGGTCGTGTGGCTGTTTCTGTTCCGACCATCATCTCTGGTCGTCTGAAACCGCTGGTGCAAC
TGCTGAACGAAGAAGAACAGGAGCGTCTGAAGCGTGCAGCGAAAGCCATCCGCAATGTTTACGAAAGCATTCTGACCCATCATCACCACCATCA
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