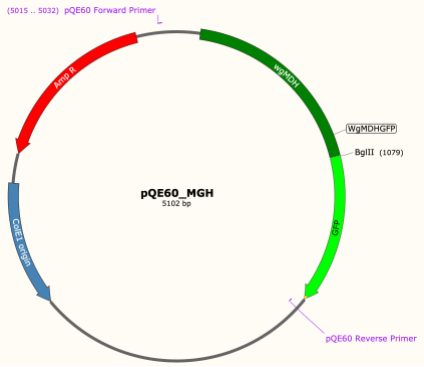
MCC Protein/Clone Information Sheet MGH

Protein Name: Watermellon glyoxylate – GFP fusion (MGH)
Organism: Watermelon (*Citrullus lantus*) **Plasmid Name**: pQE60 MGH

Clone/Plasmid History: The wg MDH was into pQE60 vector with the His affinity tag on the C terminus of MDH. This is the mature watermelon glyoxyl MDH (wgMDH) without presequence. This construct does NOT have the transit peptide (mqpipdvnqr iarisahlhp pksqmeessa lrrancr) and instead has an added Met as the start codon after removal of transit peptide sequence. wgMDH, was prepared by PCR using the NcoI- and BglII-site and cloned into the same vector. wgMDH is cloned between the restriction sites NcoI and BglII; the NcoI-site also provided the start codon. The necessary restriction sites at the 5'-end and 3'-end of the cDNA sequence were added by PCR. Either during cloning or in subsequent manipulations, the NcoI site (C/CATGG) was mutated to CTATGG, destroying the NcoI cut site. The wild type gene GFP was amplified by PCR using GFP-specific oligonucleotides. The fragment was cloned into pQE60 plasmid containing watermelon glycoxyl (no precursor) using BgLII and BamHI/BglII restriction sites.

NCBI / Gene Accession Number: N/A see wgMDH and GFP

Plasmid Map: SnapGene file of this construct is available to members of the MCC. Features annotated on the file include the ampicillin resistance gene, bacterial promotors, the ribosome binding site (RBS), sequencing primers, start and stop codons, the His-tag, the TEV sequence and cleavage site, and the cloning history.



NCBI Protein Sequence Accession: N/A see wgMDH and GFP

UniProt Protein Page: N/A see wgMDH and GFP

RCSB PDB Page: N/A see wgMDH and GFP

Key Publications: Bringing the excitement and motivation of research to students; Using inquiry and research-based learning in a year-long biochemistry laboratory.

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

Protein Notes: This protein was created to provide a colorimetric way to teach/train students on expression, inclusion bodies and to follow purification of an enzymatically functional MDH fusion protein. The cells will become bright green if expression is moderate to high. The lysate will be green and the GFP portion of the protein is easily visualized in room light. The enzyme remains functional even with the fusion tag. No work on the impact on the kinetics or allostery of the protein have been published.

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.

Clone FAQ and Important Points: The protein expresses at very high levels at 370C and 20oC. However most of the protein will be located in the inclusion bodies. To achive soluble protein expression, the overnight culture should not grow beyond OD of 0.6, cooled to 16oC and only when cooled can the culture be induced with 0.5 mM IPTG. Express for 48 hours at 16oC. 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, pH 8.0). 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.

atggctaaaggcggagctcccgggttcaaagtcgcaatacttggcgctgccggtggcattggccagccccttgcgatgttgatgaagatgaatcctctggtttctgttctacatctatatgatgt
agtcaatgcccctggtgtcaccgctgatattagccacatggacacgggtgctgtggtgcgtggattcttggggcagcagcagctggaggctgcgcttactggcatggatcttattatagtccct
gcaggtgttcctcgaaaaccaggaatgacgagggatgatctgttcaaaataaacgcaggaattgtcaagactctgtgtgaagggattgcaaagtgttgtccaagagccattgtcaacctgat
cagtaatcctgtgaactccaccgtgcccatcgcagctgaagttttcaagaaggctggaacttatgatccaaagcgacttctgggagttacaatgctcgacgtagtcagagccaatacctttgtg
gcagaagtattgggtcttgatcctcgggatgttgatgttccagttgttggcggtcatgctggtgtaaccattttgccccttctatctcaggtgaagcctccaagttctttcacacaagaagagatt
agttacctgactgataggattcaaaatggtggaacagaagttgtcgaggccaaagcaggagctggttcagcaactctctcaatggcttatgctgccgttaagtttgcagatgcatgcctcagg
ggcttaagaggagatgctggtgtcattgaatgcgcgtttgtgtcttctcaggtgactgaacttccattctttgcatcaaaagtacgacttggtcgcaatggtatcgaagaagtatactccctttggc
ccgctaaatgagtatgagaggattggattggagaaagcgaagaaagagttggcaggaagcattgagaagggagtttccttcatcagaagcagatctatggctagcaaaggagaagaactc
ttcactggagttgtcccaattcttgttgaattagatggtgatgttaacggccacaagttctctgtcagtggagagggtgaaggtgatgcaacatacggaaaacttaccctgaagttcatctgcac
tactggcaaactgcctgttccatggccaacactagtcactactctgtgctatggtgttcaatgcttttcaagatacccggatcatatgaaacggcatgactttttcaagagtgccatgcccgaag
gttatgtacaggaaaggaccatcttcttcaaagatgacggcaactacaagacacgtgctgaagtcaagtttgaaggtgataccttgttaatagaatcgagttaaaaggtattgacttcaagg
aagatggcaacattctgggacacaaattggaatacaactataactcacacaatgtatacatcatggcagacaaacaaaagaatggaatcaaagtgaacttcaagacccgccacaacattg
aagatggaagcgttcaactagcagaccattatcaacaaaatactccaattggcgatggccctgtccttttaccagacaaccattacctgtccacacaatctgccctttcgaaagatcccaacg
aaaagagagaccacatggtccttcttgagtttgtaacagctgctgggattacacatggcatggatgaactgtacaacGGATCTCATCACCATCACCATCAC

MAKGGAPGFKVAILGAAGGIGQPLAMLMKMNPLVSVLHLYDVVNAPGVTADISHMDTGAVVRGFLGQQQLEAALTGMDLIIVPAGVPRKPGMTR
DDLFKINAGIVKTLCEGIAKCCPRAIVNLISNPVNSTVPIAAEVFKKAGTYDPKRLLGVTMLDVVRANTFVAEVLGLDPRDVDVPVVGGHAGVTILPLLSQ
VKPPSSFTQEEISYLTDRIQNGGTEVVEAKAGAGSATLSMAYAAVKFADACLRGLRGDAGVIECAFVSSQVTELPFFASKVRLGRNGIEEVYSLGPLNEYE
RIGLEKAKKELAGSIEKGVSFIRSRSMASKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLVTTLCYGVQCFSRYP
DHMKRHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKTRHNIED
GSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYNGSHHHHHH