MDH Protocol:

1.5 ml Continuous Assay (OAA -> Malate)

This is the basic protocol for running a 1.5 ml, continuous enzyme assay (AKA real time) measuring the reduction of NAD^+ at 340 nm. One unit oxidizes one µmole of NADH per minute at 25°C and pH 8.0 under the specified conditions. See the introduction to enzyme assay handout for a more complete description of the background of and the performing an enzymatic assay.

$$\text{Oxaloacetate} + \text{NADH} + H^+ <-> \text{Malate} + NAD^+$$

Protocol:

Stock solutions

NADH, 6mM in H_2O

Oxaloacetate, i) 60mM in H_2O

ii) 6mM in H_2O

(Make up the 60mM and dilute some of it 10 fold with H_2O)

Keep all stock solutions on ice in an ice bucket

100mM Phosphate Buffer at pH 8: keep at room temperature or whatever temperature you wish if using some other temperature

H_2O, keep at room temperature or whatever temperature you wish if using some other temperature

NADH Varied: OAA fixed at 200 µM, NADH Varied 10 µM to 200 µM

Oxaloacetate Varied: NADH fixed at 100 µM, OAA Varied from 20 µM to 2 mM

Recommended Approach

In 3mL cuvettes:

Add 1.5mL stock buffer

Add appropriate volume of H_2O so that the final volume in the cuvette after addition of NADH and Oxaloacetate is 3mL.

Add appropriate volume of stock NADH, mix, run a 'No enzyme" control for 30 seconds- this allows you to calculate the actual NADH concentration in the cuvette.

Add appropriate volume of stock Oxaloacetate solution and 10µL (to give a final concentration in the cuvette of about 0.005-0.01µg/mL wildtype enzyme (for mutants you will have to establish the appropriate concentration to give linear kinetics), mix and start data collection for 0.5 minutes.

Typically we analyze the acquired data from 0.05 to 0.25 minutes since the first 0.05 minutes often reflects mixing etc.

Save the primary data as a plain text or csv file for later input into an excel file for data archiving.

Record the rate obtained over the 0.05-0.25 minute time frame

Repeat to give a total of three to five rate measurements to allow an average and standard deviation of the rate to be calculated.

Caveat!! Mutants of Malate Dehydrogenase exist that give distinct hysteretic effects where the rate changes dramatically during a time course. Since such effects can lead to inconsistent data it is important to check during determination of specific activity for such effects using an extended time course.