



Malate Dehydrogenase Enzyme Assay Protocol



The reaction velocity is determined by measuring the decrease in absorbance at 340 nm resulting from the oxidation of NADH. One unit oxidizes one μmole of NADH per minute at 25°C and pH 7.4 under the specified conditions.

For SPECIFIC instructions - use the Cox et al paper "*Organelle and translocatable forms of glyoxysomal malate Dehydrogenase. The effect of the N-terminal presequence.*" Use this paper to determine the parameters for the assay. Use the information below to help organize your work and calculations.

Stock Solutions: For solutions that need to be made fresh each day make 50% more than you calculate you will need. **For all powders stored at -20°C allow to equilibrate at room temperature 10 min** before opening as not to let water condense on the material. There is stock MDH in the freezer to test your assay.

PBS pH 7.4	Can be made and stored at RT the semester
20 mM OAA in PBS	Formula Weight = 131.1 Must be made fresh each day (20 ml)
10 mM NADH in PBS	Formula Weight = 704 Must be made fresh each day (5 ml)

Store all of the above compounds except PBS on ICE to prevent breakdown of solutions. Prepare 1 to 5 ml of OAA and NADH for each day.

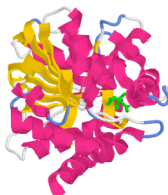
Procedure/Important notes:

- Prepare the following additions and allow the temperature to equilibrate prior to addition of enzyme.
- Zero spectrophotometer at 340 nm with PBS.
- Use plastic cuvetts / Use the VIS lamp.
- After all components are added, the final volume should be 1.0 ml
- Pipet the PBS/NADH/OAA mixture into the cuvette and check the absorbance. Is it more than 0.5 but less than 2.8? Record this absorbance as starting absorbance.
- Remember that a flat line means either there is no activity or all of the substrate is gone. In the first case, the absorbance will remain the same as before enzyme addition, in the latter, the absorbance should be significantly decreased (much more than half of the initial absorbance).

	Volume of Stock	Final Concentration
PBS	USE PBS to QS to final vol of 1.0 ml μl	
OAA		2.0 mM
NADH		0.200 mM
Enzyme		

Initiate Assay by addition of enzyme.

Be careful to mix well. Pipetting is not mixing. The assay should be linear for at least 1 min. If not dilute enzyme with PBS as necessary and re-assay. The change in absorbance per minute should be from 0.05 to 0.5. A rate less than 0.05 is generally considered no activity (0 $\Delta\text{OD}/\text{min}$) and a rate greater than 0.5 is not likely to be linear, meaning it is not in first order kinetics.



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Calculating enzyme units

1 Unit of enzyme catalyzes the conversion of 1 μ mole of substrate to product per minute

To calculate the units in any spectrophotometric based assay, Beer's law is used.

$$A = \epsilon l C$$

Where A = absorbance ($M^{-1} cm^{-1}$), b = pathlength of the cell (1 cm), c = concentration of the absorbing species (M) and ϵ = the molar extinction coefficient.

When assaying enzyme activity we use $\Delta A / \text{min}$ (change in absorbance per time). So $\Delta A = \epsilon l (\Delta C)$ - as the concentration of chromophore changes so will the absorbance

$$\Delta A / \text{min} = \epsilon l (\Delta C / \text{min}) - \text{adds in the time factor}$$

$$\Delta C / \text{min} = (\Delta A / \text{min}) / \epsilon l - \text{rearrange factors}$$

$$\Delta C / \text{min} = (\Delta A / \text{min}) / (6.2 \times 10^3 \times 1)$$

Example of enzymes that use NADH

NADH has an extinction coefficient of $6.2 \times 10^3 m^{-1}cm^{-1}$

$$\Delta C / \text{min} = (\Delta A / \text{min} \times 0.161 \times 10^{-3}) M / \text{min}$$

- inverse of the denominator

$$\Delta C / \text{min} = (\Delta A / \text{min} \times 0.161 \times 10^{-3}) (\text{moles/liter}) / \text{min}$$

- convert M to mole/liter

$$\Delta C / \text{min} = (\Delta A / \text{min} \times 0.161 \times 10^3) (\mu\text{moles/liter}) / \text{min}$$

- convert to μ mole

$$\Delta C / \text{min} = (\Delta A / \text{min} \times 0.161) (\mu\text{moles/ml}) / \text{min}$$

- convert to ml

This is the Units /ml of enzyme in the assay itself. But you only measure a few μ l of actual enzyme from the test tube.....

Suppose: X ml of enzyme in a final volume of Y ml gives a $\Delta A / \text{min}$.

Then: Units of enzyme / ml of the enzyme in your test tube. Using a 0.5 ml total assay volume with 0.020 ml of enzyme sample

$$\Delta A / \text{min} \times 0.161 \times (Y/X)$$

$$\Delta A / \text{min} \times 0.161 \times (0.5/0.01)$$

then $8.05 \times \Delta A / \text{min}$ is the U/ml in your assay cuvet

This will be difference for an assay that uses a different chromophore with a different molar extinction coefficient.