Malate Dehydrogenase
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Summary

Malate dehydrogenase catalyzes the reversible reduction of oxaloacetate to malate using NADH as a co-substrate. This protocol describes a direct enzyme assay for determining malate dehydrogenase activity.

Solutions Required

1. 200 mM tricine pH = 8.1
   adjust with 20% KOH to a pH of 8.1

2. 2 mM NADH
   must be prepared fresh

3. 40 mM oxaloacetate
   must be prepared fresh

Preparation of Cell Extract

Follow general protocol **Preparation of Cell Extract**.

1. Centrifuge sufficient cells so that the volume diluted down to 5 mL would give an optical density of 20-30. For example, for a broth of OD=1, use 100 mL. For a broth of OD=10, use 10 mL.
2. After first pelletization of cells, resuspend in 5-15 mL of tricine buffer.
3. After second pelletization of cells, resuspend in 5 mL of tricine buffer, and break with French Press.

Spectrophotometer

Turn on the ultraviolet bulb on the spectrophotometer (Beckman DU50) and wait 30 minutes for warm-up. Select the kinetics-time window on the instrument. Load the method "A:/nadh". This method has a run-time of 60 s, a temperature of 37°C, a wavelength of 340 nm and uses 2 autosamplers.

Procedure

1. For each assay, prepare the two cocktails shown in the following table into two separate UV-translucent cuvettes, and keep them on ice.

	Volume (µL) added to:	
Solution	Control	Experimental
DI H_2O	350	300
Potassium Tricine	500	500
NADH	100	100
Oxaloacetate	0	50

2. Directly from the ice when ready to commence the assay, place the two cuvettes (each containing 950 µL†) into the spectrophotometer holder (position #1 for control, position #2 for experimental).
3. Wait 10 minutes to allow the temperature of the solutions in the cuvettes to equilibrate.
4. "Blank" and then depress "Read Samples" on the monitor.
5. Simultaneously add 50 µL† of the cell extract to the cuvettes.
6. To mix solutions, immediately and simultaneously aspirate and dispense the contents of the cuvettes with a pipettor. Mix the solutions in this way ten times. (Count!)
7. Promptly depress "start" on the monitor.
8. Record the rates for the two (control and experimental) cuvettes.

† Dilution of the cell extract may be adjusted so that change in absorbance is between about 0.05 and 0.7 AU in one minute. This dilution should be accomplished externally in a microcentrifuge tube (for example, by adding 50 µL of cell extract to 950 µL DI water to achieve a dilution of 20). The volume of 50 µL should always be used in the enzyme assay mixture. Commonly, cell extracts have high malate dehydrogenase activity. In order to achieve a linear rate, the cell extract may have to diluted significantly (e.g., 100 times). For example, 50 µL of cell extract could be diluted in 4.5 mL buffer (a dilution of 91). After mixing this solution of total volume 4.55 mL, 50 µL could be used in the assay above.

Calculation of Activity

One unit (U) of malate dehydrogenase is defined as the amount of enzyme required to produce 1.0 μmole of malate in one minute.

1. $dA/dt\ (min^{-1}) = [Rate]_{experimental} - [Rate]_{control} = dA/dt$

2. $$\text{Activity} = \frac{1000 \times TV \times D \times dA/dt}{\varepsilon \times V \times CF}$$

Activity:	Volumetric Activity (U/L)
TV:	Total volume in cuvette (1000 μL)
D:	Dilution of the cell extract. (For example, if 50 μL of cell extract were add to 950 μL DI water prior to using a volume of cell extract in the assay, then D=20)
V:	Volume of cell extract used (50 μL)
ε:	Molar extinction coefficient for NADH (6.22 L/mmol for a path length of 1.0 cm)
CF:	Concentration Factor of cell extract (For example, if a 100 mL sample is concentrated to a 2 mL volume for the French Press, then CF=50)

3. $$\text{Specific Activity} = \frac{Activity}{Protein\ Concentration} 1$$

Activity:	Volumetric Activity, as calculated in #2 above (U/L)
Protein Concentration:	Protein concentration, as calculated in protocol **Total Protein Concentration** (mg/L)
Specific Activity:	(U/mg protein)

Reference

J. G. Zeikus, G. Fuchs, W. Kenealy, R. K. Thauer (1977) Oxidoreductases Involved in Cell Carbon Synthesis of *Methanobacterium thermoautotrophicum*. Journal of Bacteriology 132, 604-613.