

Malic Enzyme

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Summary

Malic enzyme (Malate dehydrogenase decarboxylating) catalyzes the oxidative carboxylation of L-malate to pyruvate using NAD as a co-substrate. This protocol describes a direct enzyme assay for determining malic enzyme activity.

Solutions Required

1. 100 mM tris HCl buffer pH = 7.4
adjust with 20% NaOH to a pH of 7.4
2. 250 mM tris HCl buffer pH = 7.4
adjust with 20% NaOH to a pH of 7.4
3. 50 mM MnCl₂
4. 40 mM NH₄Cl
5. 1 M KCl
6. 100 mM L-malate
can be prepared in stock solution and stored in refrigerator.
7. 20 mM NAD⁺
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 4°C 100 mM tris buffer.
3. After second pelletization of cells, resuspend in 5 mL of 4°C 100 mM tris buffer, and break with French Press.

Spectrophotometer

Turn on the ultraviolet bulb on the spectrophotometer (Beckman DU50) and wait 10 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 (or the temperature of the fermentation), 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.

Solution	Volume (μL) added to:	
	Control	Experimental
DI H ₂ O	355	255
250 mM tris	400	400
MnCl ₂	20	20
NH ₄ Cl	25	25
KCl	100	100
NAD	50	50
malate	0	100

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.

Calculation of Activity

One unit (U) of malic enzyme activity is defined as the amount of enzyme required to produce 1.0 μ mole of pyruvate in one minute.

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

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

Activity: Volumetric Activity (U/L)

TV: Total volume in cuvette (1000 μ L)

D: Dilution of the cell extract. (For example, if 50 μ L of cell extract were added to 950 μ L DI water prior to using a volume of cell extract in the assay, then D=20)

V: Volume of cell extract used (50 μ L)

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)

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

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. Spina, H. J. Bright, J. Rosenbloom (1966) Purification and Properties of L-malic enzyme from *Escherichia coli*, *Biochemistry* 9, 3794-29-39.