Pyruvate Decarboxylase
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Summary

Pyruvate decarboxylase catalyzes the oxidation of decarboxylation of pyruvate to acetaldehyde. This assay is an indirect method in which the conversion is linked to the activity of the subsequent enzyme alcohol dehydrogenase, which supplied in excess, converts the product acetaldehyde effectively into NAD and ethanol.

Solutions Required

1. 400 mM tris-HCl buffer pH = 6.0
   adjust to a pH of 6.0 with 20% KOH.

2. 10 mM thiamine pyrophosphate

3. 10 mM $MgCl_2 \cdot 2H_2O$

4. 1.0 M sodium pyruvate

5. 4.0 mM NADH
   must be prepared fresh

6. Alcohol dehydrogenase solution
   prepare a solution having activity of 6 U/mL

Preparation of Cell Extract

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

1. After first pelletization of cells, resuspend at 4°C in tris buffer.
2. After second pelletization of cells, resuspend at 4°C in tris buffer.

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:/nadh37". These methods each have a run-time of 60 s, a temperature of 37°C, a wavelength of 340 nm and use 2 autosamplers.

Procedure

1. After the second pelletization, to each 800 μL of cell pellet solution add 100 μL thiamine pyrophosphate and 100 μL $MgCl_2$.

2. Heat in a water bath to 60°C for 30 minutes. Allow to cool to 37°C.

3. 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	250	150
1.0 M tris-HCl	500	500
alcohol dehydrogenase	100	100
NADH	100	100
Pyruvate	0	100

2. Directly from the ice when ready to commence the assay, place the two quartz cuvettes (each containing 900 μL) into the spectrophotometer holder (position #1 for control, position #2 for experimental). Use cuvette lid caps to mix 3 or 4 times then insert in instrument.
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 pyruvate decarboxylase activity is defined as the amount of enzyme required to produce 1.0 μmole of acetaldehyde in one minute.

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

2. $$\text{Activity} = \frac{1000 \times TV \times D1 \times D2 \times dA/dt}{\varepsilon \times V \times CF}$$

Activity: Volumetric Activity (U/L)
TV: Total volume in cuvette (1000 μL)
D1: Dilution of the cell extract prior to boiling as a result of adding thiamine pyrophosphate and $MgCl_2$. (D1=1000/800 = 1.25)
D2: Dilution of the cell extract prior to assay. (For example, if 50 μL of cell extract were add to 950 μL DI water *prior* to using 50 μL of cell extract in the assay, then D2=20)
V: Volume of cell extract used in the assay (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

T. C. Hoppner, H. W. Doelle (1983) "Purification and kinetic characteristics of pyruvate decarboxylase and ethanol dehydrogenase from *Zymomonas mobilies* in relation to ethanol production," European Journal of Applied Microbiology and Biotechnology, 17, 152-157.
T. Conway, Y. A Osman, J. I. Konnan, E. m. Hoffmann, L. O. Ingram (1987) "Promoter and nucleotide sequences of the *Zymomonas mobilis* pyruvate decarboxylase," Journal of Bacteriology, 169(3):949-954.