

Pyruvate Dehydrogenase

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Summary

Pyruvate dehydrogenase is a component of the enzyme complex which catalyzes the conversion of pyruvate to acetyl CoA and CO₂ using NAD⁺ as the co-substrate. This protocol describes a coupled assay to measure the pyruvate dehydrogenase activity.

Solutions Required

1. 0.25 M Tris-HCl Buffer (pH 8.0)
Prepare in stock and store at 4°C.
2. 0.2 M Sodium pyruvate
Prepare in stock and store at -20°C.
3. 4 mM Sodium CoA
Sigma C3144 (Sigma C3019 can be substituted)
Must be prepared fresh on ice.
4. 40 mM NAD⁺
Must be prepared fresh on ice.
5. 40 mM Thiamine pyrophosphate (TPP)
Sigma C8754
Must be prepared fresh on ice.
6. 10 mM MgCl₂
Prepare in stock and store at room temperature
7. 200 mM Dithiothreitol (DTT)
Must be prepared fresh on ice.
8. 25 mM Oxaloacetate (OAA)
Prepare in stock and store at -20°C.
9. 0.05 g 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) in 10 mL 100% ethanol
Must be prepared fresh on ice.
10. Citrate synthase
Sigma C3260
Solution as purchased from Sigma (250 U/mL)

Preparation of Cell Extract

Wash 100 mL cells grown overnight ($OD_{600\text{nm}} \sim 10$) using Tris-HCl buffer (pH 8.0) and concentrate them to 2 mL. Follow the protocol for preparing the cell extract from the webpage and always keep the extract on ice.

Spectrophotometer

Turn on the UV light on the spectrophotometer 5-10 minutes before using it. Select the A:/pdh method in the kinetics-time frame. This method has a run time of 100 s at 30°C at a wavelength of 412 nm. Make sure quartz (or any other UV sensitive) cuvettes are used for the assay.

Procedure

1. For each assay, prepare the following reagent mixture in two separate clean microfuge tubes.

Solution	Volume (μL) added to:	
	Control	Experimental
DI H ₂ O	325	275
Tris-HCl	200	200
Sodium pyruvate	50	50
Sodium CoA	0	50
NAD ⁺	50	50
TPP	50	50
MgCl ₂	100	100
DTT	50	50

2. To each above reagent mixture add 100 μL of the ice-cold cell extract and incubate the microfuge tubes at 37°C in a waterbath for fifteen minutes.
3. Transfer the contents of the two microfuge tubes into quartz cuvettes. Add 50 μL OAA and 25 μL DTNB to both cuvettes and lightly vortex. Place the cuvettes in the spectrophotometer (Control cuvette in position 1 and experimental cuvette in position 2). Wait 10 minutes for the mixtures to equilibrate and blank the spectrophotometer with the control cuvette. Press “read samples” and then add 5 U of citrate synthase.
4. Promptly press “start” on the monitor.
5. Record the reaction rates for the two cuvettes.

Calculation of Activity

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

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

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

Activity: Volumetric Activity (U/L)

TV: Total volume in cuvette (1000 μ L)

V: Volume of cell extract used (100 μ L)

ϵ : Molar extinction coefficient for reduced DTNB (13.6 mM^{-1} for a unit path length)

CF: Concentration Factor of cell extract (For example, if a 100 mL sample is concentrated to a 5 mL volume for the French Press, then CF=20)

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

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)

References

1. Szutowicz, A., Stepien, M., Piec, G. 1981. Determination of pyruvate dehydrogenase and acetyl coA synthetase activities using citrate synthase. *Anal. Biochem.* 115:81-87.
2. Payne, J., Morris, J.G. 1969. Pyruvate carboxylase in *Rhodopseudomonas sphaeroides*. *J. Gen. Microbiol.* 59:97-101.