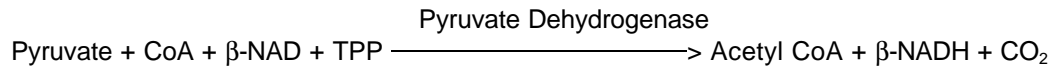


Enzymatic Assay of PYRUVATE DEHYDROGENASE

PRINCIPLE:



Abbreviations used:

CoA = Coenzyme A

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

TPP = Thiamine Pyrophosphate

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

Acetyl CoA = Acetyl Coenzyme A

CONDITIONS: T = 30°C, pH = 7.4, $A_{340\text{nm}}$, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 150 mM MOPS HCl Buffer, pH 7.4 at 30°C
(Prepare 100 ml in deionized water using MOPS, Sodium Salt, Sigma Prod. No. M-9381.
Adjust to pH 7.4 at 30°C with 1 M HCl.)
- B. 12 mM Magnesium Chloride Solution (MgCl_2)
(Prepare 5 ml in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250.)
- C. 0.6 mM Calcium Chloride Solution (CaCl_2)
(Prepare 5 ml in deionized water using Calcium Chloride, Dihydrate, Sigma Prod. No. C-3881.)
- D. 18 mM Thiamine Pyrophosphate (Coccarboxylase) Solution (TPP)
(Prepare 5 ml in deionized water using Coccarboxylase, Sigma Prod. No. C-8754. **PREPARE FRESH.**)
- E. 0.72 mM Coenzyme A Solution (CoA)
(Prepare 5 ml in deionized water using Coenzyme A, Sodium Salt, Sigma Prod. No. C-3144. **PREPARE FRESH.**)

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REAGENTS: (continued)

- F. 20 mM β -Nicotinamide Adenine Dinucleotide Solution (β -NAD)
(Prepare 4 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)
- G. 15.6 mM L-Cysteine Solution, pH 7.4 at 30EC (L-Cys)
(Prepare 20 ml in deionized water using L-Cysteine, Hydrochloride, Monohydrate, Sigma Prod. No. C-7880. Adjust to pH 7.4 at 30°C with 1 M NaOH. **PREPARE FRESH.**)
- H. 75 mM Pyruvic Acid Solution (PYR)
(Prepare 10 ml in deionized water using Pyruvic Acid, Sodium Salt, Sigma Prod. No. P-2256. **PREPARE FRESH.**)
- I. 50 mM MOPS HCl Buffer, pH 7.4 at 30EC (Enzyme Diluent)
(Prepare 100 ml in deionized water using MOPS, Sodium Salt, Sigma Prod. No. M-9381. Adjust to pH 7.4 at 30°C with 1 M HCl.)
- J. Pyruvate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.4 - 0.6 unit/ml of Pyruvate Dehydrogenase in cold Reagent I.)

PROCEDURE:

Pipette (in milliliters) the following reagents into suitable containers:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	1.00	1.00
Reagent B ($MgCl_2$)	0.05	0.05
Reagent C ($CaCl_2$)	0.05	0.05
Reagent D (TPP)	0.05	0.05
Reagent E (CoA)	0.50	0.50
Reagent F (β -NAD)	0.30	0.30
Reagent G (L-Cys)	0.50	0.50
Deionized Water	0.30	0.30
Reagent J (Enzyme Solution)	0.05	-----
Reagent I (Enzyme Diluent)	-----	0.05

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PROCEDURE: (continued)

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent H (PYR)	0.20	0.20
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Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(\text{df})(3.00)}{(6.22)(0.05)}$$

df = Dilution factor

3.00 = Total volume (in milliliters) of assay

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

0.05 = Volume (in milliliters) of enzyme used

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will convert 1.0 μmole of β -NAD to β -NADH per minute at pH 7.4 at 30°C in the presence of saturating levels of Coenzyme A.

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FINAL ASSAY CONCENTRATION:

In a 2.958 ml reaction mix, the final concentrations are 51 mM MOPS, 0.20 mM magnesium chloride, 0.01 mM calcium chloride, 0.30 mM cocarboxylase, 0.12 mM coenzyme A, 2.0 mM β -NAD, 2.64 mM L-cysteine hydrochloride, 5.1 mM pyruvate and 0.003 - 0.005 unit pyruvate dehydrogenase.

REFERENCES:

Brown, J.P., and Perham, R.N. (1976) *Biochem. J.* **155**, 419-427.

NOTES:

1. This assay is a modification of the assay procedure described in the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.