



乙醛脱氢酶

PRINCIPLE:

Acetaldehyde + B-NAD $\xrightarrow{\text{Aldehyde Dehydrogenase}}$ Acetic Acid + β -NADH

Abbreviations used:

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

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

CONDITIONS:

T = 25°C, pH = 8.0, A340nm, Light path = 1 cm

METHOD:

Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 1 M Tris HCl Buffer, pH 8.0 at 25°C

(Prepare 50 ml in deionized water using Trizma Base, Adjust to pH8.0 at 25°C with 1 M HCl.)

B. 20 mM B-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (B-NAD)

(Prepare 1 ml in deionized water using β -Nicotinamide Adenine Dinucleotide, or dissolve the contents of one 20 mg vial of B-Nicotinamide Adenine Dinucleotide, in the appropriate volume of deionized water. PREPARE FRESH.)

C. 100 mM Acetaldehyde Solution (AcetalD)

(Prepare 10 ml in deionized water from a 2 M stock solution of Acetaldehyde, . PREPARE FRESH.)

D. 3 M Potassium Chloride Solution (KCl)

(Prepare 1 ml in deionized water using Potassium Chloride,.)

E. 1 M 2-Mercaptoethanol Solution (2-ME)



(Prepare 1 ml in deionized water using 2-Mercaptoethanol.PREPARE FRESH.)

F. 100 mM Tris HCl Buffer with 0.02% (w/v) Bovine Serum Albumin, pH 8.0 at 25°C (Enz Dil)(Prepare 25 ml in deionized water using Trizma Base, and Albumin,Bovine,. Adjust the pH to 8.0 at 25°C with 1 M HCl.)

G. Aldehyde Dehydrogenase Enzyme Solution

(Immediately before use, prepare a solution containing 0.25 - 0.5 unit/ml of Aldehyde Dehydrogenase in cold Reagent F.)

PROCEDURE:

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

	Test	Blank
Deionized Water	2.32	2.32
Reagent A (Buffer)	0.30	0.30
Reagent B (β -NAD)	0.10	0.10
Reagent D (KCl)	0.10	0.10
Reagent C (Acetald)	0.05	0.05
Reagent C (Acetald)	0.03	0.03
Mix by inversion and equilibrate to 25° C. Monitor the A340nm until constant, using a suitably thermostatted spectrophotometer. Then add:		
Reagent F (Enz Dil)	-----	0.10
Reagent G (Enzyme Solution)	0.10	-----

Immediately mix by inversion and record the increase in A340nm for approximately 5 minutes. Obtain 340nm/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) of enzyme used



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

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

UNIT DEFINITION:

One unit will oxidize 1.0 umole of acetaldehyde to acetic acid per minute at pH 8.0 at 25°C in the presence of β-NAD, potassium and thiols.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 103 mM Tris, 0.67 mM B-nicotinamide adenine dinucleotide, 100 mM potassium chloride, 10 mM 2-mercaptoethanol, 2 mM acetaldehyde, 0.0007% (w/v) bovine serum albumin and 0.025 - 0.05 unit aldehyde dehydrogenase.

NOTES:

1. Prepare fresh from a 2 M stock solution of acetaldehyde. The 2 M stock solution of acetaldehyde is prepared by adding 1.0 ml of 17.9 M acetaldehyde to 7.9 ml of deionized water. In case of any problems with the assay, redistillation of the 17.9 M Acetaldehyde, (from which the 2 M stock is made) should be done to alleviate problems with the substrate.

2. Because of the volatility of these reagents, they should be added to the cuvette immediately before running the assay.

3. The enzyme is inhibited by acetaldehyde. Higher rates may be obtained by using lower concentrations of Reagent C (Acetald).

4. Where Yuanye Product or Stock numbers are specified, equivalent reagents may be substituted.