SIGMA QUALITY CONTROL TEST

ProductInformation

Enzymatic Assay of ALDEHYDE DEHYDROGENASE
(EC 1.2.1.5)

PRINCIPLE:

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

Abbreviations used:
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 8.0, A_{340nm}, 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, Sigma Prod. No. T-1503. Adjust to pH 8.0 at 25°C with 1 M HCl.)

B. 20 mM β-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (β-NAD)
(Prepare 1 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004, or dissolve the contents of one 20 mg vial of β-Nicotinamide Adenine Dinucleotide, Sigma Stock No. 260-120, 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, Sigma Prod. No. A-5076. **PREPARE FRESH**.)[1]

D. 3 M Potassium Chloride Solution (KCl)
(Prepare 1 ml in deionized water using Potassium Chloride, Sigma Prod. No. P-4504.)

E. 1 M 2-Mercaptoethanol Solution (2-ME)
(Prepare 1 ml in deionized water using 2-Mercaptoethanol, Sigma Prod. No. M-6250. **PREPARE FRESH**.)

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PROCEDURE: (continued)

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, Sigma Prod. No. T-1503, and Albumin, Bovine, Sigma Prod. No. A-4503. 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)[2,3]		0.05	0.05
Reagent E (2-ME)[2]		0.03	0.03

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} 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 A_{340nm} for approximately 5 minutes. Obtain the ΔA_{340nm}/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

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

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
0.1 = Volume (in milliliter) of enzyme used

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CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole 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 β-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.

REFERENCE:

Bostian, K.A. and Betts, G.F. (1978) *Biochemical Journal* **173**, 773-786

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, Sigma Prod. No. A-5076, (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 Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.