SIGMA QUALITY CONTROL TEST PROCEDURE

ProductInformation

Enzymatic Assay of ALCOHOL DEHYDROGENASE[1] (EC 1.1.1.1)

PRINCIPLE:

$$\text{Ethanol} + \beta\text{-NAD} \xrightarrow{\text{Alcohol Dehydrogenase}} \text{Acetaldehyde} + \beta\text{-NADH}$$

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

CONDITIONS: T = 25°C, pH = 8.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 50 mM Sodium Pyrophosphate Buffer, pH 8.8 at 25°C
(Prepare 100 ml in deionized water using Sodium Pyrophosphate, Decahydrate, Sigma Prod. No. S-9515. Adjust to pH 8.8 at 25°C with 8% (v/v) Phosphoric Acid.)

B. 95% (v/v) Ethanol
(Prepare 10 ml in deionized water using 200 Proof USP Ethyl Alcohol, available from Quantum Chemical Company.)

C. 15 mM β-Nicotinamide Adenine Dinucleotide Solution (β-NAD)
(Prepare 15 ml in deionized water using β-Nicotinamide Adenine Dinucleotide, Sigma Prod. No. N-7004. **PREPARE FRESH.**)

D. 10 mM Sodium Phosphate Solution
(Prepare 100 ml in deionized water using Sodium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. S-0751.)

E. 10 mM Sodium Phosphate Buffer, pH 7.5 at 25°C
(Prepare 100 ml in deionized water using Sodium Phosphate, Dibasic, Anhydrous, Sigma Prod. No. S-0876. Adjust to pH 7.5 at 25°C with Reagent D.)

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REAGENTS: (continued)

F. 10 mM Sodium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 7.5 at 25°C (Enzyme Diluent)
(Prepare 25 ml in Reagent E with Albumin, Bovine, Sigma Prod. No. A-7906.)

G. Alcohol Dehydrogenase Enzyme Solution (ADH)[2]
(Immediately before use, prepare a solution containing 1 mg/ml of Alcohol Dehydrogenase in cold Reagent E. Further dilute to 0.75 unit/ml with cold Reagent F.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	1.30	1.30
Reagent B (Ethanol)	0.10	0.10
Reagent C (β-NAD)	1.50	1.50

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent G (Enzyme Solution)	0.10	------
Reagent F (Enzyme Diluent)	------	0.10

Immediately mix by inversion and record the increase in A_{340nm} for approximately 6 minutes. Obtain the ΔA_{340nm}/minute using the one to six minute range 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

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

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CALCULATIONS: (continued)

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

UNIT DEFINITION:

One unit will convert 1.0 μmole of ethanol to acetaldehyde per minute at pH 8.8 at 25°C.

FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 22 mM sodium pyrophosphate, 3.2% (v/v) ethanol, 7.5 mM β-nicotinamide adenine dinucleotide, 0.3 mM sodium phosphate, 0.003% (w/v) bovine serum albumin, and 0.075 unit alcohol dehydrogenase.

REFERENCE:

Kägi, J.H.R. and Vallee, B.L. (1960) *Journal of Biological Chemistry* **235**, 3188-3192

NOTES:

1. This assay procedure is not to be used to assay Alcohol Dehydrogenase, Insoluble enzyme attached to beaded agarose, Sigma Prod. No. A-2529.

2. Alcohol Dehydrogenase is very unstable when diluted, and should be assayed immediately following preparations of solutions.

3. For an example of determining the Coefficient of Variation (CV), see attachment.

4. This assay is based on the cited reference.

5. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

CV VALUE CALCULATION
(Attachment)

Immediately before use, prepare three independent solutions containing 1 mg solid/ml of Alcohol Dehydrogenase (ADH) in cold Reagent E (10 mM Sodium Phosphate buffer with 0.1% Bovine Serum Albumin, pH 7.5 at 25°C). Further dilute 50 µl of these solutions to either 25 ml or 50 ml with cold Reagent F (Enzyme Diluent). Then assay 100 µl of each dilution and one blank. This is in contrast to the Standard Sigma procedure, which calls for one level of 100 µl and one blank. To calculate the CV Value, do as follows:

1. Determine the rate (ΔA/min) from one minute to six minutes.

2. Multiply each sample and the blank by 2412^{A} or 4823^{B}, depending on the dilution used. The resulting value will be units/mg solid.

3. Sample (units/mg solid) - Blank (units/mg solid) = Corrected sample (units/mg solid).

4. Determine the mean of the corrected samples.

5. CV Value =

$$\frac{[\Sigma\ (\text{Mean corrected sample} - \text{corrected sample})^2]^{(1/2)}}{\text{Mean corrected sample}} \times 100$$

Dilution factors are as follows:

A.

$$2412 = \frac{(3.0)(500)}{(0.1)(6.22)}$$

3.0 = Total volume (in milliliters) in cuvette
0.1 = Volume (in milliliter) of enzyme used
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
500 = Dilution factor (0.05 ml ADH to 25 ml Reagent F)

B.

$$4823 = \frac{(3.0)(1000)}{(0.1)(6.22)}$$

3.0 = Total volume in cuvette
0.1 = Volume (in milliliter) of enzyme used
6.22 = Millimolar extinction coefficient of β-NADH at 340 nm
1000 = Dilution factor (0.05 ml ADH to 50 ml Reagent F)