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Alcohol Dehydrogenase (ADH)

(Alcohol:NAD+ oxidoreductase; EC 1.1.1.1)

Alcohol dehydrogenase (ADH) catalyzes the oxidation of alcohol and the reduction of aldehydes as shown below:

$$RCH_2OH + NAD \xrightarrow{ADH} RCHO + NADH + H^+$$

ASSAY

The increase in absorbance at 340 nm caused by the reduction of NAD+, is a measure of the catalytic activity of ADH (Vallee, B.L. and Hoch, F.L., Proc. Nat. Acad. Sci. USA, 41, 327, 1955).

REAGENTS

1. 0.05 M Sodium pyrophosphate buffer, pH 8.8.
2. 96% Ethanol (substrate).
3. 0.025 M NAD+ (16.7 mg/ml) in 0.01 M Tris-HCL buffer, pH 7.5. Prepare fresh.
4. 0.01 M Tris-HCL buffer, pH 7.5, containing 0.1% bovine serum albumin (BSA).
5. Alcohol dehydrogenase (enzyme) - Dissolve sufficient amount of enzyme in 0.01 M Tris-HCL buffer containing 0.1% BSA, pH 7.5, to give a concentration of 0.1-0.5 U/ml. Prepare fresh immediately prior to assay.

PROCEDURE

1 Set spectrophotometer (equipped with temperature control) at 340 nm and 25°C.

2 In a cuvette pipette the following reagents in the amounts indicated:

Sodium pyrophosphate buffer	1.4 ml
NAD+	1.4 ml
Ethanol (substrate)	0.1 ml

3 Incubate cuvette in spectrophotometer, at 25°C for 5 min. to achieve temperature equilibration and then record absorbance at 340 nm (blank).

4 Initiate the reaction by adding 0.1 ml of ADH (enzyme) solution to the cuvette. Record the increase in absorbance at 340 nm for 5 min.

5 Calculate the ΔE340nm/min

CALCULATION

$$\text{Activity (U/mg)} = \frac{(\Delta E_{340nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$