Tribioscience

Alcohol Dehydrogenase, Enzyme Activity

Catalog	Unit
TBP0053-5KU	5000 U
TBP0053-10KU	10000 U

Product Details

Form: Freeze-dried powder

Solubility: Distilled water or dilute buffer

Stability: Store at -20° C (-4° F)

Activity: 300 U/mg protein

Protein: 90-95%

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

Unit Definition

The amount of enzyme which catalyzes the reduction of one micromole NAD+ per minute at 25°C and pH 8.8.

Assay Method

The increase in absorbance at 340 nm caused by the reduction of NAD+, is a measure of the catalytic activity of ADH.

Applications

The enzyme occurs in various mammalian tissues but is found in relatively high concentrations in liver and kidney. It is a zinc-containing enzyme and is activated by glutathione and EDTA and inhibited by heavy metals. ADH from equine liver has a molecular weight of 80,000, while the yeast enzyme has a molecular weight of 141,000.

Alcohol dehydrogenase (EC 1.1.1.1) is widely used for the determination of ethanol in biological fluids. It can also be used in coupled enzyme reactions for determination of metabolites in biological fluids. It's optimum pH is 8.6 to 9.0 and it has an extension coefficient of 12.6 and isoelectric point is 5.4. The enzyme is activated with sulfhydryl reagents, like mercapethanol and dithiotreitol.

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

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

3. 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.
4. Calculate the E340nm/min

Calculation

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

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