Tribioscience

Lipoamide Dehydrogenase, Enzyme Activity

Catalog	Unit
TBP0078-100U	100 U
TBP0078-500U	500 U

Product Details

Form: Freeze-dried

Solubility: Soluble in distilled water or dilute buffer

Stability: -20° C; -4° F

Activity: 25 U/mg protein

Protein: 19-22%

Catalog No.: 153A0025

Unit Definition

The amount of enzyme which catalyzes the oxidation of one micromole NADH per minute at pH 5.65 and 25°C.

Assay Method

The assay of lipoamide dehydrogenase is based on the method of Massey, et al. The decrease in the absorbance at 340 nm, caused by the oxidation of NADH, is a measure of the enzyme activity. Note: DL-8,6-Thioctic Acid is the same as DL-a-Lipoic Acid and the same as DL-1,2-Dithiolane-3-pentanoic Acid.

Applications

Lipoamide dehydrogenase (or diaphorase) (EC 1.6.4.3) catalyzes the following reaction:

$$\text{Lipoamide} + \text{NADH} + \text{H+} \xrightarrow{\text{LADH}} \text{Dihydrolipoamide} + \text{NAD+}$$

The enzyme occurs in mammalian and microbial cells and it catalyzes a number of reactions which involve NAD+ or NADH. Lipoamide dehydrogenase from porcine heart contains two polypeptide chains which are similar. It has two molecules of tightly bound flavin adenine dinucleotide (FAD). The molecular weight of the porcine heart enzyme is between 100,000 and 114,000.

Reagents

1. 1.0 M Sodium citrate buffer, pH 5.65.
2. 2% Bovine serum albumin (BSA) in 0.03 M EDTA disodium salt, pH 7.0.
3. 0.002 M NAD+ (1.33 mg/ml) in buffer.
4. 0.0015 M NADH disodium salt (1.0 mg/ml) in buffer.
5. 0.02 M DL-6,8-Thioctic acid (4.24 mg/ml), pH 7.0. Suspend 80 mg of DL-6,8-Thioctic acid in 10 ml distilled water. Adjust pH to 12.0 with 2 M NaOH. Stir for 15 minutes, maintaining pH at 12.0. Adjust pH to 7.0 with citrate buffer. Add distilled water to bring the final concentration to 0.02 M Thioctic acid. Prepare fresh daily.
6. Lipoamide dehydrogenase (enzyme). Dissolve and dilute in citrate buffer to a final concentration of 0.1-0.2 U/ml. Prepare fresh prior to assay.

Calculation

$$\text{Activity (U/mg)} = \frac{(\Delta E_{304nm/min})(\text{Total Vol.})(\text{Enz. Diln.})}{(6.22)(\text{Enz. Vol.})(\text{mg Enz./ml})}$$

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