H-010

Bulk Enzyme Production

β-Hydroxybutyrate Dehydrogenase

E.C. 1.1.1.30

β-HBD catalyzes oxidation of D-3-Hydroxybutyrate to acetoacetate:

D-3-Hydroxybutyrate + NAD → Acetoacetate + NADH + H^+

Used for the enzymatic determination of ketone bodies in blood and serum

β-HBD determined by measuring increase in NADH at 340 nm wavelength

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Specifications

Form
Off-white to light gray lyophilized powder.

Activity
≥ 20 U/mg powder.

Unit
One unit is defined as the amount of enzyme, which catalyzes the formation of 1 µmole of NADH per minute at 37˚C under the conditions given in the assay procedure.

Assay Method

Reagents
1. Tris-HCl buffer: 0.1 M, pH 8.5.
2. Substrate Solution: Dissolve 200 mg of DL-3-Hydroxybutyrate Na salt in 10.0 mL of 0.1 M Tris-HCl buffer, pH 8.5.
3. NAD Solution: Dissolve 80 mg of NAD in 4.0 mL of 0.1 M Tris-HCl buffer, pH 8.5.
4. Enzyme Diluent: Prepare a 1 mg/mL solution of bovine serum albumin in 0.1 M Tris-HCl buffer, pH 8.5.
5. Enzyme Solution: Prepare a 1 mg/mL enzyme solution in enzyme diluent. Dilute the enzyme in same to yield an activity of approximately 0.2 to 0.4 U/mL. Keep the diluted enzyme chilled.

Procedure
Combine 2.3 mL of 0.1 M Tris-HCl buffer, pH 8.5, 0.5 mL of 158 mM DL-3-Hydroxybutyrate* Na salt, 0.2 mL of 27.9 mM NAD solutions at 37°C with 0.1 mL of diluted enzyme in a cuvette.

Mix and measure the rate of increase in absorbance at 340 nm in a spectrophotometer controlled at 37°C.

The change in absorbance should be between 0.03 and 0.09 per minute.

Test and subtract a reagent blank by substituting enzyme diluent for diluted enzyme.

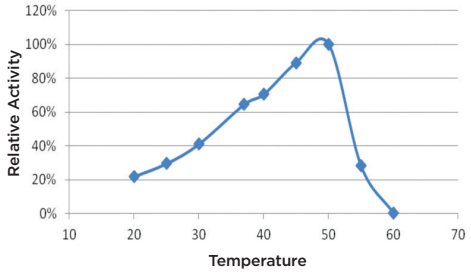
* Note: DL-3-Hydroxybutyrate is used as a substrate; however, the H-010 enzyme is specific to the D-3-Hydroxybutyrate isomer

Properties

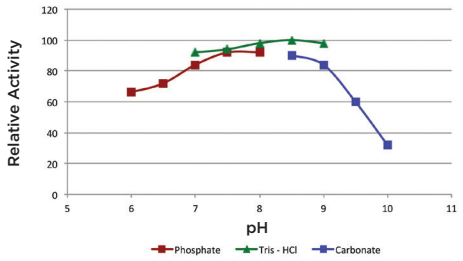
Solubility
β-Hydroxybutyrate dehydrogenase is soluble in water and buffers.

Optimum pH and Temperature
The graphs below show the relative activity of β-Hydroxybutyrate dehydrogenase at various temperatures and pH under the assay conditions:

H-010 Temperature Effect



H-010 pH Effect



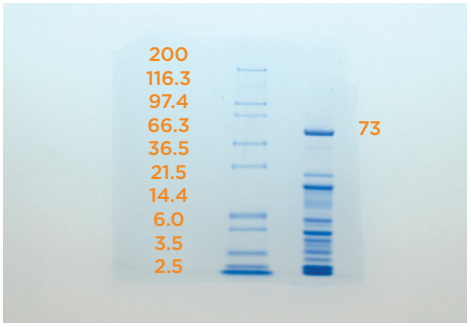
Michaelis-Menten Constant
β-Hydroxybutyrate dehydrogenase has an apparent K_M of:
9.1×10^{-3} M for D-3-Hydroxybutyrate
2.2×10^{-3} M for NAD.

pI
β-Hydroxybutyrate dehydrogenase has an apparent pI of 4.9.

Molecular Weight
The molecular weight of β-Hydroxybutyrate dehydrogenase was determined to be ≈ 70 kDa via size exclusion chromatography and subsequent enzyme analysis.

The image below demonstrates the electrophoretic separation of a sample from a lot of β-Hydroxybutyrate dehydrogenase. Protein standard markers are shown on the left.

The major protein migrates as a single polypeptide chain of 80 kDa. Mass spectrophotometric analysis confirms the presence of a 74 kDa protein.



Calculation
Calculate β-Hydroxybutyrate dehydrogenase activity as follows:

$$U/mg = \frac{(\Delta A_{340}\ \text{test} - \Delta A_{340}\ \text{blank}) \times cv \times \text{dilution}}{6.22 \times sv}$$

where,

cv = reaction volume in mL
sv = enzyme sample volume in mL

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