Enzymatic Assay of ß-HYDROXYBUTYRATE DEHYDROGENASE (EC 1.1.1.30)

PRINCIPLE:

$_{D}$-ß-Hydroxybutyrate + ß-NAD $\xrightarrow{\text{ß-HBDB}}$ Acetoacetate + ß-NADH

Abbreviations used:
ß-NAD = ß-Nicotinamide Adenine Dinucleotide
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
ß-HBDB = ß-Hydroxybutyrate Dehydrogenase

CONDITIONS: T = 37°C, pH = 7.8, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Tris HCl Buffer, pH 7.8 at 37°C
(Prepare 50 ml in deionized water using Trizma Base, Prod. No. T-1503. Adjust to pH 7.8 at 37°C with 1 M HCl.)

B. 160 mM ß-Hydroxybutyric Acid Solution (ß-HB)
(Prepare 5 ml in Reagent A using $_{DL}$-ß-Hydroxybutyric Acid, Sodium Salt, Prod. No. H-6501.)

C. 30 mM ß-Nicotinamide Adenine Dinucleotide Solution (ß-NAD)
(Dissolve the contents of one 50 mg vial of ß-Nicotinamide Adenine Dinucleotide, Stock No. 260-150, in 2.5 ml of deionized water **or** prepare 2 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Prod. No. N-7004. **PREPARE FRESH.**)

D. ß-Hydroxybutyrate Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.3 - 0.6 units/ml of ß-Hydroxybutyrate Dehydrogenase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.20	2.30
Reagent B (ß-HB)	0.50	0.50
Reagent C (ß-NAD)	0.20	0.20

Mix by inversion and equilibrate to 37°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reagent D (Enzyme Solution)	0.10	------

Immediately mix by inversion and record the increase in A_{340nm} for approximately 5 minutes. Obtain the $r\ A_{340nm}$/minute using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/mg enzyme} = \frac{r\ A_{340nm}/\text{min Test} - r\ A_{340nm}/\text{min Blank}}{(6.22)\ (\text{mg enzyme/ml RM})}$$

6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
RM = Reaction Mix

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of D-ß-hydroxybutyrate to acetoacetate per minute at pH 7.8 at 37° C.

FINAL ASSAY CONCENTRATION:

In a 3 ml reaction mix, the final concentrations are 93 mM Tris, 27 mM D-ß-hydroxybutyrate, 2 mM ß-NAD and 0.03 - 0.06 units ß-hydroxybutyrate dehydrogenase.

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NOTES:

1. All product and stock numbers, unless otherwise indicated, are Sigma product and stock numbers.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.