**Enzymatic Assay of β-HYDROXYBUTYRATE DEHYDROGENASE**  
(EC 1.1.1.30)

**PRINCIPLE:**

\[ \text{D-β-Hydroxybutyrate} + \beta-\text{NAD} \rightarrow \text{Acetoacetate} + \beta-\text{NADH} \]

Abbreviations used:

\( \beta-\text{NAD} = \beta-\text{Nicotinamide Adenine Dinucleotide} \)
\( \beta-\text{NADH} = \beta-\text{Nicotinamide Adenine Dinucleotide, Reduced Form} \)
\( \beta-\text{HBDB} = \beta-\text{Hydroxybutyrate Dehydrogenase} \)

**CONDITIONS:**  
\( T = 37^\circ C, \; \text{pH} = 7.8, \; A_{340nm}, \; \text{Light path} = 1 \; \text{cm} \)

**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 β-Hydroxybutyric Acid Solution (β-HB)  
(Prepare 5 ml in Reagent A using DL-β-Hydroxybutyric Acid, Sodium Salt, Prod. No. H-6501.)

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.20</td>
<td>2.30</td>
</tr>
<tr>
<td>Reagent B (β-HB)</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Reagent C (β-NAD)</td>
<td>0.20</td>
<td>0.20</td>
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</tbody>
</table>

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

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

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

CALCULATIONS:

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of β-β-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 β-β-hydroxybutyrate, 2 mM β-NAD and 0.03 - 0.06 units β-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.