

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

PRINCIPLE:

D- β -Hydroxybutyrate + β -NAD $\xrightarrow{\beta\text{-HBDB}}$ Acetoacetate + β -NADH

Abbreviations used:

β -NAD = β -Nicotinamide Adenine Dinucleotide, Oxidized Form

β -NADH = β -Nicotinamide Adenine Dinucleotide, Reduced Form

β -HBDB = β -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. 100 mM β -Hydroxybutyric Acid Solution (β -HB)
(Prepare 5 ml in deionized water using DL- β -Hydroxybutyric Acid, Sodium Salt, Prod. No. H-6501.)
- C. 50 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 1.5 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:

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.60	2.70
Reagent B (β -HB)	0.10	0.10
Reagent C (β -NAD)	0.20	0.20

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	-----
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Immediately mix by inversion and record the increase in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $r A_{340\text{nm}}$ /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 μ mole of D- 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 90 mM Tris, 3.3 mM D- β -hydroxybutyrate, 3.3 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.