Enzymatic Assay of ß-HYDROXYACYL-COA DEHYDROGENASE (EC 1.1.1.35)

PRINCIPLE:

S-Acetoacetyl-CoA + ß-NADH $\xrightarrow{\text{HOADH}}$ ß-Hydroxybutyryl-CoA + ß-NAD

Abbreviations used:
ß-NADH = ß-Nicotinamide Adenine Dinucleotide, Reduced Form
HOADH = ß-Hydroxyacyl-CoA Dehydrogenase
ß-NAD = ß-Nicotinamide Adenine Dinucleotide, Oxidized Form
CoA = Coenzyme A

CONDITIONS: T = 37°C, pH = 7.3, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Potassium Phosphate Buffer, pH 7.3 at 37°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.3 at 37°C with 1 M KOH.)

B. 5.4 mM S-Acetoacetyl Coenzyme A Solution (SAAC)
(Prepare 1 ml in Reagent A using S-Acetoacetyl Coenzyme A, Sodium Salt, Sigma Prod. No. A-1625. Store on ice.)

C. 6.4 mM ß-Nicotinamide Adenine Dinucleotide, Reduced Form (ß-NADH)
(Prepare 1 ml in cold Reagent A using ß-Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH**.)

D. ß-Hydroxyacyl-CoA Dehydrogenase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 - 0.7 unit/ml of ß-Hydroxyacyl-CoA Dehydrogenase in cold Reagent A.)

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PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	2.80	2.80
Reagent B (SAAC)	0.05	0.05
Reagent C (ß-NADH)	0.05	0.05

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	------
Deionized Water	------	0.10

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

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340nm}/\text{min Test} - \Delta A_{340nm}/\text{min Blank})(3)(df)}{(6.22)(0.1)}$$

3 = Total volume (in milliliters) of assay
df = Dilution factor
6.22 = Millimolar extinction coefficient of ß-NADH at 340 nm
0.1 = Volume (in milliliters) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will convert 1.0 µmole of acetoacetyl-CoA to ß-hydroxybutyryl-CoA per minute at pH 7.3 at 37°C in the presence of ß-NADH.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 97 mM potassium phosphate, 0.09 mM S-acetoacetyl-coenzyme A, 0.1 mM ß-nicotinamide adenine dinucleotide, reduced form and 0.02 - 0.07 unit ß-hydroxyacyl-CoA-dehydrogenase.

REFERENCE:

Lynen, F. and Wieland, O. (1955) *Methods in Enzymology*, Volume I, 566-573.

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.