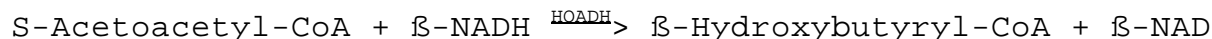


**Enzymatic Assay of  $\beta$ -HYDROXYACYL-COA DEHYDROGENASE  
(EC 1.1.1.35)**

**PRINCIPLE:**



Abbreviations used:

$\beta$ -NADH =  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form

HOADH =  $\beta$ -Hydroxyacyl-CoA Dehydrogenase

$\beta$ -NAD =  $\beta$ -Nicotinamide Adenine Dinucleotide, Oxidized Form

CoA = Coenzyme A

**CONDITIONS:** T = 37°C, pH = 7.3, A<sub>340nm</sub>, 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  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form ( $\beta$ -NADH)  
(Prepare 1 ml in cold Reagent A using  $\beta$ -Nicotinamide Adenine Dinucleotide, Reduced Form, Disodium Salt, Sigma Prod. No. N-8129. **PREPARE FRESH.**)
- D.  $\beta$ -Hydroxyacyl-CoA Dehydrogenase Enzyme Solution  
(Immediately before use, prepare a solution containing 0.2 - 0.7 unit/ml of  $\beta$ -Hydroxyacyl-CoA 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)         | 2.80        | 2.80         |
| Reagent B (SAAC)           | 0.05        | 0.05         |
| Reagent C ( $\beta$ -NADH) | 0.05        | 0.05         |

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

Immediately mix by inversion and record the decrease 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:**

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of  $\beta$ -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  $\mu\text{mole}$  of acetoacetyl-CoA to  $\beta$ -hydroxybutyryl-CoA per minute at pH 7.3 at 37°C in the presence of  $\beta$ -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  $\beta$ -nicotinamide adenine dinucleotide, reduced form and 0.02 - 0.07 unit  $\beta$ -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.

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