Enzymatic Assay of ß-HYDROXYSTEROID DEHYDROGENASE (EC 1.1.1.51)

PRINCIPLE:

$$\text{Testosterone} + \text{ß-NAD} \xrightarrow[\text{Dehydrogenase}]{\text{ß-Hydroxysteroid}} \text{5-a-Androsterone-3, 17-dione} + \text{ß-NADH}$$

Abbreviations used:
ß-NAD = Nicotinamide Adenine Dinucleotide, Oxidized Form
ß-NADH = Nicotinamide Adenine Dinucleotide, Reduced Form

CONDITIONS: T = 25°C, pH = 8.9, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

A. 100 mM Sodium Pyrophosphate Buffer, pH 8.9 at 25°C
(Prepare 100 ml in deionized water using Tetrasodium Pyrophosphate Decahydrate, Prod. No. P-9146. Adjust to pH 8.9 at 25°C with 1 M HCL.)

B. 6 mM ß-Nicotinamide Adenine Dinucleotide, Oxidized Form, Solution (ß-NAD)
(Prepare 5 ml in deionized water using ß-Nicotinamide Adenine Dinucleotide, Oxidized Form, Prod. No. N-7004.)

C. 0.015% (w/v) Testosterone Substrate Solution
(Prepare 5 ml in Reagent F using Testosterone, Prod. No. T-1500.)

D. 10 mM Potassium Phosphate Buffer, pH 7.2 at 25°C
(Prepare 50 ml in deionized water using Potassium Phosphate, Monobasic, Prod. No. P-5379. Adjust to pH 7.2 with 1 M KOH.)

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

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REAGENTS: (continued)

F. Absolute Methanol (MeOH)
(Use Methanol, Absolute, Stock No. 17-5.)

PROCEDURE:

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

	Test	Blank
Reagent A (Buffer)	0.60	0.60
Reagent B (ß-NAD)	0.20	0.20
Reagent E (Enzyme Solution)	0.10	0.10
Deionized Water	2.00	2.00

Mix by inversion and equilibrate to 25°C. Monitor the A_{340nm} until constant, using a suitably thermostatted spectrophotometer. Then add:

Reaction C (Testosterone)	0.10	------
Reagent F (MeOH)	------	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:

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

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

UNIT DEFINITION:

One unit will oxidize 1.0 µmole of testosterone per minute at pH 8.9 at 25°C, in the presence of ß-NAD^+.

FINAL ASSAY CONCENTRATION:

In a 3.0 ml reaction mix, the final concentrations are 20 mM sodium pyrophosphate, 0.4 mM ß-NAD, 0.3 mM sodium phosphate, 0.0005% testosterone, and 0.03 units ß-hydroxysteroid 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.