

**Enzymatic Assay of 3a,20β-HYDROXYSTEROID DEHYDROGENASE
(EC 1.1.1.53)**

PRINCIPLE:

Cortisone + β-NADH $\xrightarrow{3a,20\beta\text{-HSDH}}$ 20-Dihydrocortisone + β-NAD

Abbreviations used:

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

3a,20β-HSDH = 3a,20β-Hydroxysteroid Dehydrogenase

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

CONDITIONS: T = 25°C, pH 7.6, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 50 mM Triethanolamine HCl Buffer, pH 7.6 at 25°C
(Prepare 100 ml in deionized water using Triethanolamine Hydrochloride, Sigma Prod. No. T-1502. Adjust to pH 7.6 at 25°C with 1 M NaOH.)
- B. Methanol
(Use Methanol, Absolute, Sigma Stock No. 17-5.)
- C. 28 mM Cortisone Solution (Cortisone)
(Prepare 5 ml in Reagent B using Cortisone, Sigma Prod. No. C-2755.)
- D. 6.4 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (β-NADH)
(Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- E. 3a,20β-Hydroxysteroid Dehydrogenase Enzyme Solution (3a,20β-HSDH)
(Immediately before use, prepare a solution containing 0.17 - 0.35 unit/ml of 3a,20β-Hydroxysteroid Dehydrogenase in cold deionized water.)

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PROCEDURE:

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

	<u>Test</u>	<u>Blank</u>
Reagent A (Buffer)	2.75	2.75
Reagent C (Cortisone)	0.10	0.10
Reagent D (β-NADH)	0.05	0.05

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

Reagent E (3a,20β-HSDH)		0.10
Deionized Water	-----	----- 0.10

Immediately mix by inversion and record the decrease 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:

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

3 = Total volume (in milliliters) of assay

df = Dilution factor

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

0.1 = Volume (in milliliter) 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 reduce 1.0 μmole of cortisone to 20-dihydrocortisone per minute at pH 7.6 at 25°C, in the presence of β-NADH.

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FINAL ASSAY CONCENTRATION:

In a 3.00 ml reaction mix, the final concentrations are 46 mM triethanolamine, 0.93 mM cortisone, 0.1 mM β -nicotinamide adenine dinucleotide, reduced form, 0.017 - 0.035 unit 3 α ,20 β -hydroxysteroid dehydrogenase, and 3.3% (v/v) methanol.

REFERENCE:

Edwards, C.A.F. and Orr, J.C. (1978) *Biochemistry* **17**, 4370-4376

NOTES:

1. This assay is based on the cited reference.

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