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

PRINCIPLE:

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

Abbreviations used:
β-NADH = β-Nicotinamide Adenine Dinucleotide, Reduced Form
3α,20β-HSDH = 3α,20β-Hydroxysteroid Dehydrogenase
β-NAD = β-Nicotinamide Adenine Dinucleotide, Oxidized Form

CONDITIONS:  T = 25°C, pH 7.6, A$_{340\text{nm}}$, 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. 
Adjust to pH 7.6 at 25°C with 1 M NaOH.)

B. Methanol  
(Use Methanol, Absolute.)

C. 28 mM Cortisone Solution (Cortisone)  
(Prepare 5 ml in Reagent B using Cortisone)

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, in the appropriate volume of deionized water. PREPARE FRESH.)

E. 3α,20β-Hydroxysteroid Dehydrogenase Enzyme Solution (3α,20β-HSDH)  
(Immediately before use, prepare a solution containing 0.17 – 0.35 unit/ml of 3α,20β-Hydroxysteroid Dehydrogenase in cold deionized water.)
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PROCEDURE:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (Buffer)</td>
<td>2.75</td>
<td>2.75</td>
</tr>
<tr>
<td>Reagent C (Cortisone)</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Reagent D (β-NADH)</td>
<td>0.05</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent E (3α,20β-HSDH)</td>
<td>0.10</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>------</td>
</tr>
</tbody>
</table>

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

CALCULATIONS:

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

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 3α,20β-hydroxysteroid dehydrogenase, and 3.3% (v/v) methanol.

REFERENCE:


NOTES:

1. This assay is based on the cited reference.

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