INTENDED USE
For the direct quantitative determination of 3α-Diol G by an enzyme immunoassay in human serum.

PRINCIPLE OF THE TEST
The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabelled antigen (present in standards, controls and patient samples) and an enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microplate microwells. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the amount of free 3α-Diol G in the sample. A set of standards is used to plot a standard curve from which the concentration of 3α-Diol G in patient samples and controls can be directly read.

CLINICAL APPLICATIONS
5α-Androstane-3α,17β-diol glucuronide (3α-Diol G) is a C19 steroid and is either abbreviated as 3α-Diol G, 5α-Diol G or simply, Diol G. It is produced primarily as a metabolic product of testosterone and dihydrotestosterone (DHT). It is largely produced in target peripheral tissues such as the skin, especially around hair follicles. The stimulation by large amounts of 3α-Diol G leads to excessive hair formation, notably where hair is not normally present in women.

In recent years the interest in the measurement of this steroid has increased among clinical investigators studying women suffering from idiopathic hirsutism.

Among the steroids known to be precursors for 3α-Diol G are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), dihydrotestosterone (DHT), androstenedione and testosterone. Only 3α-Diol G has been shown to increase with hirsutism and decreased with treatment. Elevated 3α-Diol G has also been demonstrated in patients with polycystic ovarian syndrome (PCO) and idiopathic hirsutism. These alterations have therefore proved to be a useful indicator in a variety of ways including monitoring the response of treatment of idiopathic hirsutism and women with PCO.

Furthermore, diabetic patients (both men and women) under cyclosporine A therapy have shown increased 3α-Diol G levels, a side effect resulting in the appearance of hair in previously hairless areas.

PROCEDURAL CAUTIONS AND WARNINGS
1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce the risk of potential hazardous substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. All controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage, may be indicated when assay values for the controls do not establish the expected level.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any air bubbles before starting the reading step.
10. A calibrator curve (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
12. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with metal parts.
13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
14. Do not mix various lot numbers of kit components within a test and do not use component beyond the expiration date printed on the label.
15. Kit reagents must be regarded as hazardous waste and handled with the same precautions as applied to human specimens.

DIRECTIONS FOR USE

1. Rabbit Anti-3α-Diol G Antibody-Coated-Break-Apart Well Microplate – Ready To Use
   - Contents: One 96-well (12x) polyclonal antibody-coated microwell in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months or as indicated on label.

2. 3α-Diol G-Horseradish Peroxidase (HRP) Conjugate – Requires Preparation
   - Contents: 3α-Diol G-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months or as indicated on label.

3. Microplate Reader – Ready To Use
   - Contents: Six vials containing 3α-Diol G in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of 3α-Diol G.
   - Storage: Refrigerate at 2–8°C.
   - Stability: 12 months or as indicated on label.
**CALCULATIONS**

1. Calculate the mean optical density of each calibrator and specimen sample into corresponding labelled wells in duplicate.
2. Pipette 100 μL of the conjugate working solution into each well. (We recommend using a multi-channel pipette.)
3. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
4. Wash the wells 3 times with 300 μL of diluted wash buffer.
5. Pipette 150 μL of TMB substrate into each well at timed intervals.
6. Wash the wells (4 × 300 μL) for desired OD.
7. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
8. Stop incubation by adding 100 μL of stop solution.
9. Read the plate on a microplate reader at 450 nm filter (if unavailable, a 405 or 415 nm filter may be substituted). The optical densities will be lower, however, this will not affect the results of patient/control samples.

**INTER-ASSAY PRECISION**

Three samples were assayed ten times each over a period of four weeks. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.98</td>
<td>0.10</td>
<td>10.4</td>
</tr>
<tr>
<td>2</td>
<td>7.05</td>
<td>0.46</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>20.92</td>
<td>2.26</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**EXPECTED VALUES**

As for any laboratory, all assays each laboratory should collect data and establish their own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>1.53–14.82</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>0.22–4.64</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>0.61–3.71</td>
</tr>
<tr>
<td>Puberty</td>
<td>0.51–4.03</td>
</tr>
</tbody>
</table>

**REFERENCES**


**SYMBOLS**

- **SSM**: Specificity (Cross-Reactivity)
- **CV**: Coefficient of Variation
- **SD**: Standard Deviation
- **%**: Percentage
- **ng/mL**: Nanograms per Milliliter