Testosterone

Enzyme immunoassay for the quantitative determination of Testosterone in human serum or plasma

Only for in-vitro diagnostic use

Product Number: DNOV002 (96 Determinations)
## CONTENTS

1. INTRODUCTION 3  
2. INTENDED USE 3  
3. PRINCIPLE OF THE ASSAY 3  
4. MATERIALS 3  
   4.1. REAGENTS SUPPLIED 3  
   4.2. MATERIALS SUPPLIED 3  
   4.3. MATERIALS AND EQUIPMENT NEEDED 3  
5. STABILITY AND STORAGE 4  
6. REAGENT PREPARATION 4  
   6.1. COATED SNAP-OFF STRIPS 4  
   6.2. TESTOSTERONE-HRP CONJUGATE 4  
   6.3. TESTOSTERONE STANDARDS 4  
   6.4. TMB SUBSTRATE SOLUTION 4  
6.5. STOP SOLUTION 4  
7. SPECIMEN COLLECTION AND PREPARATION 4  
   7.1. PRECAUTION 4  
8. ASSAY PROCEDURE 4  
   8.1. TEST PREPARATION 4  
   8.2. MEASUREMENT 5  
9. RESULTS 5  
   9.1. CALCULATION OF RESULTS 5  
   9.2. REFERENCE VALUES 5  
10. QUALITY CONTROL 5  
11. SPECIFIC PERFORMANCE CHARACTERISTICS 5  
   11.1. PRECISION 5  
   11.2. SPECIFICITY 6  
   11.3. SENSITIVITY 6  
   11.4. ACCURACY 6  
   11.5. CORRELATION WITH RIA 6  
12. LIMITATIONS OF THE PROCEDURE 6  
13. PRECAUTIONS AND WARNINGS 6  
   13.1. DISPOSAL CONSIDERATIONS 7  
14. LITERATURE 7  
15. ORDERING INFORMATION 7
1. INTRODUCTION

Testosterone (17β-Hydroxy-4-androstene-3-one) is a steroid hormone from the androgen group. In postpubertal males, testosterone is secreted primarily by the testes with only a small amount derived from peripheral conversion of androstenedione. In adult women over 50% of serum testosterone is derived from peripheral conversion of androstenedione secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands. The majority of circulating testosterone is bound by SHBG and a smaller portion is bound by albumin. Only 1-2% exists in circulation as unbound or free testosterone.

Testosterone effects can be classified as virilizing and anabolic effects, although the distinction is somewhat artificial, as many of the effects can be considered both. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Virilizing effects include maturation of the sex organs, and after birth (usually at puberty) a deepening of the voice, growth of the beard and axillary hair (male secondary sex characteristics).

Testosterone levels decline gradually with age in men (andropause). The signs and symptoms are non-specific, and are generally associated with aging such as loss of muscle mass and bone density, decreased physical endurance, decreased memory ability and loss of libido.

In females of all ages, elevated testosterone levels can be associated with a variety of virilizing conditions, including adrenal tumors and polycystic ovarian disease.

2. INTENDED USE

Competitive immunoenzymatic colorimetric method for quantitative determination of Testosterone in human serum or plasma.

3. PRINCIPLE OF THE ASSAY

Microtiter strip wells are precoated with anti-Testosterone antibodies (solid-phase). Testosterone in the sample competes with added horseradish peroxidase labelled Testosterone (enzyme-labelled antigen) for antibody binding. After incubation a bound/free separation is performed by solid-phase washing. The immune complex formed by enzyme-labelled antigen is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of Testosterone in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Anti-Testosterone IgG Coated Wells**: 12 breakapart 8-well snap-off strips coated with anti-Testosterone IgG; in resealable aluminium foil.
- **Stop Solution**: 1 bottle containing 15 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Testosterone conjugate**: 1 bottle containing 12 ml of horseradish peroxidase labelled Testosterone (enzyme-labelled antigen) for antibody binding.
- **TMB Substrate Solution**: 1 bottle containing 15 ml 3, 3’, 5, 5’-tetramethylbenzidine (H2O2-TMB 0.26 g/l) (avoid any skin contact).
- **Testosterone Standards**: 5 bottles, 1 ml each
  - Standard 0: 0.0 ng/ml
  - Standard 1: 0.2 ng/ml
  - Standard 2: 1.0 ng/ml
  - Standard 3: 4.0 ng/ml
  - Standard 4: 16.0 ng/ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes
- Rotating mixer
- Distilled water
- Timer
5. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22...28°C) before starting the test run!

6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti-Testosterone IgG antibodies. Store at 2...8 °C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.

6.2. Testosterone-HRP Conjugate

Mix gently for 5 minutes with a vortex mixer. After first use the conjugate is still stable for another 6 months if stored at 2...8 °C.

6.3. Testosterone Standards

The standards are ready to use and have the following concentration of Testosterone:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0</td>
<td>0.0</td>
</tr>
<tr>
<td>Standard 1</td>
<td>0.2</td>
</tr>
<tr>
<td>Standard 2</td>
<td>1.0</td>
</tr>
<tr>
<td>Standard 3</td>
<td>4.0</td>
</tr>
<tr>
<td>Standard 4</td>
<td>16.0</td>
</tr>
</tbody>
</table>

The solutions have to be stored at 2...8 °C. After first use the standards are still stable for another 6 months if stored at +2...+8°C.

6.4. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first use the TMB substrate solution is still stable for another 6 months if stored at 2...8 °C.

6.5. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C. After first use stable until expiry date.

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma samples with this assay. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

7.1. Precaution

- The reagent contains Proclin 300® as preservative
- Do not use heavily haemolysed or lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- This method allows the determination of Testosterone from 0.2 ng/ml to 16.0 ng/ml.
- Treatment of the patient with cortisone, natural or synthetic steroids can impair Testosterone determination.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

<table>
<thead>
<tr>
<th>Well</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 well (e.g. A1)</td>
<td>for the substrate blank</td>
</tr>
<tr>
<td>2 wells (e.g. B1+C1)</td>
<td>for standard 0</td>
</tr>
<tr>
<td>2 wells (e.g. D1+E1)</td>
<td>for standard 1</td>
</tr>
<tr>
<td>2 wells (e.g. F1+G1)</td>
<td>for standard 2</td>
</tr>
<tr>
<td>2 wells (e.g. H1+A2)</td>
<td>for standard 3</td>
</tr>
<tr>
<td>2 wells (e.g. B2+C2)</td>
<td>for standard 4</td>
</tr>
</tbody>
</table>
It is recommended to determine controls and patient samples in duplicate. Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each standard and each patient sample. Adjust the incubator to 37°± 1°C.

1. Dispense 25 µl standards and samples into their respective wells. Add 100 µl conjugate to each well. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour at 37°C.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well twice with 300µl distilled water. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl TMB Substrate Solution into all wells.
6. Incubate for exactly 15 min at room temperature (22…28°C) in the dark.
7. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Shake the microplate gently.

Any blue colour developed during the incubation turns into yellow.
8. Measure the absorbance (E) of the specimen at 450 nm within 30 min after addition of the Stop Solution.

8.2. Measurement
Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and patient sample in the distribution and identification plan.
Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS
9.1. Calculation of results
Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (Four Parameter Logistic).
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

9.2. Reference values
The serum or plasma Testosterone reference values are:
WOMAN: 0.2 - 1.2 ng/ml
CHILDREN: 0.1 - 0.4 ng/ml
MEN: 1.8 - 9.0 ng/ml

10. QUALITY CONTROL
Each laboratory should assay controls at normal, high and low levels range of Testosterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

11. SPECIFIC PERFORMANCE CHARACTERISTICS
11.1. Precision
Intra Assay Variation
Within run variation was determined by replicate determination (16x) of three different control sera in one assay. The within assay variability is ≤5.8%.
Inter Assay Variation
Between run variation was determined by replicate measurements (16x) of three different control sera in different lots. The between assay variability is ≤ 10.5 %.

11.2. Specificity
The cross reaction of the antibody calculated at 50% according to Abraham is:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross Reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>100.0 %</td>
</tr>
<tr>
<td>DHT</td>
<td>16.0 %</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.8 %</td>
</tr>
<tr>
<td>Androsterone</td>
<td>0.0 %</td>
</tr>
<tr>
<td>DHEA-S</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Cortisone</td>
<td>0.0 %</td>
</tr>
<tr>
<td>17α Estradiol</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.0 %</td>
</tr>
<tr>
<td>Prednisone</td>
<td>0.0 %</td>
</tr>
</tbody>
</table>

11.3. Sensitivity
The lowest detectable concentration of testosterone that can be distinguished from the zero standard is 0.07 ng/ml at the 95 % confidence limit.

11.4. Accuracy
The recovery of 0.2, 1.0, 4.0, 16 ng/ml of Testosterone added to sample gave an average value (±SE) of 105.5 % with reference to the original concentrations.

11.5 Correlation with RIA
The NovaTec Testosterone ELISA was compared to another commercially available Testosterone assay. Serum samples of 25 females and 27 males were analysed according in both test systems.

The linear regression curve was calculated

\[(\text{Testosterone NovaTec}) = 0.97 \times (\text{Testosterone RIA}) + 0.13 \]

\[r^2 = 0.99\]

12. LIMITATIONS OF THE PROCEDURE
Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed.
- The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING: Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!
13.1. Disposal Considerations
Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE
Turkes, A. et al.; J Endocrinol. 1979, 81 (2), P165
Rajkowski K.M, Cittanova N, Desfosses B, and Jayle M.F., Steroids 29 no 5 1977
Widsdom G. B.; Clin. Chem. 1976, 22/8, 1243 - 1255

15. ORDERING INFORMATION
Prod. No.: DNOV002 Testosterone Determination (96 Determinations)
### SCHEME OF THE ASSAY

**Testosterone**

#### Test Preparation

Prepare reagents and samples as described.  
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.  
Select the required number of microtiter strips or wells and insert them into the holder.

#### Assay Procedure

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Standard 0</th>
<th>Standard 1</th>
<th>Standard 2</th>
<th>Standard 3</th>
<th>Standard 4</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank</td>
<td>25 µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Standard 0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 1</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard 3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
<td>-</td>
</tr>
<tr>
<td>Standard 4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25 µl</td>
</tr>
<tr>
<td>Conjugate</td>
<td>-</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

- Cover wells with foil supplied in the kit  
- **Incubate for 1 h at 37 °C**  
- Wash each well twice with 300 µl distilled water

<table>
<thead>
<tr>
<th>TMB Substrate</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>

- **Incubate for exactly 15 min at room temperature (22…28°C) in the dark**

<table>
<thead>
<tr>
<th>Stop Solution</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
<th>100 µl</th>
</tr>
</thead>
</table>

- Photometric measurement at 450 nm

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