Testosterone [125I] RIA KIT
(Ref: RK-61M)

Description
The Testosterone [125I] radioimmunooassay system provides the quantitative in vitro determination of testosterone in human serum. Testosterone can be assayed in the range 0.35 nmol/l, using 50 µl serum sample. Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and the assay of 41 unknowns and 1 control in duplicate.

Introduction
The blood level of testosterone is an important indicator of a wide variety of pathologic conditions.

Elevated concentration of testosterone in male is characteristic of: precocious puberty, congenital 21-hydroxylase deficiency, adrenal hyperplasia (Cushing’s syndrome), testicular tumours in females with: ovarian or endometrium tumours, Stein-Leventhal syndrome, Adrenal hyperplasia (Cushing’s syndrome), Hirsutism

Decreased concentration of testosterone in male is associated with: Klinefelter’s syndrome, agonadism, anorchidism, chryp
torchidism, Kallman’s syndrome, Leydig cell aplasia, defects of the pituitary functions and in females at postmenopausal.

Principle of the method
This assay is based on the competition between unlabelled testosterone and a fixed quantity of 125I-labelled testosterone for a limited number of binding sites on testosterone specific antibody. Allowing to react a fixed amount of tracer and antibody with different amounts of unlabelled ligand the amount of tracer bound by the antibody will be inversely proportional to the concentration of unlabelled ligand. Upon addition of magnetisable immunosorbent the antigen-antibody complex is bound on solid particles which are then separated by either magnetic sedimentation or centrifugation. Counting the radioactivity of solid phase enables a standard curve to be constructed and samples to be quantitated.

Contents of the kit
1 TRACER, concentrated solution.
   vial < 150 kBq 125I-testosterone in 1 ml organic solution
6 STANDARDS (±6), lyophilised.
   vials 0.5 ml per vial, containing 0 (S1), 0.43 (S2), 1.73 (S3), 4.3 (S4), 17.3 (S5), 35 (S6) nmol/l in 0.5 ml serum with 0.1% NaN3
1 ANTISERUM, lyophilised. Con
   vial tains polyclonal anti-testosterone (rabbit) IgG in buffer.

Materials and equipment required
Round bottom polystyrene or polycarbonate assay tubes (about 12 x 75 mm), plastic film to cover tubes, precision pipettes (50, 100, 200, and 500 µl disposable tips), vortex mixer, magnetic separator, (or alternatively centrifuge), decanting racks, gamma counter

Recommended tools and equipment
orbital shaker, repeating pipettes

Preparation of reagents
Add 2-4 ml Tracer Buffer into the Tracer Concentrate and transfer the content into the Tracer Buffer bottle. Reconstitute antiserum with 10 ml distilled water. Add exactly 500 µl distilled water to each standard vial. Add exactly 500 µl distilled water to the lyophilised control serum. Ensure that complete dissolution is achieved, and allow the solutions to equilibrate at room temperature for at least 20 minutes.

Specimen collection and storage
Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Sera can be stored at 2-8 °C for two days after collection. For later analysis they should be stored deep-frozen. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens. Samples with a testosterone concentration higher than that of the most concentrated standard should be diluted and reassayed. Use the zero standard as diluent.

Assay procedure
(For a quick guide refer to Table 1)

1) Equilibrate all reagents to room temperature.
2) Label duplicate tubes for total counts (T), zero standard (Standard 1 = B0), standards (S2–6), control (C) and samples (Sx).
3) Mix all reagents and samples thoroughly before use. Avoid excessive foaming.
4) Pipette 50 µl each of standards, control and samples into the properly labelled tubes.
5) Pipette 200 µl of tracer solution into all tubes.
6) Pipette 100 µl of antiserum into all tubes except T.
7) Thoroughly vortex mix all tubes except T for 2-5 seconds. When having an orbital shaker, leave all tubes in the rack holder, fix the holder onto the plate of the shaker, and shake it gently for a few seconds.
8) Cover tubes with a plastic foil, and allow them to incubate at room temperature for 3 hours.
9) Place T tubes on a separate tube rack. Gently shake and swirl the bottle containing magnetic immunosorbent until homogeneity. Add 500 µl to each tube except T. When using a single pipette, swirl the bottle of MIS after every 15-20 tubes. With the use of a repeating pipette (e.g. Eppendorf), there is no need for repeated homogenisation of MIS reagent.
10) Thoroughly vortex mix all tubes and incubate them for 5 minutes at room temperature.
11) Separate the bound fraction by using one of the following procedures. Magnetic separation
   Attach the rack on to the magnetic separator base and ensure that every tube is in contact with the base plate. Let the MIS particles settle for 5 minutes. Do not remove the rack from the separator base after the separation of the solid and liquid phases. Pour off and discard the supernatant. Keeping the separator inverted, place the tubes on a pad of absorbent tissue and allow to drain for 2 minutes.
   Centrifugation
   Centrifuge all tubes for 15 minutes at 1500 g or greater. Aspirate the supernatant. Take care of not disturbing the precipitate.
12) Count the radioactivity of each tube for at least 60 seconds or longer in a gamma counter.
13) Calculate the concentrations as described under Calculation of results.
Conversion of SI units can be performed according to the following formula:

\[ 1 \text{ nmol/l} = 0.29 \text{ ng/ml} \]

Characterization of the assay

**Assay parameters**

- **NSB/T**: < 3%
- **B0/T**: 45.0 ± 10%
- **ED-50**: 4.2 ± 1.1 nmol/l

**Specificity**

Cross reactivity was defined by weight at the 50% displacement level in per cent.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cross reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>100</td>
</tr>
<tr>
<td>5α-dihydrotestosterone</td>
<td>35</td>
</tr>
<tr>
<td>5β-dihydrotestosterone</td>
<td>0.8</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>0.01</td>
</tr>
<tr>
<td>Cortisol</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Expected reference values**

- Male: 9 – 38 nmol/l (2.6 – 11 ng/ml)
- Female: 0.9 – 4.5 nmol/l (0.26 – 1.3 ng/ml)

It is recommended that each laboratory establishes its own reference intervals. The results obtained should only be interpreted in the context of the overall clinical picture. None of in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

Additional information

**Storage**

Store the reagents between 2 and 8 °C. At this temperature each reagent is stable until expiry date. Pay special attention to preventing magnetic immunosorbent suspension from freezing.

**Availability**

From stock.

**Shelf life**

The minimum shelf life of kit reagents is usually 8 weeks from the date of manufacturing. The actual expiry date is given on the package label and in the quality certificate. To make the maximum benefit of long-term stability it is recommended to adjust the date of ordering to new-batch manufacturing calendar issued each year. Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions and warnings

**Radioactivity**

This kit contains radioactive material. Receipt, acquisition, possession, or use of radioactive materials are subject to regulations, and a licence of (inter)national authorizing bodies. It is the responsibility of the user to ensure that local regulations or codes of practice are satisfied.

**Potentially infectious materials**

Human blood products provided as components of this product have been obtained from donors tested individually and found negative for Human Immunodeficiency Virus antibody (HIV-Ab) as well as for Hepatitis B surface Antigen (HBsAg) using approved EIA methods.

Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV), or other infectious agents are absent, and all human blood samples should be considered potentially infectious.

**Chemical and other hazard**

Some components contain sodium azide (0.1 % w/v) as an antimicrobial agent. Dispose the waste by flushing it with copious amounts of water to avoid build up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 82.5 mg.