Testosterone [I-125] RIA KIT

(Ref: **RK-61CT)**

Description

The Testosterone [^{125}I] radioimmunoassay system provides the quantitative in vitro determination of testosterone in human serum. Testosterone can be assayed in the range 0-60 nmol/L, using 25 µL serum sample. Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and the assay of 42 unknowns and 2 controls in duplicate.
The test is suitable for processing on automated RIA equipment..

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: ovarium or endometrium tumours, Stein-Leventhal syndrome, Adrenal hyperplasia (Cushing's syndrome), Hirsutism Glycocorticoid therapy
Decreased concentration of testosterone:
in male is associated with: Klinefelter's syndrome, agonadism, anorchism, chryptorchidism, Kallman's syndrome, Leydig cell aplasia, defects of the pituitary functions
in females at postmenopausa

Principle of the method

This assay is based on the competition between unlabelled testosterone and a fixed quantity of ^{125}I-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. The antigen-antibody complex is bound on solid phase (antibody coated tubes). Counting the radioactivity of solid phase enables a standard curve to be constructed and samples to be quantitated.

Contents of the kit

1 bottle	TRACER, ready to use. 44 mL per vial, containing < 260 kBq Testosterone-[^{125}I] in buffer with red dye and 0.1% NaN_3.
6 vials	STANDARDS$_{(1-6)}$, ready to use. 0.5 mL per vial, containing 0 (S1), 0.6 (S2), 2 (S3), 6 (S4), 20 (S5), 60 (S6) nmol/L in 0.5 mL human serum with 0.1% NaN_3.
2 vials	CONTROL SERA, ready to use 0.5 mL per vial, containing human serum with 0.1% NaN_3. *The concentration of control sera are specified in the quality certificate enclosed.*
2 boxes	COATED TUBES, ready to use. 2x50 plastic tubes, 12x75 mm, coated with monoclonal anti- testosterone antibody.
1 pc	Quality certificate
1 pc	Pack leaflet

Materials and equipment required

Test tube rack, plastic foil, precision pipettes with disposable tips (25 and 400 µL, 2 mL), vortex mixer, shaker, absorbent tissue, gamma counter, distilled water
Recommended tools and equipment
repeating pipette for 400 µL, dispenser with reservoir instead of the 2-mL pipette

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 ^{o}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.

CAUTION!
Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Assay procedure

(For a quick guide)

1. Label coated tubes in duplicate for each standard (S1-S6), control serum (CI, CII) and sample (Px). Optionally, label two test tubes for total count (T).
2. Pipette **25 µL** each of STANDARDS, CONTROLS and SAMPLES into the properly labelled tubes.
3. Pipette **400 µL** of TRACER into each tube.
4. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube.
5. Incubate tubes for 2 hours at room temperature (20-25 ^{o}C).
6. Aspirate or decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Add **2.0 mL** of DISTILLED WATER to each tube and repeat step 6..
8. Count each tube for at least 60 seconds in a gamma counter.
9. Calculate the Testosterone concentrations of the samples as described in calculation of results or use special software.

Attention! Higher incubation temperature can cause false results. please keep the recommended temperature range during incubation.

Table 1. Assay Protocol, Pipetting Guide
(all volumes in microlitres)

	T	S_{1-6}	P_X	C_{I-II}
Standard		25		
Sample			25	
Control				25
Tracer	400	400	400	400
Shake for 2 hours at room temperature (20-25 ^{o}C)				
Decant the fluid and blot on filter paper				
Distilled water		2000	2000	2000
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. Data obtained should be similar to those shown in Table 2.
Calculate the average counts per minute (CPM) for each pair of assay tubes.
Calculate the percent B_0/T for zero standard (S_1) by using the following equation:

$$B_0/T\ \% = 100 * S_1 / T$$

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0\ \% = 100 * (S_{2\text{-}6}\ ;\ CI\text{-}II\ ;\ P_x) / S_1$$

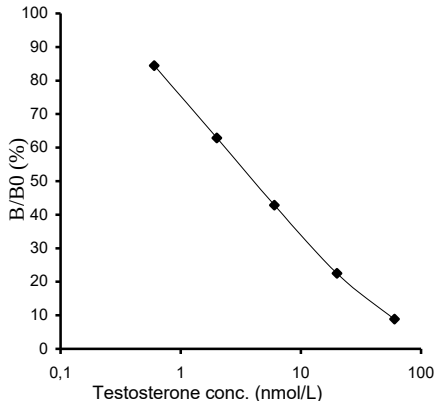
Using a semi-logarithmic graph paper plot B/B_0 % for each standard versus the corresponding concentration of testosterone. Figure 1 shows a typical standard curve.
Determine the testosterone concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Table 2. Typical Assay Data

Tubes	Counts CPM1	Counts CPM2	Mean CPM	Bo/T %	B/Bo %
T	81805	81299	81552		
S1	33103	32440	32772	40.2	
S2	28228	27079	27654		84.4
S3	21193	20005	20599		62.9
S4	13432	14645	14039		42.8
S5	7413	7334	7374		22.5
S6	2853	2940	2897		8.8
CI	23129	22780	22955		70.0
CII	9044	8967	9006		27.5

Figure 1.
A typical standard curve
(Do not use to calculate sample values)



Conversion of SI units **can be performed according to the following formula:**

1 ng/mL = 3.47 nmol/L
1 nmol/L = 0.29 ng/mL

Performance characteristics

Sensitivity

For the analytical sensitivity 0.12 nmol/L has been obtained by assaying 20 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the sum of the mean cpm and its double standard deviation.

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidlines in CLSI document EP17.

Limit of Blank (LoB): 0.075 nmol/L

Limit of Detection (LoD): 0.14 nmol/L

Limit of Quantation (LoQ): 0.21 nmol/L

The functional sensitivity is equal to the Limit of Quantitation (LoQ).

Specificity

Different hormones were added to the "0" standard at two concentration levels (A=70 nmol/L, B=700 nmol/L). The apparent Testosterone concentrations measured are reported below.

Added steroid concentration	70 nM	700 nM
	Measured Testosterone nM	
Estriol	0.1	0.31
11-Deoxicortisol	0.04	0.66
Androstandiol	0.1	0.73
Androstendiol	0	1.03
Cortisol	0.2	1.55
17-β-Estradiol	0.17	1.58
5-β-dihydro-testosterone	0.39	8.52
5-α-dihydro-testosterone	0.83	12.66
Androstendione	1	15.9
Noretisterone	1.34	16.79

Nonmeasurable concentrations were obtained for the following hormones added at 700 nmol/L: 17-α-OH-Progesterone, DHEA, Progestrerone, Aldosterone, Cortisone, Prednisolon, Dexamethasone, Pregnenolone, Pregnenolone-sulphate, Corticosterone, Deoxycorticosterone, Noretisterone-acetate, Estrone. The DHEA-S cross-reactivity was evaluated at 30 µmol/L, the apparent Testosterone concentration measured was 0.43 nmol/L.

Precision and reproducibility

Five serum pools were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 20 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
Mean (nmol/L)	CV%	Mean (nmol/L)	CV%
0.99	7.3	0.98	12.0
2.17	8.9	2.20	10.8
6.76	7.2	6.52	8.3
12.87	3.0	13.02	7.1
33.54	2.8	34.94	6.4

Linearity – dilution test

Four individual serum samples were serially diluted with human serum with low Testosterone concentration and measured according to kit protocol. Mean recovery after dilution was 81.8 %. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$Y = 0.9496X - 0.8119$ $R^2 = 0.9961$ $n = 12$

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of Testosterone. The average per cent recovery for 5 serum samples spiked with Testosterone at 3 levels each was 92.7 %, with a range of 81.8 % to 103.3 %.

Reference interval

Male range: 5.6 - 30.8 nmol/L (2.5 - 97.5%)
mean±SD: 15.14 ± 5.7 nmol/L
median: 15.12 nmol/L

Female range: 0.3 - 3.8 nmol/L (2.5 - 97.5%)
mean±SD: 1.47 ± 0.66 nmol/L
median: 1.49 nmol/L

It is recommended that each laboratory establish 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.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

Additional information

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.

Biohazard

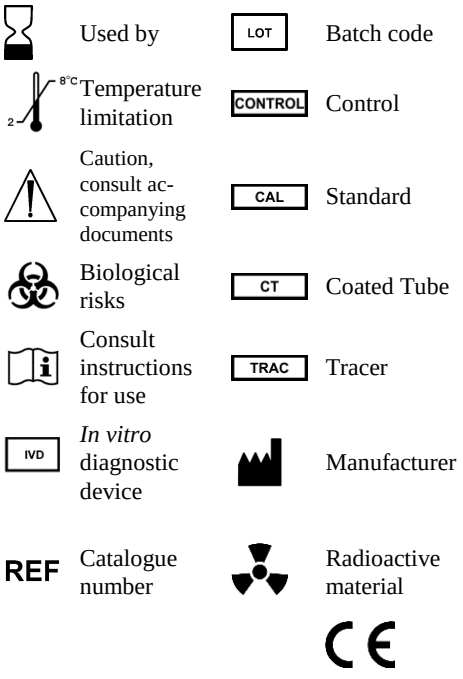
Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). 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 infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 48 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 60 days from availability.



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