



## 1. NAME AND INTENDED USE

TESTO-CT2 is a radioimmunoassay kit for the quantitative determination of Testosterone in human serum and plasma.

## 2. INTRODUCTION

Testosterone (PM 288,4) is a steroid hormone mainly synthesized by the Leydig cells of the testes in males and by ovaries and adrenal cortex in female. Testosterone production in both sexes is regulated by LH. Circulating testosterone is 60 % bound to a globulin : Sex Hormone Binding Globulin (SHBG). Only 1 % of Testosterone is free. It's the active form.

At the time of puberty, pituitary gonadotrophins increase, stimulating testicular maturation : LH acts directly on the interstitial mesenchymal elements, causing them to secrete testosterone and estrogens, as they are developing into mature Leydig cells. FSH acts on the seminiferous tubules to induce and maintain normal spermatogenesis. At the onset of puberty, testosterone stimulates the development of male sex characteristics (such as external genitalia, accessory sex organs, hair growth, linear growth, voice timbre, psyche, muscle and bone tissue mass) which it will maintain throughout life. The age at which maximum testosterone levels are observed varies from 15-20 to 25 years according to the theories. Testosterone is secreted episodically throughout the day, so that multiple peaks can be observed.

Testosterone measurement is used in following cases :

- Delayed puberty and androgen deficiency in men.
- Androgen excess in female (Hirsutism).
- Androgen excess in children.
- Monitoring of endocrine treatment.

The Testosterone measurement is not alone sufficient in the assessment of gonadal function. In the male it must be supplemented at least with careful clinical examination of the patient, evaluation of the ejaculate as well as determination of gonadotrophins. In females with hirsutism-virilism syndrome, additional tests are needed to differentiate between ovarian and adrenal origins of elevated testosterone. These tests may include, eg serum cortisol, ACTH and gonadotrophins.

## 3. PRINCIPLE

The principle of the assay is based on the competition between the labelled testosterone and testosterone contained in standards or specimens to be assayed against a fixed and limited number of antibody binding sites bound to the solid phase (coated tubes).

After incubation, the unbound tracer is easily removed by a washing step.

The amount of labelled testosterone bound to the antibody is inversely related to the amount of unlabelled testosterone present in the sample.

## 4. REAGENTS

Each kit contains enough reagents for 100 tubes. The expiry date is marked on the external label.

REAGENTS	QUANTITY	STORAGE
<b>COATED TUBES</b> : ready to use. Rabbit testosterone antibodies coated to the bottom of the tube.	100 tubes	2-8°C until the expiry date. Take only the amount of tubes needed in one run. Once opened keep the box in the resealable plastic bag with the dessicant bag
<b><sup>125</sup>I-TESTOSTERONE</b> : ready to use. 125I labelled testosterone, buffer, yellow dye and preservative. ≤ 200 kBq (≤ 5,4μCi)	1 55 ml vial	2-8°C until the expiry date.
<b>STANDARD "0"</b> : freeze-dried. Human serum, Sodium Azide and preservative.	1 vial reconstitute with 0.5 ml distilled water	2-8°C until the expiry date. 2-8°C 12 weeks after reconstitution.
<b>STANDARDS 1 to 5</b> : freeze-dried. Synthetic testosterone, human serum, sodium azide and preservative. 0.5 - 1.5 - 5 - 15 - 50 nmol/l	5 vials reconstitute with 0.5 ml distilled water	2-8°C until the expiry date. 2-8°C 12 weeks after reconstitution.

## 5. PRECAUTIONS FOR USE

### 5.1. Safety measures

Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.

Do not pipette by mouth.

Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.

Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.

Avoid splashing.

Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.

Sodium azide may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.



## 5.2. Basic radioprotection rules

This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstance be administered to humans or to animals.

The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.

The enforcement of the basic rules for handling radioactive products ensures adequate security.

A summary of these is given below :

Radioactive products must be stored in their original containers in a suitable area.

A record of the reception and storage of radioactive products must be kept up to date.

Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).

Do not eat, drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.

Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.

Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.

Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.

All radioactive waste disposal must be carried out according to the regulations in force.

## 5.3. Handling precautions

Do not use kit components beyond their expiry date.

Do not mix reagents from different batches.

Avoid any microbial contamination of the reagents or of the water used for washing.

Fully respect the incubation times and the washing instructions indicated.

## 6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed on sera or plasma (heparin or EDTA). If the test is to be carried out within 3 days, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, deep frozen (-20°C) until needed (6 months) and must be thawed only just before using and used thoroughly. Do not refreeze samples from the pool for later use.

### Dilutions

If elevated testosterone levels are suspected samples may be diluted using the standard 0.

It is recommended that disposable plastic tubes be used when carrying out the dilutions.

## 7. ASSAY PROCEDURE

### 7.1. Material required

Precision micropipettes or similar, with disposable tips, capable of dispensing 25 µl, 500 µl and 1 ml. Their calibration should be checked regularly.

Freshly distilled water.

Vortex type mixer.

Parafilm.

Aspirating device.

Water bath (37°C) .

Disposable plastic test-tubes.

Gamma scintillation counter calibrated for 125 Iodine.

### 7.2. Reconstitution of the standards

Reconstitute the standards with 0.5 ml of distilled water. Recap the vial. Mix gently by inversion to assure complete dissolution of the freeze-dried material.

N.B. : The reconstituted standards should stand at least 30 minutes after reconstitution before proceeding.

### 7.3. Protocol

All reagents should be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of reagents is also carried out at room temperature.

The assay requires the following groups of tubes :

T group, for the total activity determination.

Standard groups, to establish the standard curve.

Reference group for the external controls.

Sx groups, for the test samples.

It is recommended to perform the assay in duplicate for the standard groups, controls and samples.

Observe the order in which reagents are to be added :

**Dispense** 25 µl of standards, controls and samples to be assayed into the correspondingly-labeled coated tubes.

**Add** 500 µl of <sup>125</sup>I -Testosterone to each tube (and T group).

**Mix** each tube gently with a Vortex-type mixer.

**Cover** tubes with plastic film.

**Incubate** 1 hours at 37°C in a water bath.

**Aspirate** liquid from each assay tube (except T tubes).

**Rinse** each coated tube (except T tubes) with 1 ml of distilled water.

**Aspirate** immediately (except T tubes).

**Measure** the remaining radioactivity bound to the tubes with a gamma scintillation counter calibrated for 125 Iodine.



### 8. QUALITY CONTROL

Good laboratory practices require the use of quality control samples in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

### 9. RESULTS

For each group of tubes compute the mean counts.

Draw up the standard curve by plotting the standards'cpm against their concentration.

Read sample values directly from the standard curve, and correct the read value for the dilution factor, if necessary. The kit was validated using reference samples measured by gas chromatography mass spectrometry with isotopic dilution.

Conversion nmol/l to ng/ml may be accomplished by :

$$\text{testosterone (ng/ml)} = \text{testosterone (nmol/l)} \times 0.288$$

**Typical standard curve** (example only) : these data must not be substituted for results obtained in the laboratory.

Groups of Tubes	Mean CPM	B/Bo x 100	Concentration nmol/l
T	52287	-	
Standard 0 0 nmol/l	27931	100	
Standard 1 0.5 nmol/l	23394	83.7	
Standard 2 1.5 nmol/l	18774	67.2	
Standard 3 5 nmol/l	12540	44.9	
Standard 4 15 nmol/l	8071	28.9	
Standard 5 50 nmol/l	4031	14.4	
Sample A	5674	20.3	30.2
Sample B	18944	67.8	1.4

### 10. PROCEDURAL LIMITATIONS

Strict adherence to the exact procedures described within this package insert and careful technique should be exercised to obtain reliable results with the TESTO-CT2 kit.

The measurement of serum testosterone is not sufficient in the assessment of gonadal function. The testosterone RIA kit measures total unconjugated testosterone, ie protein-bound and non protein-bound. If the carrier plasma proteins ( SHBG, Albumin ) are likely to be abnormally low (eg in liver disease) or high (eg during oestrogen therapy, pregnancy, before puberty, in old age in males), the respective changes in the fraction of the free testosterone must be considered when the results shall be interpreted.

Total testosterone values cannot be interpreted accurately in female patients treated with Danazol.

### 11. EXPECTED VALUES

Each laboratory must establish its own range of normal values. Testosterone levels from healthy, fasting individuals obtained utilizing the TESTO-CT2 kit are presented in table below (indicative values) :

	n	Range ( nmol/l )
MALE	87	8.2 - 34.6
FEMALE	160	0.3 - 3.0

### 12. SPECIFIC CHARACTERISTICS OF THE ASSAY

#### 12.1. Imprecision

This was evaluated with samples with different concentrations assayed either 12 times in the same series or once in 10 different series.

Samples	Within-run		Samples	Between-run	
	Mean (nmol/l)	(%) CV		Mean (nmol/l)	(%) CV
1	1.6	7.5	5	1.2	7
2	4.8	4.5	6	3.5	5.1
3	11.4	3.8	7	9.1	4.8
4	26.5	5.5	8	23.3	4.8

#### 12.2. Recovery test

Known quantities of Testosterone were added into different serum base pools, including both normal male and normal female samples (n = 6). The recovery percentage of Testosterone obtained were between 84.2 % and 121.7 %.

#### 12.3. Dilution

Serum samples with high testosterone concentrations may be diluted up to 1:10 using the 0 standard of the kit.

#### 12.4. Interfering substances

Serum bilirubin concentration  $\leq 340 \mu\text{mol/l}$  does not interfere. Serum hemoglobin concentration  $\leq 2 \text{ g/l}$  does not interfere. The use of highly lipemic samples is not recommended.



### 12.5. Specificity

Determined from equivalent displacement measurements at 50 % binding. The antiserum used in the test shows the following cross-reactions :

Compound	Cross-reactivity %
Testosterone	100
5 $\alpha$ -Dihydrotestosterone	4.5
5 $\alpha$ -Androstadiol-3 $\beta$ , 17 $\beta$	0.01
Androstandione	0.03
5-Androstendiol	0.02
Androsterone	0.005
Androstendione	0.007
5 $\alpha$ -Androstadiol, 3 $\alpha$ , 17 $\beta$	0.01
Dehydroepiandrosterone	0.003
Dehydroepiandrosteronesulphate	0.00
Methyltestosterone	0.45
Cortisol	0.006
17 $\beta$ -Estradiol	0.012
Progesterone	0.01
Danazol	0.02
17OH-Progesterone	< 0.001

### 12.6. Detection limit

The sensitivity of the method defined as being the detectable concentration equivalent to twice the standard deviation of the zero-binding value, is approximately 0.1 nmol/l.

### ASSAY FLOW-CHART

TUBES	Standards Controls samples $\mu$ l	<sup>125</sup> I Testosterone $\mu$ l		Distilled water ml	
<b>T</b>	-	500		-	
<b>Standards</b>	25	500	Mix ---- Cover the tubes ---	1	Decant liquid ----
<b>Controls and samples</b>	25	500	Incubate 1h at 37 $^{\circ}$ C ---- Decant liquid	1	Count