# PATHOZYME® TESTOSTERONE Ref OD497

## Enzyme Immunoassay for the quantitative determination of Testosterone in human serum.

## Store at 2°C to 8°C. DO NOT FREEZE.

## For in-vitro use only.

### INTRODUCTION

Testosterone (17β-hydroxyandrost-4-ene-3-one) is a C19 steroid with an unsaturated bond between C-4 and C-5, a ketone group in C-3 and a hydroxyl group in the β position at C-17. This steroid hormone has a molecular weight of 288.4 daltons.
Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in females about 50% of circulating testosterone is derived from peripheral conversion of androstenedione, about 25% from the ovary and about 25% from the adrenal glands.
Testosterone is responsible for the development of secondary male sex characteristics and its measurements are helpful in evaluating the hypogonadal states.
In women, high levels of testosterone are generally found in hirsutism and virilisation, polycystic ovaries, ovarian tumours, adrenal tumours and adrenal hyperplasia.
In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumours, congenital adrenal hyperplasia and prostate cancer.
Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminisation, Orchidectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases.

### INTENDED USE

**PATHOZYME TESTOSTERONE** is an Enzyme Immunoassay (EIA) for the the quantitative determination of total Testosterone in human serum.
For professional use only.

### PRINCIPLE OF THE TEST

**PATHOZYME TESTOSTERONE** is based on the principle of competitive binding between Testosterone in the test sample and Testosterone-HRP Conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with Testosterone standards, controls, patient samples, Testosterone-HRP Conjugate Reagent and rabbit anti-Testosterone Reagent. During the incubation, a fixed amount of HRP-labelled Testosterone competes with the endogenous Testosterone in the standard, sample or control serum for a fixed number of binding sites of the specific Testosterone antibody. Thus, the amount of Testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases.
Unbound Testosterone HRP conjugate is then removed and the wells washed. A solution of TMB is then added, resulting in the development of blue colour.
The colour development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabelled Testosterone in the sample. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The Testosterone concentration of the specimens and controls run concurrently with the standards can be calculated from the standard curve.
This test has been calibrated against in house standards. There is no International standard for this test.

### CONTENTS

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**Microtitre Plate** — **12 x 8 wells x 1**
Breakable wells coated with Goat anti Rabbit IgG contained in a resealable foil bag with a desiccant.

**Cal A 0 ng/ml** — **0.5 ml**
Reference Standard: BSA Buffer free of Testosterone.
Ready to use. (Colourless)

**Cal B 0.1 ng/ml** — **0.5 ml**
Reference Standard: Testosterone diluted in BSA Buffer.
Ready to use. (Colourless)

**Cal C 0.5 ng/ml** — **0.5 ml**
Reference Standard: Testosterone diluted in BSA Buffer.
Ready to use (Colourless)

**Cal D 2.0 ng/ml** — **0.5 ml**
Reference Standard: Testosterone diluted in BSA Buffer.
Ready to use. (Colourless)

**Cal E 6.0 ng/ml** — **0.5 ml**
Reference Standard: Testosterone diluted in BSA Buffer.
Ready to use. (Colourless)

**Cal F 18.0 ng/ml** — **0.5 ml**
Reference Standard: Testosterone diluted in BSA Buffer.
Ready to use. (Colourless)

**Control 1 Level as stated on vial** — **0.5 ml**
Known level of Testosterone diluted in human serum.
Ready to use. (Colourless)

**Control 2 Level as stated on vial** — **0.5 ml**
Known level of Testosterone diluted in human serum.
Ready to use. (Colourless)

**Ab REAG Testosterone** — **7 ml**
Rabbit anti Testosterone reagent.
Ready to use. (Pink)

**Conj** — **11 ml**
Testosterone conjugated to horseradish Peroxide
Ready to use. (Blue)

**Subs TMB** — **11 ml**
Substrate Solution: 3,3ô 5,5ôTetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)

**Soln Stop HCl 1M** — **11ml**
Stop Solution: Hydrochloric Acid diluted in purified water.
Ready to use. (Colourless)
Instruction leaflet and EIA Data Recording Sheet — **1 + 1**

### MATERIAL REQUIRED BUT NOT PROVIDED

Micropipettes: 100µl, 200µl and 1000µl
Disposable pipette tips
Incubator: Temperature of 37°C +/- 1°C
Absorbent paper
Microplate reader fitted with a 450nm filter
Graph paper
Thoroughly clean laboratory glassware.

### PRECAUTIONS

**PATHOZYME TESTOSTERONE** contains materials of human origin which have been tested and confirmed negative for HCV, HIV I and II antibodies and HBsAg by FDA approved methods at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within this kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

**PATHOZYME TESTOSTERONE** Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

**PATHOZYME TESTOSTERONE** Stop Solution is dilute Hydrochloric Acid and is therefore corrosive. Handle with care. In case of contact, rinse thoroughly with water.

**PATHOZYME TESTOSTERONE** reagents contain 1.0% Proclin™ 300* as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with water and seek medical advice.

* Proclin™ 300 is a Trade Mark of ROHM and HAAS Ltd.

### STORAGE

Reagents must be stored at temperatures between 2°C to 8°C.

Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

### SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect clear serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversly affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at ï 20°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Azide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

### REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

### LIMITATIONS OF USE

The use of samples other than serum have not been validated in this test. There is no reuse protocol for this product. When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

## ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assy.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.
4. Dispense 10µl of standards, test serum and controls into the appropriate wells.
5. Dispense 100µl of Testosterone HRP Conjugate reagent into each well.
6. Dispense 50µl of rabbit anti-Testosterone Reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely. Place in a wet box with some moist paper.
7. Incubate at 37°C for 90 minutes.
8. At the end of the incubation period, discard the contents of the wells by flicking plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the Biohazard container.
9. Hand Washing: Fill the wells with a minimum of 300µl of distilled water per well. Flick plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper..
10. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
11. Machine Washing: Ensure that 300µl of distilled water is dispensed per well and that an appropriate disinfectant is added to the waste collection bottle. Wash the empty wells 5 times. After washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
12. Dispense 100µl of Substrate solution into each well. Gently mix for 5 seconds.
13. Incubate in the dark at room temperature for 20 minutes (20°C to 25°C)
14. Stop the reaction by adding 100µl of Stop Solution to each well.
15. Gently mix for 30 seconds. It is important to make sure that all the blue colour changes to yellow colour immediately.
16. Read the absorbance at 450 nm with a microtitre well reader within 10 minutes.

## TROUBLESHOOTING

For use by operatives with at least a minimum of basic laboratory training.

Do not use damaged or contaminated kit components.

Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.

It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used, since pipetting of all Standards and specimens should be completed within 3 minutes. A full plate of 96 wells may be used if automated pipetting is available.

Replace caps on all reagents immediately after use.

Avoid repeated pipetting from stock reagents as this is likely to cause contamination.

Do not mix reagents or antibody coated strips from different kits. When dispensing, care should be taken not to touch the surface of the well.

Do not allow reagent to run down the sides of the well. Prior to the start of the assay bring all reagents to room temperature (20°C to 25°C). Gently mix all reagents by gentle inversion or swirling.

Once an assay has been initiated, the wells should not be allowed to become dry during the assay.

Do not contaminate the Substrate Solution as this will render the whole kit inoperative.

Check the precision and accuracy of the laboratory equipment used during the procedure to ensure reproducible results.

The unused strips should be resealed in the foil bag, containing the desiccant, using the resealing zip-lock before being replaced at 2°C to 8°C.

## CALCULATION OF RESULTS

Calculate the mean absorbance value ($A_{450}$) for each set of Standards, Controls and samples.
Construct a point to point standard curve by plotting the mean absorbance obtained for each Standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y-axis and concentrations horizontal or the X-axis.
Use the mean absorbance values for each specimen to determine the corresponding concentration of Testosterone in ng/ml from the standard curve.
If levels of controls or users known samples do not give expected results, test results must be considered invalid.
If using a software package choose a polygon with data extrapolation curve fit.

## EXPECTED VALUES AND SENSITIVITY

The graph produced by the calibrators should be Hyperbolic in shape with the OD450 of the calibrators inversely proportional to their concentration. The OD of calibrator A should be greater than 1.5 and the OD of calibrator F less than 0.75 for the assay results to be valid.

Each laboratory should establish its own normal range based on the patient population. **PATHOZYME TESTOSTERONE** was performed on randomly selected outpatient clinical laboratory samples. The results of these determinations are as follows:

| | | |
|---|---|---|
| Males: | Prepubertal (late) | 0.1-0.2ng/ml |
| | Adult | 3.0-10.0ng/ml |
| Females: | Prepubertal (late) | 0.1-0.2ng/ml |
| | Follicular phase | 0.2-0.8ng/ml |
| | Luteal phase | 0.2-0.8ng/ml |
| | Post menopausal | .08-0.35ng/ml |

## SENSITIVITY

The lowest detectable level of Testosterone in this test is 0.06ng/ml.

## SPECIFICITY

The following materials have been checked for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to Testosterone.
Data on the cross-reactivity for several endogenous and pharmaceutical steroids are summarised in the following table:

$$\text{Cross-reactivity(\%)} = \frac{\text{Observed Testosterone Concentration} \times 100}{\text{Steroid Concentration}}$$

| Steroid | Cross-reactivity |
|---|---|
| Testosterone | 100% |
| Dihydrotestosterone | 0.86% |
| Androstenedione | 0.89% |
| Androsterone | 1.0% |
| 17β Oestradiol | 0.05% |
| Progesterone | <0.05% |
| Epitestosterone | <0.05% |
| 17-OH-Progesterone | <0.05% |
| Oestriol | <0.05% |
| Cortisol | <0.05% |
| DHEA-Sulphate | <0.05% |

## EVALUATION DATA

Calibrated to major competitors and in house standards.
The co-efficient of variation of **PATHOZYME TESTOSTERONE** is less than or equal to 10%.

In an evaluation between the Omega Pathozyme Testosterone kit and the DRG AURICA Kit for samples with levels between 0.18ng/ml and 14.96 ng/ml the following data was generated.

| | |
|---|---|
| Number of Samples | 106 |
| Correlation Co-efficient | 0.8224 |
| Slope | 0.9794 |
| Intercept | 0.6724 |
| Omega Mean | 3.57 ng/ml |
| DRG Mean | 3.57 ng/ml |

These kits were shown to give good correlation.

## REFERENCES


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2. **Yen S.S.C.** Chronic anovulation caused by peripheral endocrine disorders. In, Yen S.C.C. and Jaffe R.B. (eds): Reproductive Endocrinology Chapter 17, W.B. Saunders. Philadelphia, 1991.
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4. **Bricaire, C., Raynaud, A., Benotmane, A., Clair, F., Paniel, B., Mowszowicz, I., Wright, F., Moreau, J.F., Kutten, F., Mauvais-Jarvis, P.** Selective venous catheterisation in the evaluation of hyperandrogenism. J. Endocrinol. Invest. 14(11): 949-956; 1991.
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## QUICK REFERENCE TEST PROCEDURE

1. Dispense 10µl of Standards, test serum or controls into each well.
2. Dispense 100µl of Testosterone HRP Conjugate into each well.
3. Dispense 50µl of Rabbit anti-Testosterone. Gently mix for 30 seconds.
4. Incubate for 90 minutes 37°C.
5. Discard well contents and wash 5 times with distilled water.
6. Add 100µl of Substrate solution to each well and gently shake for 5 seconds.
7. Incubate the plate in the dark for 20 minutes at room temperature (20°C to 25°C).
8. Add 100µl of Stop Solution to each well and gently shake for 30 seconds.
9. Read Optical Densities immediately (no later than 10 minutes) using a microplate reader with a 450nm filter.


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