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**CHEMILUMINESCENCE**  
ENZYME IMMUNOASSAY (CLIA)  
**TESTOSTERONE**

REF

Catalog No. C29-852

IVD

For In Vitro Diagnostic Use Only  
Enzyme Immunoassay for the Quantitative Determination of  
**TESTOSTERONE** in Human Serum or plasma

**INTRODUCTION OF CHEMILUMINESCENCE  
IMMUNOASSAY**

Chemiluminescence Immunoassay (CLIA) detection using microplate luminometers provides a sensitive, high throughput, and economical alternative to conventional colorimetric methodologies, such as Enzyme-linked immunosorbent assays (ELISA).

ELISA employs a label enzyme and a colorimetric substrate to produce an amplified signal for antigen, haptens or antibody quantitation. This technique has been well established and considered as the technology of choice for a wide variety of applications in diagnostics, research, food testing, process quality assurance and quality control, and environmental testing. The most commonly used ELISA is based on colorimetric reactions of chromogenic substrates, (such as TMB) and label enzymes.

Recently, a chemiluminescent immunoassay has been shown to be more sensitive than the conventional colorimetric method(s), and does not require long incubations or the addition of stopping reagents, as is the case in some colorimetric assays. Among various enzyme assays that employ light-emitting reactions, one of the most successful assays is the enhanced chemiluminescent immunoassay involving a horseradish peroxidase (HRP) labeled antibody or antigen and a mixture of chemiluminescent substrate, hydrogen peroxide, and enhancers.

The CLIA Kits are designed to detect glow-based chemiluminescent reactions. The kits provide a broader dynamic assay range, superior low-end sensitivity, and a faster protocol than the conventional colorimetric methods. The series of the kits covers Thyroid panels, such as T3, T4, TSH, Hormone panels, such as hCG, LH, FSH, and other panels. They can be used to replace conventional colorimetric ELISA that have been widely used in many research and diagnostic applications. Furthermore, with the methodological advantages, Chemiluminescent immunoassay will play an important part in the Diagnostic and Research areas that ELISAs can not do.

Immunospec CLIA Kits have been validated on the **MPL** and **MPL2** microplate luminometers from Berthold Detection System, **Lus2** microplate luminometer from Anthos, **Centro LB960** microplate luminometer from Berthold Technologies, and **Platelumino** from Stratec Biomedical Systems AG. We got acceptable results with all of those luminometers.

**INTRODUCTION OF MMUNOASSAY**

Immunospec Testosterone (17 $\beta$ -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  $\beta$  position at C-17. This steroid hormone has a molecular weight of 288.4.

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 ca. 50% of circulating testosterone is derived from peripheral conversion of androstenedione, ca. 25% from the ovary and ca. 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 virilization, polycystic ovaries, ovarian tumors, adrenal tumors and adrenal hyperplasia. In men, high levels of testosterone are associated to the hypothalamic pituitary unit diseases, testicular tumors, congenital adrenal hyperplasia and prostate cancer.

Low levels of testosterone can be found in patients with the following diseases: Hypopituitarism, Klinefelter's syndrome, Testicular feminization, Orchiectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases.

The Testosterone EIA kits are designed for the measurement of total Testosterone in human serum.

**PRINCIPLE OF THE TEST**

Immunospec Testosterone Chemiluminescence Immunoassay is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of rabbit anti-Testosterone. In the incubation, goat anti-rabbit IgG-coated wells are incubated with 10  $\mu$ l of Testosterone standards, controls, patient samples, 100  $\mu$ l Testosterone-HRP conjugate reagent and 50  $\mu$ l rabbit anti-Testosterone reagent at 37°C for 90 minutes. During the incubation, a fixed amount of HRP-labeled Testosterone competes with the endogenous Testosterone in the standard, sample, or quality 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 peroxidase conjugate is then removed and the wells washed. Next, A solution of chemiluminescent substrate is then added and read relative light units (RLU) with a Luminometer. The intensity of the emitting light is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled TESTOSTERONE in the sample. By reference to a series of TESTOSTERONE standards assayed in the same way, the concentration of TESTOSTERONE in the unknown sample is quantified.

**MATERIALS AND COMPONENTS**

**Materials Provided with Test Kit**

1. Goat Anti-Rabbit IgG-coated microtiter wells, 96 wells
2. Testosterone Reference Standards: 0, 0.1, 0.5, 2.0, 6.0 and 18.0 ng/ml. Liquids, 0.50 ml each, ready to use.
3. Rabbit Anti-Testosterone Reagent, 7.0 ml
4. Testosterone-HRP Conjugate Reagent, 12 ml
5. 50x Wash Buffer Concentrate, 15 ml
6. Chemiluminescence Reagent A, 6.0 ml
7. Chemiluminescence Reagent B, 6.0 ml
8. Controls, Level 1&2 (Optional)

**Materials Required but not Provided**

1. Distilled water.
2. Precision pipettes: 5~40  $\mu$ l, 50~200  $\mu$ l, 1.0ml
3. Disposable pipette tips.
4. Glass tube or flasks to mix Chemiluminescence Reagent A and B.
5. Microtiter well luminometer.
6. Vortex mixer or equivalent.
7. Absorbent paper.
8. Graph paper.

**REAGENT PREPARATION**

1. To prepare substrate solution, make a 1:1 mixing of Reagent A with Reagent B right before use. Mix gently to ensure complete mixing. Discard excess after use.
2. Dilute 1 volume of Wash Buffer (50x) with 49 volumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

**ASSAY PROCEDURE**

1. Secure the desired number of coated wells in the holder.
2. Dispense 10 µl of standards, specimens and controls into appropriate wells.
3. Dispense 50 µl of rabbit anti-Testosterone reagent to each well.
4. Dispense 100 µl of Testosterone-HRP Conjugate Reagent into each well.
5. **Thoroughly mix for 30 seconds. It is very important to mix them completely.**
6. Incubate at 37°C for 90 minutes.
7. Rinse and flick the microwells 5 times with washing buffer(1X).
8. Dispense 100 µl Chemiluminescence substrate solution into each well. Gently mix for 5 seconds.
9. Read wells with a chemiluminescence microwell reader 5 minutes later. (between 5 and 20 min. after dispensing the substrates).

**Important Note:**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. If there are bobbles existing in the wells, the false readings will be created. Please use distilled water to remove the bobbles before adding the substrate.

**CALCULATION OF RESULTS**

Calculate the average read relative light units (RLU) for each set of reference standards, control, and samples.

We recommend to use a proper software to calculate the results. The best curve fitting used in the assays are 4-parameter regression or cubic spline regression. If the software is not available, construct a standard curve by plotting the mean RLU obtained for each reference standard against TESTOSTERONE concentration in Ng/ml on linear graph paper, with RLU on the vertical (y) axis and concentration on the horizontal (x) axis.

Using the mean absorbance value for each sample, determine the corresponding concentration of TESTOSTERONE in ng/ml from the standard curve.

**EXAMPLE OF STANDARD CURVE**

Results of a typical standard run are shown below. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. It is required that running assay together with a standard curve each time. The calculation of the sample values must be based on the particular curve, which is running at the same time.

TESTOSTERONE (ng/ml)	Relative Light Units (RLU) (10 <sup>3</sup> )
0	92.6
0.1	57.0
0.5	35.2
2	23.0
6	11.5
18	2.40

**EXPECTED VALUES AND SENSITIVITY**

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

Males: prepubertal (late) 0.1 – 0.2 ng/ml  
 Adult 3.0 – 10.0 ng/ml

Females: prepubertal (late) 0.1 – 0.2 ng/ml  
 follicular phase 0.2 – 0.8 ng/ml  
 luteal phase 0.2 – 0.8 ng/ml  
 post menopausal 0.08 – 0.35 ng/ml

The minimum detectable concentration of the Testosterone ELISA assay as measured by 2 SD from the mean of a zero standard is estimated to be 0.05 ng/ml.

**CLINICAL APPLICATION**

**In Male:**

In man, the determination of testosterone is used as an indicator for the function of the testes: low hormone levels are found in cases with Klinefelter's syndrome, cryptorchism or anorchia. Male with testosterone deficiency often present with a number of symptoms such as decreased libido, as well as decreased muscle strength, gynecomastia and infertility.

**In Female:**

**1. Virilizing Disorders:**

Testosterone measurements are frequently utilized in the evaluation of virilizing disorders. Testosterone concentrations >2.0 ng/ml may indicate androgen secreting ovarian or adrenal neoplasms.

**2. Monitoring of Androgen Suppressing Drugs:**

Testosterone measurements may be utilized in women for the adjustment of androgen suppressing drugs and their dosages.

**3. Pregnancy:**

Testosterone concentrations are relatively consistent during the pregnancy.

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