ORIGENE

OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200, Rockville, MD 20850
Phone: 1.888.267.4436 Fax: 301-340-9254
Email: techsupport@origene.com Web: www.origene.com

Product Information

Mouse/Rat Testosterone ELISA kit

Catalog Number: EA100867
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Testosterone ELISA is for the quantitative determination of Testosterone concentration in mouse/rat serum or plasma.

Background

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 Testosterone is the most important androgen secreted into the blood. In males, testosterone is secreted primarily by the Leydig cells of the testes; in female 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, Orchidectomy and Cryptorchidism, enzymatic defects and some autoimmune diseases.

Principle of the Test

The Testosterone EIA is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone-HRP conjugate for a constant amount of mouse anti-Testosterone. In the incubation, mouse anti-Testosterone coated wells are incubated with 25 µl of Testosterone standards, controls, patient samples, and 100 µl Testosterone-HRP conjugate reagent at room temperature for 60 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 TMB Reagent is then added and incubated at room temperature for 15 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450nm.

Components

MATERIALS PROVIDED	96 Tests
1. Microwell coated with Goat Anti-Rabbit IgG	12x8x1
2. Standard: 6 vials (ready to use)	0.5 ml
3. Rabbit Anti-Testosterone Reagent (ready to use)	7 ml
4. Assay Diluent: 1 bottle (Ready to Use)	12 ml
5. Enzyme Conjugate Conc. (20X): 1 Vial	0.7 ml
6. TMB Substrate: 1 bottle (ready to use)	12 ml
7. Stop Solution: 1 bottle (ready to use)	12 ml
8. Wash Buffer (20X): 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Plasma should be treated with EDTA.
3. Plasma samples may be stored at 2-8°C for up to 8 hours, and should be frozen at -20°C or lower for up to 4 months.
4. Avoid multiple freeze-thaw cycles.
5. Prior to assay, frozen sera should be completely thawed and mixed well.
6. Do not use grossly hemolyzed or lipemic specimens.

Reagent Preparation

1. **20X Enzyme conjugate**: Prepare 1X working solution at 1:20 with assay diluent (e.g. Add 0.1ml of the Testosterone enzyme conjugate concentrate to 1.9ml of assay diluent)
2. **Prepare 1X Wash buffer**: Add the Wash Buffer (20X, 25 ml) to 475 ml of distilled or de-ionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Secure the desired number of coated wells in the holder.
2. Dispense 25 µl of standards, specimens and controls into appropriate wells.
3. Dispense 100 µl of working dilution of Testosterone-HRP Conjugate Reagent into each well.
4. Dispense 50 µl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature 60 minutes.
6. Rinse and flick the microwells 3 times with 1x wash buffer water.
7. Dispense 100 µl of TMB Reagent into each well. Gently mix for 5 seconds.
8. Incubate at room temperature (18-26°C) for 15 minutes.
9. Stop the reaction by adding 50 µl of Stop Solution to each well.
10. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

Calculation of Results

The standard curve is constructed as follows:
1. Check Total Estrogens standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the OD (Optical Density) for each Total Estrogens standard point (Y-axis) versus the Total Estrogens standard concentrations (X-axis) on a linear graph paper. Draw the best curve through the points.
3. Read the concentration (ng/ml) for controls and each unknown sample from the curve. Record the value for each control or unknown sample
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.

Example of Standard Curve

Standard	Conc.ng/mL	OD450
Std 1	0	2.38
Std 2	0.1	1.75
Std 3	0.5	1.02
Std 4	2.0	0.59
Std 5	6.0	0.34
Std 6	18.0	0.17

References

1. Chen, A., Bookstein, J.J., Meldrum, D.R., Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. Fertil. Steril., 1991; 55: 1202-1203.
2. Granoff, A.B. and Abraham, G.E., Peripheral and adrenal venous levels of steroids in a patient with virilizing adrenal adenoma. Obstet. Gynecol., 1979; 53:111-115.
3. Bricaire, C., Raynaud, A., Benotmane, A., et al., Selective venous catheterization in the evaluation of hyperandrogenism. J. Endocrinol Invest., 1991; 14: 949-956.
4. Heinonen, P.K., Androgen production by epithelial ovarian tumours in post-menopausal women. Maturitas, 1991; 13: 117-117-122

5. Tietz, N.W. ed., Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Co., Philadelphia, 1995: 578-580.
6. USA Center for Disease Control/National Institute of Health Manual, “Biosafety in Microbiological and Biomedical Laboratories"”84
7. ICN Guide to Endocrine Testing. Diagnostic Division, ICN Biomedicals, Inc. pp. 2:33-35; 3:4-6.

Version 3, last updated October 18, 2015