Human Testosterone (free) ELISA Kit

Enzyme Immunoassay for the quantitative determination of Human Free Testosterone in serum and plasma

Catalog number: ARG80855

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Testosterone is a steroid hormone from the androgen group. Testosterone is primarily secreted in the testes of males and the ovaries of females although small amounts are secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In both males and females, it plays key roles in health and well-being.

Due to its insolubility in aqueous solutions, for the most part Testosterone circulates in the blood bound to transport proteins. Only a small percentage (< 1%) of circulating Testosterone exists as unbound or free Testosterone. The majority, approximately 60%, is bound to SHBG with high affinity, while the remainder is loosely bound to albumin. Both the albumin-bound and free fractions may be biologically active, while SHBG effectively inhibits Testosterone action. Testosterone effects can be classified as virilizing and anabolic effects. Anabolic effects include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation. Virilizing effects include maturation of the sex organs. Testosterone levels decline gradually with age in men.

Measurement of the free or unbound fraction of serum Testosterone has been proposed as a means of estimating the physiologically bioactive hormone. Free Testosterone levels are elevated in women with hyperandrogenism associated with hirsutism in the presence or absence of polycystic ovarian disease. In addition, free Testosterone measurements may be more useful than total Testosterone in situations where SHBG is increased or decreased (e.g. hypothyroidism and obesity).

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. Testosterone in the blood is bound to SHBG (60%) and in lower quantity to other proteins (for example albumin). This ELISA kit only the measurement of Free Testosterone (< 1% of Total Testosterone) permits the estimating of the hormone biologically active. A highly specific Testosterone antibody has been pre-coated onto a microtiter plate. Testosterone containing samples or standards and a Testosterone-HRP conjugate are given into the wells of the microtiter plate. Enzyme labeled Testosterone and free Testosterone compete for the antibody binding sites. After incubation at room temperature, the wells are washed with diluted washing solution to remove unbound material. A substrate solution is added and incubated, resulting in the development of a blue color. The color development is inhibited by the addition of a stop solution, and the color turns yellow. The yellow color is measured at 450 nm. The concentration of Testosterone is indirectly proportional to the color intensity of the test sample.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date. Open the bag of antibody-coated microplate only when it is at room temperature and close it immediately after use, once opened, it is stable until the expiry date of the kit.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-Testosterone Conjugate	15 ml (ready to use)	4°C
Standards 0-5 (0, 0.2, 1, 4, 20, 100 pg/ml)	6 X 1 ml (ready to use)	4°C
Control 1 (1.33 pg/ml; acc. range: 0.71-1.95 pg/ml)	1 ml (ready to use)	4°C
Control 2 (15.01 pg/ml; acc. range: 9.99-20.03 pg/ml)	1 ml (ready to use)	4°C
10X Wash Buffer	50 ml	4°C
TMB substrate	15 ml (ready to use)	4°C (Protect from light)
STOP solution	15 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: read at 620-630 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- All reagents should be stored refrigerated at 2 °C- 8 °C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22 °C- 28 °C) and mix well prior to use.
- If crystals are observed in the 10X Wash buffer, warm to RT until the crystals are completely dissolved.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- Standards: The Standards are ready to use. Mix the vials thoroughly with a rotating mixer for 5 minutes before opening. Once opened, the Standards are stable 6 months at 2-8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 22-28°C) for at least 30 min. All reagents should be store at 2°C- 8°C immediately after used and avoid long exposure to room temperature. Standards, samples and controls should be assayed in duplicates.

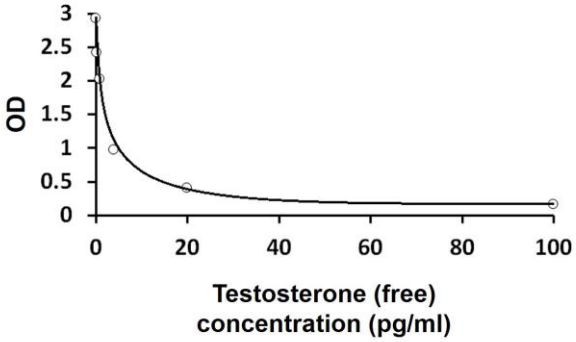
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it and stored at 2°C - 8°C.
2. Add 20 µl of standards, controls and samples in duplicate into wells. Keep one well empty as blank.
3. Add 100 µl of HRP-conjugated antibody into each well, expect blank well. Gently tap the plate to mix well. Incubate for 1 hours at 37°C.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Gently shake the plate for 5 seconds. Then complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

   Note: if you use automated equipment, wash the wells at least 5 times.
5. Add 100 µl of TMB Reagent to each well (including blank well). Incubate for 15 minutes at room temperature in dark.
6. Add 100 µl of Stop Solution to each well. Shake the microplate gently. The color of the solution should change from blue to yellow.
7. Read the OD with a microplate reader at 450 nm against a reference

wavelength of 620-630 nm or against Blank. It is recommended read the absorbance within 5 minutes after adding stop solution.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. Refer to the table below for molar conversion:

	Concentration of standards					
Standard	A	B	C	D	E	F
Testosterone (pg/ml)	0	0.2	1	4	20	100
Testosterone (pmol/L)	0	0.694	3.47	13.88	69.4	347
Conversion	Testosterone (pg/ml) x 3.47 = Testosterone (pmol/L)					

QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.04 pg/ml.

Specificity

The following substances were tested for cross reactivity of the assay:

Steroid	Cross Reaction (%)	Steroid	Cross Reaction (%)
DHT	0.0822	Danazol	0.00327
Androstenedione	0.132	Aldosterone	< 0.00001
Androsterone	0.00005	Estriol	0.00009
DHEA-S	< 0.00001	Epitestosterone	0.00001
Cortisol	< 0.00001	Progesterone	< 0.00001
Cortisone	< 0.00001	17aOH-progesterone	0.00008
17β Estradiol	0.00069	DHEA	0.00003
Estrone	< 0.00001	Prednisone	< 0.00001
Prednisone	< 0.00001	Sodium Citrate	< 0.00001
17α Ethynilestradiol	0.00002	EDTA	< 0.00001
Norgestrel	0.0059	Heparin	< 0.00001

Intra-assay and inter-assay precision

The CV value of intra-assay precision was ≤ 8.9% and the CV value of inter-assay precision was ≤ 12.4%.