Human Free Testosterone ELISA Kit (Competitive EIA)

Catalog number: EK7057

For detection of multiple analytes using one single assay.

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

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Catalog Number: EK7057

Size: 96 wells/kit

Sample Type: Serum and Plasma

Sensitivity: 0.25 pg/ml

Assay Range: 0.25-100 pg/ml

Storage: Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.)

Introduction

This ELISA kit is of competitive format. Competitive ELISA, also known as inhibition ELISA, is a surface/plate based assay, where the plate is coated with capture antibodies reactive to the molecule of interest. The sample (containing native molecule of interest) and enzyme conjugated recombinant protein (the competing molecule) are added to the coated wells. Since the amount of enzyme conjugated molecule in each well is constant, the level of native molecule in the sample will determine the binding ratio of enzyme conjugated molecule vs. native molecule. After an incubation period, any unbound antibody is washed off. Enzyme substrate (for example, TMB for HRP) is added to each well and will be transformed into a blue precipitate, the amount of which is linearly proportional to the amount of enzyme in the well. The precipitate is then turned into yellow by adding the acid stop solution and the concentration of yellow precipitate is read at 450nm for light absorbance (O.D. value). The O.D. is then used to calculate the amount of molecule of interest in each well, by comparing each sample well against the standard curve. The standard curve is generated using the same principle but instead of adding samples, a series of recombinant molecules with known concentrations are added to 6-8 wells.

Kit Components

Description	Quantity
1. Microwells coated with Goat anti-rabbit IgG	12x8x1
2. Standard: 6 vials (ready to use)	0.5 ml
3. Enzyme Conjugate (ready to use)	7 ml
4. Rabbit Anti-Testosterone Reagent (ready to use)	7 ml
5. TMB substrate (ready to use)	12 ml
6. Stop solution (ready to use)	12 ml
7. Wash Solution 20x Concentrated	25ml

Standard Concentrations and example data

	OD 450 nm	Conc. pg/mL
Std 1	2.762	0
Std 2	1.528	0.15
Std 3	0.903	1.5
Std 4	0.468	8
Std 5	0.14	25

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Std 6	0.075	60

Materials Required, but Not Provided

1. Distilled or deionized water

2. Precision pipettes

3. Disposable pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel

6. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. For laboratory use.
3. Potential biohazardous materials: The standards contain human source components which have been tested and found non- reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
4. This kit is designed for research use only.
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
6. 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 serum samples be run in duplicate.
7. This test kit is designed for Research and Development use only.
8. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

SPECIMEN COLLECTION AND HANDLING

1. Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature.
2. Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant and centrifuged immediately after collection.
3. Do not use haemolytic, icteric or lipaemic serum.
4. Testosterone can be determined in plasma as well as in serum of patients who have been fasting. The clinical significance of the determination of Free Testosterone can be invalidated if the patient was treated with cortisone or natural or synthetic steroids
5. Specimens which are not used at the same day of collection have to be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing
6. Samples with values greater than the highest standard should be diluted with standard 0 and reassayed.

PREPARATION FOR ASSAY

20X Wash Buffer: Prepare 1X Wash Buffer by adding the contents of the bottle (25ml, 20X) to 475 ml of distilled or deionized water. Store at room

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temperature (20-25°C).

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming. Once the test has been started, all steps should be completed without interruption.

1. Secure the desired number of microwells strips in the holder.
2. Dispense 25 ul Testosterone Standards, controls and samples with new disposable tips into appropriate wells.
3. Dispense 50ul anti-testosterone reagent into each well.
4. Dispense 50ul Enzyme Conjugate into each well.
5. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for 1 hour at room temperature.
7. Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted wash solution. Strike the wells sharply on absorbent paper to remove residual water droplets. NOTE: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
8. Add 100 ul of Substrate Solution to each well.
9. Incubate for 15 minutes at room temperature in the dark.
10. Stop the enzymatic reaction by adding 50 ul of Stop Solution into each well.
11. Read absorbance on ELISA Reader at 450 nm within 10 minutes after adding the stop solution.