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antibody and ELISA experts

Human Free Estriol ELISA Kit (Competitive EIA)

Catalog number: EK7048

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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Human Free Estriol ELISA Kit (Competitive EIA)

Catalog Number: EK7048

Size: 96wells/kit

Sample Type: Serum and Plasma

Sensitivity: 0.1 μg/ml

Assay Range: 0.1-10 μg/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 anti-Estriol Ab	12x8x1
2. Estriol Standard: 6 vials (ready to use)	1 ml
3. Control Low & High: 2 vials (ready to use)	2x1 ml
4. Enzyme Conjugate: 1 Bottle (ready to use)	14 ml
5. TMB Substrate reagent: One Bottle (ready to use)	14 ml
6. Wash Buffer (40X)	30 ml
7. Stop Solution: One Bottle (ready to use)	14 ml

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

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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 calibrator and controls 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, 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. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. It is recommended that standards, control and serum samples be run in duplicate.
7. 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.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Typically, specimens may be stored refrigerated at (2-8. C) for 5 days. If storage time exceeds 5 days, store frozen at (-20. C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENT PREPARATION

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

ASSAY PROCEDURE

All reagents and specimens must be allowed to come to room temperature before use.
1. Secure the desired number of coated strips in the holder.
2. Dispense 10 ul of each standards, controls and sample with new disposible tips into appropriate wells.
3. Dispense 50 ul of DHEA-S Enzyme-Reagent into each well.
4. Dispense 50 ul of Anti-DHEA-S Antibody-Reagent into each well.
5. Thoroughly mix the plate for 10 seconds. It is important to have complete mixing in this step.
6. Incubate for 60 minutes at room temperature.
7. Briskly shake out the contents of the wells.
8. Rinse the wells 3 times with diluted Wash Solution (350 ul per well). Strike the wells sharply on absorbent paper to remove residual droplets.
9. Add 100 ul of TMB Substrate into each well.
10. Incubate for 30 minutes at room temperature.
11. Stop the enzymatic reaction by adding 50 ul of Stop Solution into each well.
12. Read absorbance on ELISA Reader at 450 nm within 10 minutes of adding the Stop Solution.

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CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check DHEA-S 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 absorbance for the CEA standards (vertical versus standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

LIMITATION OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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