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antibody and ELISA experts

Human Total Estrogens ELISA Kit

Catalog number: EK7015

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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3942 B Valley Ave, Pleasanton, CA 94566
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Human Total Estrogens ELISA Kit

Catalog Number: EK7015

Size: 96wells/kit

Sample Type: Serum and plasma

Assay Range: 10-1500 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 Total Estrogens ELISA kit is based on the principle of competitive binding between Estrogens in the test specimen and enzyme labeled estrogens for a constant amount of rabbit anti-estrogens antibody. In the first incubation, goat anti-rabbit IgG-coated wells are incubated with 50ul of Total Estrogens standards, controls, patient samples, 100ul EstrogensHRP conjugate reagent and 50ul rabbit anti-Total Estrogens reagent, at room temperature, for 120 minutes, with agitation. During the incubation, HRP labeled Total Estrogens competes with the endogenous estrogens in the standard, controls and sample, for a fixed number of binding sites of the specific anti Total Estrogens antibody. Thus, the amount of HRP labeled Estrogens conjugate immunologically bound to the antibody progressively decreases as the concentration of Total Estrogens in the specimen increases. Unbound Total estrogens enzyme conjugate is then removed and the wells washed. Then substrate (TMB) reagent is added and incubated, with agitation, for 30 minutes, resulting in the development of blue color. The color development is stopped with the addition of 50µL stop solution, and the absorbance is spectrophotometrically measured at 450nm. A standard curve is prepared relating color intensity to the concentration of Total Estrogens versus the absorbance.

Kit Components

Description	Quantity
Microwells coated with Goat Anti Rabbit IgG	12x8x1 Microwells
Total Estrogens Standards	7 vials (ready to use) 0.250 ml
Anti Total Estrogens Ab Reagent	1 vial (ready to use) 7 ml
Estrogens-HRP Conjugate (20x)	1 Vial 0.7 ml
Assay Diluent	1 bottle (ready to use) 12ml
TMB Substrate	1 bottle (ready to use) 12ml
Stop Solution	1 bottle (ready to use) 12ml
20X Wash concentrate	1 bottle 25ml

Materials Required, but Not Provided

1. Distilled or deionized water

2. Ethyl acetate: Hexane (Mix ratio 3:2)

3. Precision pipettes

4. Disposable pipette tips

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5. ELISA reader

6. Disposable glass tubes

7. Compressed inert gas (nitrogen)

8. Absorbent paper or paper towel

9. Graph paper

WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.

2. 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.

3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

5. It is recommended that standards, control and serum samples be run in duplicate.

6. 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.

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

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.

2. Typically, specimens may be stored refrigerated at (2°C to 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.

EXTRACTION OF SERUM/PLASMA SAMPLES

Note: A mixture of 3:2 Ethyl acetate: Hexane is used to extract the estrogens from serum or plasma samples

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1. Add 0.2ml of serum or plasma to an appropriate size of glass tube.

2. Add 2mL of the mixture to serum or plasma.

3. Vortex vigorously for 1minute and allow the phase to separate.

4. Transfer the organic layer into a clean glass tube and evaporate under inert gas (e.g. nitrogen).

5. Reconstitute the sample pellet with 1X PBS, pH 7.0. The sample is ready for assay (50µL per well).

6. During sample analysis, multiply the outcome by a dilution factor (It is 10 in the above example).

REAGENT PREPARATION

20X Enzyme Conjugate: Prepare 1X working solution at 1:19 with assay diluent (e.g. Add 0.1mL of the estrogens enzyme conjugate concentrate to 1.9mL of assay diluent)

20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475 mL of distilled water. Store 1X wash buffer at room temperature.

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature (20-25°C).
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.

2. Add 50 ul of Total Estrogens standards, controls and patient's samples into designated microwells.

3. Add 100 ul of the working solution of Total Estrogens enzyme reagent into each well.

4. Add 50µl of anti Total Estrogens antibody reagent into each well.

5. Cover the plate and incubate for 120 minutes at room temperature with shaking (600rpm).

6. Remove liquid from all wells. Wash wells three times with 350 ul of 1X Wash Buffer. Blot on absorbent paper towels.

7. Add 100 ul of TMB Substrate into each well.

8. Cover the plate, and incubate for 30 minutes at room temperature with shaking (600rpm).

9. Uncover the plate and add 50 ul of Stop Solution into each well. Mix, gently, for 10 seconds.

10. Read the absorbance at 450 nm within 10 minutes in a microplate reader.

CALCULATION OF RESULTS

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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 (vertical axis) versus the Total Estrogens standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

3. Read the concentration (pg/ml) for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

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