Mouse Rat Estradiol ELISA Kit

Catalog number: EK7003

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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Mouse Rat Estradiol ELISA Kit

Catalog Number: EK7003

Size: 96wells/kit

Sample Type: Serum and plasma

Sensitivity: 3 pg/ml

Assay Range: 3-300 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 based on the principle of competitive binding between E2 in the test specimen and E2 enzyme conjugate for a constant amount of anti-Estradiol polyclonal antibody. In the incubation, anti-E2 antibody coated wells are incubated with E2 standards, controls, samples, and E2 enzyme conjugate at room temperature for 60 minutes. During the incubation, a fixed amount of HRPlabeled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 30 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 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance

Kit Components

Description	Quantity
Microwells coated with polyclonal anti-Estradiol Antibody	12x8x1 Microwells
Estradiol Standards	6 vials (ready to use) 0.5 ml
Estradiol Enzyme conjugate Concentrate, 20X	1 vial 0.7ml
Assay Diluent	1 bottle (ready to use) 12ml
TMB Reagent	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. 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

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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.

REAGENT PREPARATION

20X Enzyme conjugate: Prepare 1X working solution at 1:20 with assay diluent (e.g. Add 0.1ml of the E2 enzyme conjugate concentrate to 1.9ml of assay diluent)

Wash Buffer: 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

1. Bring all reagents to room temperature (20-25°C) before use.

2. Secure the desired number of coated wells in the holder.

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3. Dispense 25 ul of standards, specimens and controls into appropriate wells.

4. Dispense 100 ul of working reagent of Estradiol enzyme conjugate into each well.

5. Mix well by placing on shaker for 10 – 20 seconds.

6. Incubate at room temperature (20-25°C) for 120 minutes.

7. Remove liquid from all wells. Wash wells three times with 300 uL of 1X wash buffer. Blot on absorbance paper or paper towel.

8. Dispense 100 ul of TMB Reagent into each well. Gently mix for 10 seconds.

9. Incubate at room temperature (20-25°C) for 30 minutes.

10. Stop the reaction by adding 50 ul of Stop Solution to each well.

11. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

12. Read absorbance at 450 nm with a microplate reader within 15 minutes.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in pg/ml on a linear-linear graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.

3. Use the mean absorbance values for each specimen to determine the corresponding concentration of Estradiol in pg/ml from the standard curve.

4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.