BOSTER

antibody and ELISA experts

Androstenedione ELISA Kit (Competitive EIA)

Catalog number: EK7019

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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Androstenedione ELISA Kit (Competitive EIA)

Catalog Number: EK7019

Size: 96 wells/kit

Sample Type: Serum and Plasma

Sensitivity: 0.12 ng/mL

Assay Range: 0.12-10 ng/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
Microwells coated with Goat anti-rabbit IgG	12x8x1
Standard: 6 vials (ready to use)	0.5 ml
Enzyme Conjugate (ready to use)	7 ml
Rabbit Anti- Androstenedione Reagent (ready to use)	7 ml
TMB substrate (ready to use)	12 ml
Stop solution (ready to use)	12 ml
Wash Solution 20x Concentrated	25 ml

Standard Concentrations and example data

	OD 450 nm	Conc. ng/mL
Std 1	2.132	0
Std 2	1.705	0.12
Std 3	1.324	0.37

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Std 4	0.811	1.11
Std 5	0.314	3.33
Std 6	0.171	10

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. Not for Internal or External Use in Humans or Animals.
4. There should be no eating or drinking within work area.
5. Always wear gloves and a protective lab coat.
6. No pipetting should be done by mouth. Handle all specimens and reagents as potentially infectious and biohazardous.
7. Do not add sodium azide to samples as preservative.
8. Do not use external controls containing sodium azide.
9. Use disposable pipette tips to avoid contaminating chromogenic substrate reagent. Discard reagent if it turns blue.
10. Do not pour chromogenic substrate back into container after use.
11. Do not freeze reagents.
12. Do not mix reagents from different kit lot numbers.
13. Keep reagents out of direct sunlight.
14. Handle stop reagent with care, since it is corrosive.
15. Bring all reagents to room temperature.
16. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.
17. Ensure the bag containing the micro-plate strips and desiccant is sealed well, if only a few strips are used.

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 1 week. If storage time exceeds 1 week, 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.

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PREPARATION FOR ASSAY

20XWash 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 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 25ul Androstenedione Standards, controls and samples into appropriate wells.
3. Dispense 50ul Enzyme Conjugate into each well.
4. Dispense 50ul anti- Androstenedione reagent into each well.
5. Incubate for 60 minutes at room temperature with shaking.
6. 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.
7. Add 100 ul of Substrate Solution to each well.
8. Incubate for 15 minutes at room temperature.
9. Stop the enzymatic reaction by adding 50ul of Stop Solution into each well.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples The standard curve is constructed as follows: concentration in ng/ml 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 of
2. To construct the standard curve, plot the absorbance for DHEA-S standards (vertical axis) capability, other methods of data reduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit. For instance, if the calculated value for a urine sample from the standard curve is 2.40 .g/ml; then Androstenedione concentration higher than the concentration of the highest standard have to be diluted with zero standard. For the calculation of the concentrations this dilution factor has to be taken into account.

LIMITATION OF THE TEST

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

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