biosensis® Mouse/Rat Testosterone Competitive ELISA
Kit Protocol

Catalog No: BEK-2362-2P

A competitive ELISA for the quantitative detection of Mouse/Rat Testosterone in serum only when used as directed.

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I. Materials Provided

Kit Components	Unit Size/Volume
Recombinant standards	12 vials (ready to use) 0.5 ml each
96 wells (12x8x1) strips pre-coated Goat Anti-Rabbit IgG antibody	2 sets (12x8x1)
Rabbit anti-testosterone reagent	7 mL x 2
Assay Diluent	12ml x 2
Enzyme Conjugate Concentrate (20X)	7 mL x 2
Wash Buffer Concentrate (20X)	25 mL x 2
TMB ready to use substrate	12 ml x 2
TMB ready to use stop solution	12 ml x 2

WARNING: DO NOT MIX REAGENTS SUCH AS ANTIBODY BUFFERS ETC BETWEEN DIFFERENT ASSAYS IF USING MULTIPLE KITS. USE ONLY THE BUFFERS, STANDARDS & SOLUTIONS INCLUDED FOR YOUR SPECIFIC LOT. MIXING SOLUTIONS FROM DIFFERENT TARGET KITS CAN LEAD TO POOR KIT PERFORMANCE

II. Equipment Required but Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips. Multichannel pipettes are recommended
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

IIA. 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. Optimal results will be obtained by strict adherence to this protocol.
6. 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.

III. Technical Hints

1. Spin all kit components to get liquid to the bottom of tubes before use.

2. It is recommended that standards, control and serum samples be run in duplicate.
3. In order to avoid marginal effects of plate incubation due to temperature differences, it is suggested that TMB solutions be brought to room 30 min before use.

4. Recommended method for manual plate washing.

   - Emptying of wells on the plate:
     Place the plate on the palm of the hand in a position that enables easy flicking movement using the wrist. Holding the plate over a sink, quickly invert the plate, whilst accelerating the arm downward toward the sink. Abruptly stop the downward acceleration to force the liquid from the wells into the sink. When done correctly the technique should prevent liquid from getting on to the fingers or on the outside of the strip wells or plate holder. *Note: Retain the upside down position of the plate to avoid any back flow into the wells. DO NOT LET THE PLATE DRY OUT.*

   - Blotting the plate:
     Immediately blot the inverted plate by lightly tapping the plate 3-4 times on blotting paper.

   - Washing: Forcefully pipette Wash Buffer into each well with a multi-channel pipette. Empty the wells of wash buffer using technique described above. Repeat washing and flicking procedures thrice. Wash plate 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min before rinsing with more wash buffer as directed above. *Note: **Avoid** touching the inside surface of the wells with the pipette tips.*

   Do not let the wells dry out at any time or enzymatic activity will be lost.

IV. Storage of Kit Components

This kit may be stored for up to 6 months at 2°C to 8°C from the date of shipment. Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

V. Sample Preparation and Dilution

- **Serum:** Allow the serum to clot in a serum separator tube (4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C-80°C.Avoid multiple freeze-thaw cycles. Do not use on lipemic specimens.
- If diluting samples, use physiological saline solution, pH7.4. Optimal dilutions need to be determined by end-user.

VI. Reagent Preparation

1. Preparation of Standards

Standards are ready to use, aliquot as directed.

2. Preparation Wash Buffer and Enzyme Conjugate

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

20X 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 (18-26°C).

Sensitivity	0.1 ng/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	0.1-18 ng/ml
Cross Reactivity	There is no detectable cross-reactivity.

VII. Assay Procedure

We recommend that standard solution dilutions and each sample are plated in duplicate. Read entire protocol before beginning

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

2. Dispense 25 μl of standards, specimens and controls into appropriate wells.

3. Dispense 100 μl of working dilution of Testosterone-HRP Conjugate Reagent into each well.

4. Dispense 50 μl of rabbit anti-Testosterone reagent to each well. Thoroughly mix for 30 seconds. It is very important to mix completely.

5. Seal plate and incubate at room temperature 60 minutes, shaking optional.

6. Rinse 3X as directed above under Technical Tips with 1x wash buffer water.

7. Dispense 100 μl of TMB Reagent into each well. Gently mix for 5 seconds.

8. Incubate at room temperature (20-25°C) for 15 minutes.

9. Stop the reaction by adding 50μl of Stop Solution to each well.

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

11. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

CAUTION: Bubbles in the wells will cause inaccurate readings. Ensure that all bubbles are removed prior to taking the absorbance reading.

VIII. Calculation of Results

(a) Manual Plate Reading:

(The relative O.D.$_{450}$) = (the O.D.$_{450}$ of each well) – (the O.D.$_{450}$ of Zero well).

The standard curve can be plotted as the relative O.D.$_{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). Known concentrations of the target protein are plotted on the X-axis and the corresponding O.D.$_{450}$ on the Y-axis. The standard curve should result in a graph that shows an indirect relationship between target protein concentrations and the corresponding O.D.$_{450.}$ The greater the concentration of target protein in the sample, the lower the O.D.$_{450.}$

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Determine concentration of target protein in unknown sample:

The target protein concentration of the samples can be interpolated from the standard curve. Draw a horizontal line to intersect with the standard curve. A vertical line dropped from this point intersects the X-axis at the concentration of antibody in the unknown sample.

(b) PC Interface Plate Reading

Enter the data into computer program curve fitting software. Existing spreadsheet software can perform a good fit with a linear regression analysis.

IX: Typical Standard Curve (for reference only, not to be used for actual data)