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User's Manual

Human Prolactin ELISA Kit

REF **DEIA9562**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Prolactin ELISA kit is used for the quantitative measurement of prolactin in human serum.

General Description

Human prolactin (lactogenic hormone) is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. Prolactin is secreted from the anterior pituitary gland in both men and woman. Women normally have slightly higher basal prolactin levels than men. During and following pregnancy, prolactin, in association with other hormones, stimulates breast development and milk production. Hypersecretion of prolactin can be caused by pituitary tumors, hypothalamic diseases, hypothyroidm, renal failure, acute exercise and several medications. Hyperprolactinemia inhibits hypogonadism in men and women with accompanying low FSH and LH levels.

Principles of Testing

The Prolactin ELISA kit is a solid phase sandwich ELISA assay method, based on a streptavidin-biotin principle. The standards, samples and a reagent mixture of Anti-Prolactin Enzyme and Biotin conjugates are added into the wells, coated with Streptavidin. Prolactin in the patient's serum forms a sandwich between two highly specific Prolactin antibodies, labeled with Biotin and HRP. Simultaneously, the biotinylated antibody is immobilized onto the well through a high affinity Streptavidin-Biotin interaction. Unbound protein and excess biotin/enzyme conjugated reagent are washed off by wash buffer. Upon the addition of the substrate, the intensity of color developed is directly proportional to the concentration of Prolactin in the samples. A standard curve is prepared relating color intensity to the concentration of the Prolactin.

Reagents And Materials Provided

1. Microwells coated with Streptavidin 12 × 8 × 1
2. Prolactin Standards: 6 vials (ready to use) 0.5 mL
3. Enzyme Conjugate: 1 bottle (ready to use) 12 mL
4. TMB Substrate: 1 bottle (ready to use) 12 mL
5. Stop Solution: 1 bottle (ready to use) 12 mL
6. 20× Wash concentrate: 1 bottle 25 mL

Materials Required But Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm

5. Absorbance paper or paper towel
6. Graph paper

Storage

1. Store the kit at 2 - 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

Specimen Collection And Preparation

1. Collect blood specimens and separate the serum immediately.
2. 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

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

Assay Procedure

Prior to assay, allow reagents to stand at room temperature.

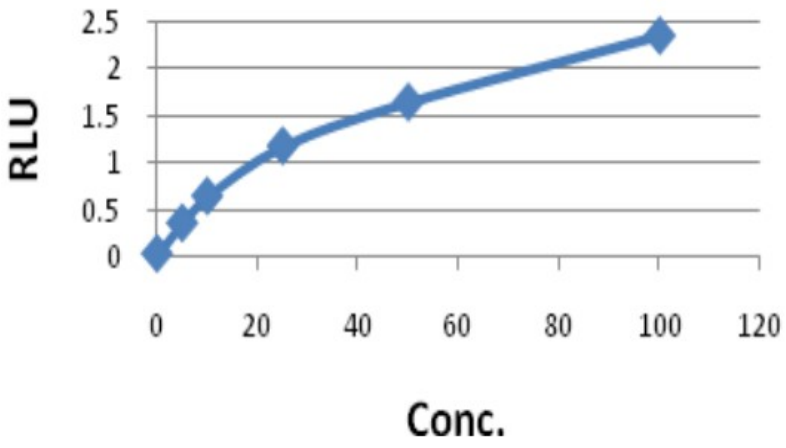
Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 25 µl of Prolactin standards, control and patient's sera.
3. Add 100 µl of enzyme conjugate to all wells.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25°C).
5. Remove liquid from all wells. Remove liquid from all wells. Wash wells three times with 300 µl of 1× wash buffer. Blot on absorbance paper or paper towel.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Typical Standard Curve

The standard curve is constructed as follows:
1. Check Prolactin standard values 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 standards (vertical axis) versus the 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.

	OD 450nm	Conc. ng/mL
Std 1	0.037	0
Std 2	0.363	5
Std 3	0.648	10
Std 4	1.181	25
Std 5	1.647	50
Std 6	2.353	100



Specificity

NA

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.

Limitations

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