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| <p>CALDON BIOTECH INC. Prolactin ELISA</p> |
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Catalog No. PR063F
(96 tests)

INTENDED USE

The CALDON BIOTECH INC. (CBI) Prolactin ELISA kit is used for the quantitative measurement of prolactin in human serum or plasma.

SUMMARY AND EXPLANATION OF THE TEST

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, hypothyroidism, renal failure, acute exercise and several medications. Hyperprolactinemia inhibits hypogonadism in men and women with accompanying low FSH and LH levels.

PRINCIPLE OF THE TEST

The prolactin ELISA is based on a solid phase direct sandwich ELISA method. The samples and diluted anti-prolactin HRP conjugate are added to the wells coated with Mab to prolactin. Prolactin in the patient's serum binds to anti-prolactin MAb on the well and the anti-prolactin HRP then binds to prolactin. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of prolactin in the samples. A standard curve is prepared relating color intensity to the concentration of the prolactin.

MATERIALS PROVIDED

1. Wells coated with prolactin MAb (12x8x1 wells). 96 wells.
2. Standard: 6 vials (0.7 mL each). Ready to use.
3. TMB Substrate: 1 bottle (12 mL). Ready to use.
4. Stop Solution: 1 bottle (8 mL). Ready to use.
5. Conjugate Diluent: 1 bottle (12 mL). Ready to use.
6. Enzyme Conjugate: 1 bottle (12mL). Ready to use.
7. 10X Wash Concentrate: 1 bottle (50 mL).

STORAGE AND STABILITY

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.

WARNINGS AND PRECAUTIONS

1. 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.
2. This test kit is designed for in vitro diagnostic use only.
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.

SPECIMEN COLLECTION HANDLING

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 (-2°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.

REAGENTS PREPARATION

10X Wash Buffer Concentrate: To prepare working wash buffer, add the contents of the bottle to 450 ml of distilled water. Store at room temperature.

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. Pipet 50 μ L of prolactin standards, control and patient's sera.
3. Add 100 μ L of enzyme conjugate to all wells.
4. Cover the plate and incubate for 30 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Fill wells with working wash buffer. Wash three times. Blot on absorbent paper towels.
6. Add 100 μ L of TMB substrate to all wells.
7. Incubate for 10 minutes at room temperature.
8. Add 50 μ L of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 20 minutes after adding the stopping solution.

CALCULATION OF RESULTS

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.

EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for prolactin may be used as initial guideline ranges only:

| Classification | Normal Range (mIU/mL) |
|-------------------------------------|-----------------------|
| Males: | 2-17 ng/mL |
| Females: | 3-25 ng/mL |
| Pregnancy 3 rd trimester | 95-480 ng/mL |

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS**1. Correlation with a Reference ELISA kit:**

A total of 110 sera were tested by this ELISA and a reference ELISA kit. Results were as follows:

| Correlation | Slope | Intercept |
|-------------|-------|-----------|
| 0.86 | 1.96 | 4.81 |

2. Precision**Intra-Assay**

| Serum | No. of Replicates | Mean IU/mL | Standard Deviation | Coefficient of Variation% |
|--------|-------------------|------------|--------------------|---------------------------|
| High | 16 | 33.2 | 2.27 | 6.8 |
| Normal | 16 | 15.7 | 0.75 | 4.8 |
| Low | 16 | 4.2 | 0.24 | 5.8 |

Inter-assay

| Serum | No. of Replicates | Mean IU/mL | Standard Deviation | Coefficient of Variation% |
|--------|-------------------|------------|--------------------|---------------------------|
| High | 10 | 30.5 | 2.7 | 6.9 |
| Normal | 10 | 14.5 | 0.98 | 6.7 |
| Low | 10 | 4.3 | 0.3 | 6.9 |

3. Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

| Serum | No. of Replicates | Mean IU/mL | Standard Deviation | Mean + 2SD (Sensitivity) |
|---------------|-------------------|------------|--------------------|--------------------------|
| Zero Standard | 20 | 1.26 | 0.208 | 0.334 ng/mL |

4. Recovery

Known quantities of prolactin were added to a serum that contained a low concentration of prolactin.

| Expected Value(mIU/mL) | Recovered (mIU/mL) | Percentage of Recovery |
|------------------------|--------------------|------------------------|
| 5 | 4.8 | 96 |
| 15 | 15.5 | 103.3 |
| 30 | 32 | 106.7 |

5. Linearity

Two different patient samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. Prolactin values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

| Serum | Original Value (mIU/mL) | Percentage of Recovery | | |
|-------|-------------------------|------------------------|-----|-----|
| | | 1:2 | 1:4 | 1:8 |
| 1 | 60 | 102 | 98 | 92 |
| 2 | 50 | 105 | 97 | 93 |

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