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Product Information

Prolactin ELISA kit

Catalog Number: EA100846
Storage Temperature: 2 – 8°C

Instruction for Use

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

Background
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.

Principle of the test
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.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
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. 20X Wash concentrate: 1 bottle	25 ml

Materials and Equipment Required but Not Provided
1. Distilled or deionized water

2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

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

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 (-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 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).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

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 (18-26°C).
5. Remove liquid from all wells. Remove liquid from all wells. Wash wells three times with 300 µl of 1X 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.

Calculation of Results

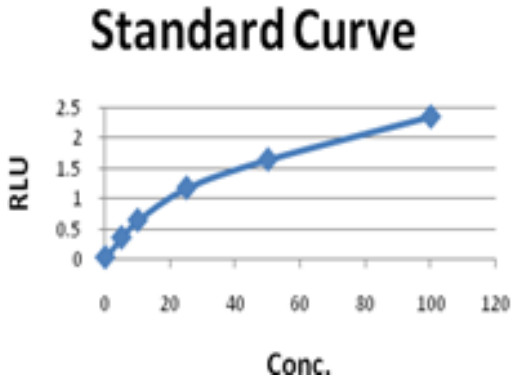
The standard curve is constructed as follows:

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.

Example of a Standard Curve

	OD 450 nm	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



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 (ng/ml)
Males	2-17
Females	3-25
Pregnancy 3rd trimester	95-480

References

1. Vanderpump MP; French JM; Appleton D; Tunbridge WM; Kendall-Taylor P. The prevalence of hyperprolactinaemia and association with markers of autoimmune thyroid disease in survivors of the Whickham Survey cohort. Clin Endocrinol (Oxf) 1998; 48(1):39-44.
2. Straub RH; Zeuner M; Lock G; Sch¨olmerich J; Lang B. High prolactin and low dehydroepiandrosterone sulphate serum levels in patients with severe systemic sclerosis. Br J Rheumatol 1997; 36(4):426-32.
3. Neidhart M. Elevated serum prolactin or elevated prolactin/cortisol ratio are associated with autoimmune processes in systemic lupus erythematosus and other connective tissue diseases. J Rheumatol 1996; 23(3):476- 81.
4. Neidhart M Serum levels of interleukin-1 beta, luteinizing hormone, and prolactin correlate with the expression of CD45 isoforms on CD4+ peripheral blood T lymphocytes in healthy women. Ann Hematol 1997; 75(4):155-9.
5. Maes M; Mommen K; Hendrickx D; Peeters D; D'Hondt P; Ranjan R; De Meyer F; Scharp´e S. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol (Oxf) 1997; 46(5):587-98.

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