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Product Information

Prolactin ELISA

Catalog Number **SE120105**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Human prolactin (lactogenic hormone) is a single chain polypeptide hormone with a molecular mass of ~23 kDa. 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.

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

Components

Materials Provided	96 Tests
Microwells coated with Prolactin Mab	12 x 8 x 1
Prolactin Standards: 6 vials (ready to use)	0.5 mL
Enzyme Conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample 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.

20x Wash Buffer Concentrate

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).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: 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.

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.

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 sera into appropriate wells.
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. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbent 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.

Results

Calculations

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 the value is checked on every kit.
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.

   Example of a Standard Curve

	OD (450 nm)	**Concentration (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

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 (ng/mL)
Males	2–17
Females	3–25
Pregnancy 3rd trimester	95–480

Product Profile

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

Precision
Intra-Assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	16	33.2	2.27	6.8
2	16	15.7	0.75	4.8
3	16	4.2	0.24	5.8

Inter-assay

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Coefficient of Variation (%)
1	10	30.5	2.7	6.9
2	10	14.5	0.98	6.7
3	10	4.3	0.3	6.9

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

Serum	No. of Replicates	Mean ng/mL	Standard Deviation	Mean + 2 SD (Sensitivity)
Zero Standard	20	0.126	0.208	0.334 ng/mL

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

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

Linearity
Two different 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 (ng/ml)	Percentage of Recovery		
		1:2	1:4	1:8
1	60	102	98	92
2	50	105	97	93

References

1. Vanderpump, M.P. et al., 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.
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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. et al., 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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