Human Prolactin ELISA Kit

Enzyme Immunoassay for the quantitative determination of human Prolactin in serum

Catalog number: ARG80852

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INTRODUCTION

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23.000 daltons. The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor.

Women normally have slightly higher basal prolactin levels than men; apparently, there is an estrogen- related rise at puberty and a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation. Prolactin also suppresses gonadal function.

During pregnancy, prolactin levels increase progressively to between 10 and 20 times normal values, declining to non-pregnant levels by 3-4 weeks post-partum. Breast feeding mothers maintain high levels of prolactin, and it may take several months for serum concentrations to return to non-pregnant levels.

The determination of prolactin concentration is helpful in diagnosing hypothalamic- pituitary disorders. Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence. High prolactin levels are commonly associated with galactorrhea and amenorrhea.

Prolactin concentrations have been shown to be increased by estrogens, thyrotropin- releasing hormone (TRH), and several drugs affecting dopaminergic mechanisms. Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia.

Additionally, the release of prolactin is episodic and demonstrates diurnal variation. Mildly elevated prolactin concentrations should be evaluated taking these considerations into account. Prolactin concentrations may also be increased by drugs such as chloropromazine and reserpine, and may be lowered by bromocyptine and L-dopa.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Prolactin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Prolactin present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for Prolactin is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Prolactin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Prolactin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP- Prolactin antibody Conjugate	11 ml (ready to use)	4°C
Standards 0-5 (0, 5, 20, 50, 100, 200 ng/ml)	6 vials	4°C, lyophilized
TMB substrate	14 ml (ready to use)	4°C (Protect from light)
STOP solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP- Prolactin antibody conjugate before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents and specimens must be allowed to come to room temperature (21-26°C) before use. All reagents must be mixed without

foaming before use.

- Once the test has been started, all steps should be completed without interruption.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Pipetting of all standards, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Samples should be frozen only once at -20 °C prior to assay. Avoid repeated freeze-thaw cycles. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION

- **Standards:** Reconstitute the lyophilized contents of the standard with 1 ml distilled water. The reconstituted standards are stable for 2 months at 2°C - 8°C. For longer storage freeze at -20°C.

  Note: The standards are calibrated against WHO 3rd International Standard for Prolactin IRP (84/500.)
- **Sample:** If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Standard 0 and reassayed as described in Assay Procedure.

  For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) dilution 1:10: 10 µL Serum + 90 µL Standard 0 (mix thoroughly)

  b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Standard 0 (mix thoroughly).

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 25 µl Standard, controls and samples in duplicate into the appropriate wells.
3. Add 100 µl HRP- Prolactin antibody into each well. Thoroughly mix for 10

seconds. It is important to have a complete mixing in this step. Incubate for 30 minutes at RT.

4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with distilled water (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl of TMB substrate to each well. Incubate for 10 minutes at room temperature in dark.
6. Add 50 µl of Stop Solution to each well.
7. Read the OD with a microplate reader at 450 nm immediately. It is recommended that read the absorbance within 10 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-logarithmic or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated

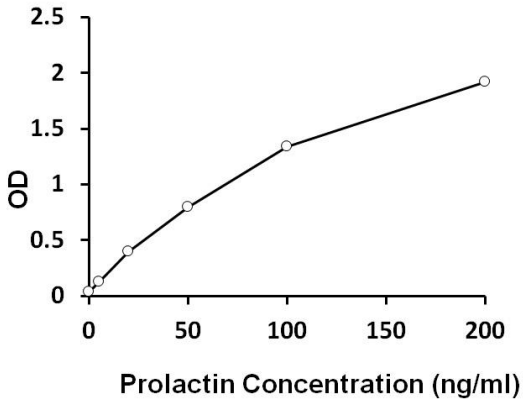
automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 200 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6. Conversion: 1 ng/mL = 21.1 mIU/L.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



The O.D. of standard 5 (200ng/ml) should ≥ 1.200.

QUALITY ASSURANCE

Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of Standard 0 and was found to be 0.35 ng/ml.

Specificity

The following substances were tested for cross reactivity of the assay:

Hormone Tested	Concentration	Produced Color Intensity Equivalent to LH in Serum (mIU/mL)
hCG (WHO 1st IRP 75/537)	62,500 mIU/ml	0
	125,000 mIU/ml	0
	250,000 mIU/ml	0
	500,000 mIU/ml	0
TSH (WHO 2nd IRP 80/558)	250 µIU/ml	0
	500 µIU/ml	0
FSH (WHO 2st IRP-HMG)	250 mIU/ml	0
	500 mIU/ml	0
LH (WHO 1st IRP 68/40)	500 mIU/ml	0
	1000 mIU/ml	0
hGH (WHO 1st IRP 66/217)	1000 µg/ml	2.5

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 4.45% and the CV value of inter-assay precision was 5.92%.

Interferences

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 0.9 mg/mL) have no influence on the assay results.

Recovery

86.3-110.2%

Linearity

87.4-111.4