

PROLACTIN (PRL) ELISA Kit

48 Tests Kit

Enzyme Immunoassay for the
Quantitative Determination of
Prolactin Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-Pro-48

PISHTAZ TEB DIAGNOSTICS

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 level 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 level increases progressively to between 10 and 20 times normal value, declining to non-pregnant level 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 level. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. 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 mechanism. 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 chlorpromazine and reserpine, and may be lowered by bromocryptine and L-dopa.

Test Principle

The Prolactin Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react with the solid phase antibodies, and after incubation and washing, the enzyme conjugate will be added, resulting in the sandwich formation of prolactin between solid phase and conjugated antibodies. After second wash step a solution of TMB is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

Materials provided with the kit

1. Antibody coated wells (1 plate, 48 wells): Microtiter wells coated with monoclonal anti Human Prolactin.
2. Enzyme conjugate (1vial, 3 ml): Monoclonal anti human Prolactin labeled with HRP in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
3. Standard set (0.5 ml / vial): Contains 0.0 (1 ml), 50, 100, 500, 1500, and 3000 mIU/L of Prolactin calibrated against 3rd International Standard code 84/500 in buffer containing protein as stabilizer and Thiomerosal as preservative, Ready to use.

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4. Control serum (1 vial, 0.5 ml): Contains certain amount of human Prolactin in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
5. Chromogen substrate reagent (1 vial, 6 ml): Contains Tetra Methyl Benzidine (TMB) and hydrogen peroxide, ready to use.
6. Wash solution (1 vial, 25 ml concentrated 20x): Contains phosphate buffer solution with 0.05 % Tween 20.
7. Stop solution (1 vial, 6 ml): Contains Hydrochloric acid (1M).
8. Cardboard sealer.

Materials required but not provided

1. Precision pipettes: 50µl, 100µl
2. Distilled water.
3. Absorbent paper or paper towel.
4. Graph paper.
5. Microtiter well reader.

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, HCV and HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 24 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

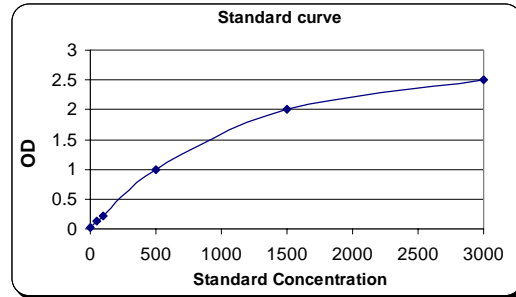
1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Assay Procedure

1. Secure the desired number of microplate wells in the holder and keep the remaining with desiccant in tightly closed original bag.
2. Dispense 50 µl of each standard, control serum and specimen into appropriate wells.
3. Cover the microtiter wells with cardboard sealer. Leave them for 30 minutes at room temperature (22-28°C).
4. Remove contents of the wells by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution). Strike the wells sharply onto absorbent paper or paper towel to remove all residual wash solution droplets.
5. Add 50 µl of Anti-prolactin-HRP conjugate to the wells.
6. Cover the microtiter wells with cardboard sealer. Leave them for 30 minutes at room temperature (22-28°C).



7. Repeat step 4.
8. Dispense 100 µl of chromogenic substrate solution to the microplate wells.
9. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
10. Add 100 µl of stop solution to the wells to stop reaction.
11. Read the absorbance of the wells at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available).



Results Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mIU/L on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Use the mean absorbance value for each sample, determine the corresponding concentration of prolactin in mIU/L from the standard curve.

Note: All absorbances shown in above curve is for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curves.

Expected Values

Each laboratory must establish its own normal ranges based on patient population. Based on a limited number of healthy adult blood specimens, the mean prolactin concentrations are shown in table below:

	Mean (mIU/L)	Ref. Interval (mIU/L)
Male	170	31-433
Females Post-menopause	195	33-413
Females Pre-menopause	251	118-555

Example of Standard curve

Standards (mIU/L)	OD
0	0.02
50	0.12
100	0.22
500	1.0
1500	2.0
3000	2.5

Ref.: Reference
 $ng/ml = 0.0472 mIU/L$
 $mIU/L = 21.2 ng/ml$

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of zero standard + 3SD. The minimal detectable concentration of human prolactin by this assay is estimated to be 15 mIU/L.

2. Precision

To determine intra-assay and inter-assay of this assay kit, replicated ELISA tests were done on three different serum samples with different serum hPRL concentrations. Results are shown in table 1 and 2:

Table 1: Intra-assay

Specimen No.	No. of tests performed	Means mIU/L	SD mIU/L	CV %
1	24	35	2.0	5.7
2	24	293	11.2	3.8
3	24	1250	42.3	3.4

Table 2: Inter-assay

Specimen No.	No. of tests performed	Means mIU/L	SD mIU/L	CV %
1	10	36.6	2.4	6.46
2	10	285	14.6	5.1
3	10	1297	53.1	4.1

Each test has been run in duplicate

3. Recovery

The certain amount of hPRL was added into 4 different sera with known concentrations of hPRL and their recovery were determined. The results are shown in table 3:

Table 3: Test recovery

No.	hPRL level mIU/L	hPRL added mIU/L	Exp. Value mIU/L	Obs. Value mIU/L	Rec. (%)
1	140	50	95	90	95
1	140	100	120	117.5	97
1	140	500	320	327	102
2	370	50	435	425	98
2	370	100	685	660	96
2	370	500	935	970	104
3	950	50	525	560	106
3	950	100	725	750	103
3	950	500	975	950	97
4	1830	50	965	990	102
4	1830	100	1165	1250	107
4	1830	500	1415	1370	97

Exp.: Expected, Obs.: Observed,
Rec.: Recovery

4. Linearity

To verify test linearity, four different serum samples with known concentration of hPRL were diluted with 0.0 standard and tested by this ELISA kit. The results are shown below:

Table 4: Linearity test results

No.	hPRL undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	670	94	102	100	98
2	1400	96	103	96	103
3	2300	100	98	97	98
4	2800	98	98	98	96

5. Specificity

To check cross reaction between hPRL and other hormones, sera with different concentrations of hLH, hFSH, hTSH and hCG. were tested by this kit. Results are shown in table 5:

Table 5: Specificity test result, Cross reaction

Analyte name	Concentration	Presumptive concentration hPRL (mIU/L)
hFSH (IU/L)	500	<15
	250	<15
	100	<15
	50	<15
hTSH (mIU/L)	500	<15
	250	<15
	100	<15
hCG (IU/L)	200000	<15
	100000	<15
	10000	<15
hLH (IU/L)	1000	<15
	500	<15
	250	<15
	100	<15
	50	<15

6. Hook effect




The hPRL assay was done on sera with high concentrations of hPRL (up to 50,000 mIU/L) and no “hook effect” was seen.

References

- Bergh T., Nilius S.H., Wide L. (1977) Hyperprolactinemia in amenorrhea incidence and clinical significance. *Acta. Endocrinol* 86:683-694.
- Seppala, M. (1978) Prolactin and female reproduction. *Ann. Clin. Res.* 10:164-170.
- Thorner, M.O., McNeilly, A.S., Hagan C. (1974) Long-term treatment of galactorrhea and hypogonadism with bromocriptine. *Br. Med. J.* 2:4.

Prolactin test Procedure

Step 1

Reagents	Standard	Control Serum	Sample
			
Standard	50 µl	None	None
Control serum	None	50 µl	None
Sample	None	None	50 µl




Cover microplate wells with cardboard sealer and incubate them for 30 minutes at room temperature.



Step 2

Remove contents of the wells. Rinse and flick the microtiter wells 5 times with working wash buffer.

Step 3

Anti PRL-HRP conjugate	50 µl	50 µl	50 µl
			

Cover the microplate wells again with cardboard sealer and incubate them for 30 minutes at room temperature.






Step 4

Remove contents of the wells. Rinse and flick the microtiter wells 5 times with working wash buffer.






Step 5

Chromogen-substrate solution	100 µl	100 µl	100 µl
			

Incubate the microplate wells for 15 minutes at room temperature and dark.



Step 6

Stop Solution	100 µl	100 µl	100 µl
			

Read the absorbance of the wells at 450 nm. (Use a 630 nm filter as reference filter if it's available)