پادتن علم
PADTAN ELM

THE PADTAN ELM EIA KIT FOR 96 TESTS

PROLACTIN EIA Kit

Intended use:
Quantitative determination of PROLACTIN levels in human serum and plasma.

For *in vitro* diagnostic use.

I.Introduction

Prolactin is a single chain polypeptide hormone secreted by the anterior pituitary gland and placenta secretion of this polypeptide, with molecular weight of about 22 kDa, is under the control of prolactin-inhibiting factor and prolactin releasing factors. These factors are secreted by the hypothalamus [1,2]. Prolactin, in synergy with estrogen, stimulates mammary gland development and also initiates and maintains lactation in females after delivery [3]. Prolactin, also plays a role in regulating gonadal function in both women and men [4-5].
The measurement of Prolactin in serum or plasma provides useful information about disorders of the hypothalamic-pituitary- gonadal axis and helps to differentiate between various disorders in which the prolactin concentration increases or decreases:

Increased prolactin concentration is observed in: Pituitary or hypothalamic tumor, stress, anxiety, surgery, primary hypothyroidism, galactorrhea and administration of certain drugs [6-11].

Decreased prolactin is observed in: adenectomy, hypophysectomy, pituitary hypofunction. e.g. sheehan's syndrome[12,13].

II.Principle of the test

The Prolactin quantitative test kit is based on solid phase enzyme immunoassay (EIA).This assay system uses two mouse monoclonal antibodies directed against distinct antigenic determinants on the Prolactin molecule. The polystyrene wells are coated with captured antibody against Prolactin. Standards, controls and patient samples are added to the wells (solide phase) and incubate. The Prolactin present in the wells is bound to the anti-Prolactin antibodies. The unbound material is removed by aspiration and washing. After washing, the HRP labeled anti-Prolactin Mab is added to the wells. After second incubation and washing, a solution of TMB (3,3', 5,5' tetra-methylbenzidine) is added to each well, resulting in the development of a blue color. The intensity of the color is proportional to the amount of Prolactin present in the sample. The color development is stopped by addition of Stop solution, causing the blue color to change to yellow. The color intensity is determined in a microtiter plate spectrophotometer at 450 nm. Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard. The Prolactin concentrations of patient samples are then read from the standard curve.

III.Kit contents

The reagents provided with the Prolactin Kits(Cat.No.P-PRI) are sufficient for 96 wells. The expiry date of each reagent is shown on the vial label.

Store the Kit at 2-8° C.

1.Coated microtiter wells: 96 wells, coated with mouse monoclonal anti-Prolactin antibodies.

2.Zero standard: 1 vial (4 ml). The zero standard should also be used as sample diluents.

3.Standards: 5 vials (1.0 ml) of Human Prolactin in serum matrix with thimerosal as a preservative. The standards supplied with this kit were calibrated against the international standard 3rd IRP 84/500 . The exact Prolactin concentration for each standard is specified on the label of each vial.

4.Serum controls: 2 vials (1.0 ml) of human Prolactin in processed human serum containing thimerosal. The nominal Prolactin concentrations for each control is specified on the label of each vial.

5.Enzyme tracer: 1 vial (6 ml) of monoclonal anti-Prolactin antibody conjugated to horseradish peroxidase (HRP) in phosphate buffered saline (PBS) with proteins and thimerosal.

6.Assay buffer: 1 vial (6 ml) of PBS with proteins and thimerosal.

7.Wash solution (concentrated 20X): 1 vial (25 ml) of PBS- Tween 20 and thimerosal.

8.TMB HRP-Substrate: 1 vial (12 ml) of buffered H_20_2 and 3,3',5,5' tetramethylbenzidine.

9.Stop solution: 1 vial (12 ml)of 2N H_2S0_4.

IV.Materials required (but not supplied with the kit)

1.Microtiter plate spectrophotometer reader with a wavelength of 450 nm(with reference wavelength at 630 nm) and an absorbance range of 0 to 3.0.

2.Precision micropipettes to deliver 50 and 100 µL.

3.Distilled or deionized water for preparation of diluted Wash Solution.

V. Specimen collection and preparation

The assay can be performed on serum or heparinized plasma samples. Keep samples at 2-8° C for 1 week; for longer periods it is recommended to store the sample in aliquot form at -20° C. Avoid repeated freezing and thawing of samples. Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand. Do not vortex patient samples. Serum sample with Prolactin greater than the last standard, should be diluted with 'zero' standard and reassayed to give a quantitative result The value obtained must be multiplied by the dilution factor to give the correct Prolactin concentration.

VI. Assay procedures

All reagents should be brought to room temperature prior to use.

Concentrated wash solution must be diluted with distilled or deionized water (Dilute one part concentrated wash solution with 19 parts of water).Diluted wash solution is stable for 7 days at 2 to 8°C in presence of undissolved crystals, resuspend the solution by placing the vial at 37°C for a few minutes.

1.Dispense 50 µl of Prolactin standards, control serums and patient samples into appropriate wells. Pipette 50 µL of Assay Buffer to each well and thoroughly mix the plate for 15 seconds.

2.Incubate the strips for 30 minutes at room temperature.

3.After the first incubation, aspirate and wash each well 4 times with 300 µl of diluted wash solution.

4.Add 50 µl of enzyme tracer (HRP anti-Prolactin) to each well. Then incubate for 30 minutes at room temperature.

5.After the second incubation, aspirate and wash each well 4 times with 300 µl of diluted wash solution.

6.Dispense 100 µl of TMB HRP-Substrate into each well.

7.Incubate at room temperature in the dark for 10 minutes.

8.Add 100 µl of Stop Solution and mix for 10 seconds. Read absorbance at 450 nm(with reference wavelength at 630 nm) in a microtiter plate reader within 15 minutes after addition of stop solution.

SUMMARY OF ASSAY PROCEDURE

Pipette 50 µl standard, control or sample

Pipette 50 µl Assay buffer

Incubate 30 min. at RT

Wash 4 x (300 µl)

Pipette 50 µl Anti-Prolactin HRP

Incubate 30 min. at RT

Wash 4 x (300 µl)

Pipette 100 µl TMB

Incubate 10 min. at RT

Pipette 100 µl Stop Solution

Read at 450/630 nm

VII. Calculation of results

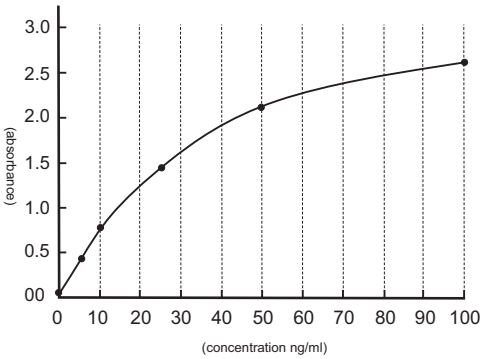
The results can be calculated by either microtiter plate, spectrophotometer reader or manual evaluation. If a microtiter plate spectrophotometer reader with data calculation program is used, refer to the plate reader and create a program using the concentration of each of the Prolactin standards in ng/ml.

For manual evaluation, a standard curve is constructed by plotting the absorbance values obtained for each Prolactin standard against the corresponding Prolactin concentrations. The unknown Prolactin concentration, in ng/ml, can then be read from the standard curve using the absorbance value of each patient specimen.

Example of calculation:

The values shown below are examples and must not be used in place of experimental data.

Specimen	Absorbance	Prolactin (ng/ml)
Standard A	0.03	0
Standard B	0.47	5
Standard C	0.78	10
Standard D	1.48	25
Standard E	2.13	50
Standard F	2.61	100
Sample	2.00	46



VIII. Normal Values

The upper limit for circulating prolactin in healthy individuals, suggested in the literature, is up to 30 ng/ml(14)

	n	mean (ng/ml)	range (ng/ml)
Normal women	207	9.6	2.8-29.2
Postmenopausal women	141	7.0	2.1-17.7
Normal men	104	6.9	1.8-20.3

The values reported above were determined using an automated chemiluminescence system[15]. These values remain only indicative. We suggest that each laboratory determine its own normal values for the interpretation of patients' results.

IX. Specific assay characteristics

1.Sensitivity: The sensitivity of this kit was calculated based upon the standard curve and expressed as the minimal concentration of Prolactin showing a significant difference from the 'zero' standard. This minimal detectable concentration of Prolactin is 0.5 ng/ml.

2.Specificity: The specificity of the Prolactin EIA test was determined by measuring the apparent prolactin response caused by high levels of LH (500mIU/mL), FSH (500mIU/mL), (TSH 100 mIU/mL), GH (500 ng/ml) and βHCG (100,000 mIU/mL). The results of these cross reaction tests are shown in the following table:

Hormone		Prolactin (ng/ml)	
Name	Concentration	Expected	Measured
LH	500 mIU/mL	22	23.8
FSH	500 mIU/mL	22	22.0
TSH	100 mIU/mL	22	22.8
GH	500 ng/ml	22	24.5
βHCG	100,000 mIU/mL	22	24.2
hPL	ND	ND	ND

3.Hook effect: Samples with very high antigen concentration are tested undiluted. With this kit, no hook effect has been noticed with samples containing Prolactin concentration up to 100,000 ng/ml.

4.Precision: Precision was evaluated for intra- and inter-assay variability.

Intra-assay reproducibility was determined by measurement of 10 replicates of 4 samples in a single run.

Inter-assay reproducibility was determined by replicate measurement of 4 samples in 10 separate runs.

Reproducibility

	Inter-assay			Intra-assay		
	Mean(ng/ml)	SD	%CV	Mean(ng/ml)	SD	%CV
Sample 1	12.0	0.2	1.7	11.2	0.2	1.8
Sample 2	29.0	0.2	6.4	29.3	1.0	3.4
Sample 3	67.8	0.8	1.2	63.6	1.7	2.7
Sample 4	121.4	3.0	2.5	129.0	6.7	5.2

5.Recovery and dilution: Recovery is defined as the increase in concentration seen when a known concentration of an analyte is added to a sample. Spiked samples were prepared by mixing the appropriate aliquot of a concentrated Prolactin solution with a serum sample containing a low Prolactin concentration. The percentage recovery is calculated as:

[(Measured conc. (ng/ml)/ Expected conc. (ng/ml)] X100

The percentage recovery ranged from 97.5 to 104.7.

Recovery Test

Added Conc. (ng/ml)	Expected Conc. (ng/ml)	Measured Conc. (ng/ml)	% Recovery
-	-	17.1	-
19.3	36.4	35.5	97.5
34.8	51.9	53.2	102.5
58.0	75.1	78.6	104.7
74.5	91.6	92.2	100.6

For dilution test, several serum samples containing elevated Prolactin concentrations were tested after serially diluting with the zero standard. The results of one of these samples are shown in the following table. The percentage recovery ranged from 96.2 to 103.3 .

Dilution test

Dilution	Expected Conc. (ng/ml)	Measured Conc. (ng/ml)	% Recovery
undiluted	-	95.7	-
1:2	47.9	49.4	103.1
1:4	23.9	23.0	96.2
1:8	12.0	11.9	99.2
1:16	6.0	6.2	103.3

6.Comparison with Prolaction electrochemiluminescence methods: Correlation studies on more than 500 random serum samples, were performed using the quantitative results from the ROCHE electrochemiluminescence (Elecsys). The correlation coefficient of test results was 0.95.

X. References

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WARNING!

This kit contains toxic materials, animal and human sourced components. Since no method can completely rule out the presence of blood-borne disease(e.g. HIV,HCV and HBV) therefore,all human sourced material must be considered potentially infectious.
In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary. Never pipette by month. Do not eat, smoke or apply cosmetics in the laboratory.

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