ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF PROLACTIN IN HUMAN SERUM

FOR IN VITRO DIAGNOSTIC USE

Store at 2 to 8°C.

INTENDED USE

The Prolactin ELISA is intended for the quantitative determination of prolactin in human serum. This assay is useful in the diagnosis and treatment of disorders of the anterior pituitary and hypothalamus.

INTRODUCTION

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women[1]. Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons.[2] The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor.[3]

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.[4,5]

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

The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders.[3,4] Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence.[6] High prolactin levels are commonly associated with galactorrhea and amenorrhea.[5]

Prolactin concentrations have been shown to be increased by estrogens, thyrotropin-releasing hormone (TRH), and several drugs affecting dopaminergic mechanisms.[7,8,9,10] 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.[5] 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.[4]

The Prolactin Enzyme Immunoassay provides a rapid, sensitive, and reliable assay for the measurement of prolactin. The antibodies developed for the test will determine a minimal concentration of human prolactin of 2 ng/ml. There is no cross-reactivity with hCG, TSH, LH, FSH, or hGH.

PRINCIPLE OF THE ASSAY

The Prolactin Quantitative Test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[11,12] The assay system utilized mouse monoclonal anti-prolactin for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-prolactin in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 45 minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of prolactin is directly proportional to the color intensity of the test sample.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (96 wells)*
   Microtiter wells coated with mouse monoclonal anti-prolactin.

2. *Enzyme Conjugate Reagent (13 mL)*
   Contains mouse monoclonal anti-prolactin conjugated to horseradish peroxidase.

3. *Reference Standard Set*
   Contains 0, 5, 15, 50, 100, and 200 ng/mL (WHO, 1st IRP, 75/504) human prolactin in bovine serum with preservatives. Lyophilized. See instructions for reconstitution under Reagent Preparation.

4. *TMB Reagent (1 bottle, 11 mL)*
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.

5. *Stop Solution - 1N HCl (11 mL)*
   Contains diluted hydrochloric acid.

Materials Required But Not Provided
1. Distilled or deionized water
2. Precision pipettes: 0.05, 0.1, 0.2, and 1 ml
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm
5. Vortex mixer or equivalent
6. Absorbent paper
7. Linear graph paper
8. QC material (e.g., BioRad LyphoCheck Controls)

WARNINGS AND PRECAUTIONS

1. CAUTION**:** This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[21]

2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.

3. Do not use the reagent when it becomes cloudy or contamination is suspected.

4. Do not use the reagent if the vial is damaged.

5. Replace caps on reagents immediately. Do not switch caps.

6. Each well can be used only once.

7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

STORAGE CONDITIONS
1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
3. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

INSTRUMENTATION
A microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD or greater at 450nm wavelength is acceptable for absorbance measurement.

SPECIMEN COLLECTION AND PREPARATION
1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

REAGENT PREPARATION
1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0 mL distilled H_20. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.

PROCEDURAL NOTES
1. Manual Pipetting: It is recommended that no more than 32 wells are used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes. A multi-channel pipette is recommended.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.

4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

ASSAY PROCEDURE
1. Secure the desired number of coated wells in the holder.
2. Dispense 50μL of standards, specimens, and controls into appropriate wells.
3. Dispense 100μL of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing.
5. Incubate at room temperature (18-25°C) for 45 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or dionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100μL of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, in the dark, for 20 minutes.
11. Stop the reaction by adding 100μl of Stop Solution to each well.
12. Gently mix for 30 seconds. **Ensure that all of the blue color changes completely to yellow.**
13. Read absorbance at 450nm with a microtiter plate reader **within 15 minutes**.

CALCULATION OF RESULTS
1. Calculate the average absorbance value (A_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on linear graph paper, with absorbance 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 of prolactin in ng/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

EXAMPLE OF STANDARD CURVE
Results of a typical standard run with absorbency readings at 450nm shown in the Y axis against prolactin concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns.

Prolactin (ng/mL)	Absorbance (450 nm)
0	0.052
5	0.166
15	0.383
50	1.047
100	1.737
200	2.644
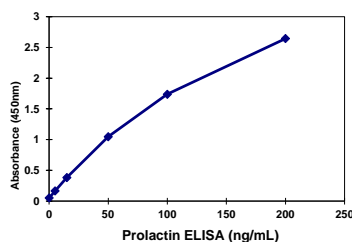
EXPECTED VALUES

Each laboratory must establish its own normal ranges based on patient population. The information provided below is cited form Reference #14.

Adult	ng/ml
Male	3.0-14.7
Female	3.8-23.2
Pregnancy, Third trimester:	95-473

PERFORMANCE CHARACTERISTICS

1. Accuracy

A statistical study using 123 patient samples with prolactin concentrations ranging from 1.2 to 266 ng/mL, demonstrated good correlation with a commercially available kit as shown below. Comparison between this kit and Serono's Serozyme kit provides the following data:

N = 123
Correlation coefficient = 0.99
Slope = 0.9344
Intercept = -2.16
Mean = 23.38 ng/mL
Serono Mean = 22.6 ng/mL

2. Sensitivity

The minimal detectable concentration of prolactin by this assay is estimated to be 2.0 ng/mL.

3. Precision

a. *Intra-Assay Precision*

Within-run precision was determined by replicate determinations of three different control sera in one assay.

Serum Sample	1	2	3
Number of Replicates	26	26	26
Mean Prolactin (ng/mL)	10.01	39.81	97.98
Standard Deviation	0.43	1.83	2.11
Coefficient of Variation (%)	4.3%	4.6%	2.1%

b. *Inter-Assay Precision*

Between-run precision was determined by replicate measurements of three different control sera over a series of individually calibrated assays.

Serum Sample	1	2	3
Number of Replicates	24	24	24
Mean Prolactin (ng/ml)	9.94	42.94	96.61
Standard Deviation	0.54	3.16	2.97
Coefficient of Variation (%)	5.4%	7.4%	3.1%

4. Recovery and Linearity Studies

a. *Recovery*

Patient serum samples of known prolactin levels were mixed and assayed in duplicate. Average recovery = 101.1%.

Expected Concentration (ng/mL)	Observed Concentration (ng/mL)	% Recovery
130.48	136.68	104.7
65.53	61.36	93.6
26.68	27.16	98.2
12.59	12.31	102.2
5.31	5.17	102.7
Mean Recovery #1 = 100.3 %		
101.12	102.40	101.0
46.87	45.95	98.0
18.70	19.48	104.1
8.74	8.61	98.5
3.48	3.41	97.9
Mean Recovery #2 = 99.9 %		

b. *Linearity*

Two patient samples were serially diluted with zero mIU/ml standards in a linearity study. The average recovery was 98.3%.

#	Dilution	Expected Conc. (ng/ml)	Observed Conc. (ng/ml)	% Recovery
1.	Undiluted	--	195.37	--
	1:2	97.68	105.12	107.6
	1:4	48.84	50.61	103.6
	1:8	24.42	23.77	97.3
	1:16	12.21	11.94	97.8
			Average =	*101.6%*
2.	Undiluted	--	166.97	--
	1:2	83.48	82.27	98.5
	1:4	41.74	39.21	93.9
	1:8	20.87	20.87	100.0
	1:16	10.43	9.49	91.0
	1:32	5.21	4.78	91.6
			Average =	*95.0%*

Prolactin Enzyme Immunoassay Test Kit

PERFORMANCE CHARACTERISTICS

5. Specificity

The following hormones were tested for cross-reactivity in the assay:

Hormone Tested	Concentration	Color Intensity Equivalent to Prolactin (ng/mL)
LH (WHO 1st IRP 68/40)	125 mIU/ml	0
	250 mIU/ml	0
	1,000 mIU/ml	0
TSH (WHO 2nd IRP 80/558)	62.5 µIU/ml	0
	125 µIU/ml	0
	250 µIU/ml	0
	500 µIU/ml	0
FSH (WHO 2nd IRP HMG)	125 ng/ml	0
	250 ng/ml	0
	500 ng/ml	0
HCG (WHO 1st IRP 75/537)	100 mIU/ml	0
	400 mIU/ml	0
	62,500 mIU/ml	0
	125,000 mIU/ml	0
	250,000 mIU/ml	0
	500,000 mIU/ml	0
HGH (WHO 1st IRP 66/217)	50 ng/ml	0
	1,000 ng/ml	0

6. Hook Effect

No hook effect is observed in this assay at prolactin concentrations up to 4,000 ng/mL.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. Controls containing sodium azide cannot be used. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

STANDARDIZATION

The Prolactin Reference Standards are calibrated against the World Health Organization's First International Reference Preparation (WHO 1st IRP 75/504).

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Pregnancy, estrogen treatment, renal disease, and hypothyroidism may affect prolactin levels.

REFERENCES

1. Shome, B. and Parlow, A.F., J. Clin. Endocrinol. Metab., 45, 1112-1115, (1977).
2. Niall, M.D. et al, "The Chemistry of Growth Hormone and the Lactogenic Hormones"; Recent Progr. Horm. Res. 29:471 (1974).
3. Friesen, H. and Hwang, P., Ann. Rev. Medicine, 24, 251-270, (1973).
4. Cowden, E.A., Ratcliffe, W.A., Beastall, G.H., and Ratcliffe, J.G., Annals Clin. Biochem., 16, 113-121, (1979).
5. Frantz, A.G., N. Engl. J. Med., 298, 201-207, (1978).
6. Thorner, M.O., Edwards, C.R.W., Hanker, J.P., Abraham, G., and Besser, G.M., "The Testes in Normal and Infertile Men"; Troen, P. and Nankin, H.R. (eds.), Raven Press, New York, 351-366, (1977).
7. Daughday, W.H., "The Adenohypophysis, Textbook of Endocrinology"; Williams, 6th Ed., Chapter 3, 87-87, (1981).
8. Tyson, J.E., Hwang, P., Guyela, H., Friesen, H.G., Am. J. Obstet. Gynecol. 113, 14-20 (1972).
9. Aubert, M.L., Grumbach, M.M. and Kaplan, S.L., Acta Endocrin. 77, 460-476 (1974).
10. Jacobs, L., Snyder, P., Wilber, J., Utiger, R., and Daughaday, W., J. Clin. Endocrin. 33:996, (1978).
11. Engvall, E., "Methods in Enzymology", Volume 70, VanVunakis H. and Langone, J.J. (eds.), Academic Press, New York, NY, 419-492, (1980).
12. Uotila, M., Ruouslahti, E. And Engvall, E., J. Immunol. Methods, 42, 11-15, (1981).
13. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
14. Clinical Guide to Laboratory Tests, 3rd Edition. Edited By Norbert W. Tietz. p. 410. W.B. Saunders Company, (1995).
15. USA Center for Disease Control/National Institute of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.

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