



Human Prolactin MICRO-ELISA Test Kit

Prod. No.: T111
Pkg. Size: 96 Tests

Description

The MICRO-ELISA PROLACTIN Diagnostic Kit is a solid phase sandwich-type enzyme immunoassay (ELISA) for the *in vitro* quantitative determination of prolactin concentration in human serum.

Summary and Explanation of the Test

Human prolactin is a product of the pituitary and has a molecular weight of approximately 23,000 daltons. Control of prolactin synthesis and secretion is provided by two hypothalamic products, thyrotropin-releasing hormone (TRH) and prolactin-inhibiting factor (PIF). TRH acts to stimulate and PIF acts to inhibit the production and release of prolactin by the pituitary.

Prolactin is present in the plasma of normal males and females and in several body fluids such as cerebrospinal fluid, semen, and amniotic fluid. The primary physiologic function of prolactin is to stimulate breast development and milk production in pregnant women. Other physiologic roles for prolactin are less well defined.

During the third trimester of pregnancy, prolactin levels increase markedly and remain elevated after delivery as long as nursing continues. Nursing stimulates the secretion of prolactin, which in turn provides for continuous milk production and for delayed resumption of menses. High levels of prolactin are associated with galactorrhea and amenorrhea in this normal physiologic function. In the absence of pregnancy, the triad of hyperprolactinemia, galactorrhea, and amenorrhea is suggestive of a pathological process. The most frequent cause of these symptoms is a prolactin secreting pituitary tumor.

Principle of the Procedure

The MICRO-ELISA PROLACTIN test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two unique antibodies (mouse monoclonal) directed against distinct antigenic determinants on the prolactin molecule.

Specifically, plastic wells are coated with anti-prolactin (mouse monoclonal). With the addition of a test sample or appropriate controls containing prolactin, immune complexes are formed between prolactin in the sample and the solid phase anti-prolactin. Anti-prolactin (mouse monoclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes at room temperature), the prolactin molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound labeled antibody. An enzyme substrate-

chromogen (hydrogen peroxide, H₂O₂, and tetramethylbenzidine, TMB) is added to the well and incubated for 15 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H₂SO₄ stops the reaction and converts the color to yellow. The intensity of the yellow color is directly proportional to the concentration of prolactin in the sample.

Reagents

Components in Each 96-Test

Micro-Elisa Prolactin Diagnostic Kit

1. 96 wells, Prolactin ANTIBODY COATED **WELLS**: Coated with anti-prolactin (mouse monoclonal); contained in a pack with silica gel desiccant.
2. 1 bottle, 22 ml, Prolactin ENZYME ANTIBODY **CONJUGATE**: anti-prolactin (mouse monoclonal) labeled with horse-radish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.
3. 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
4. 1 bottle, 12 ml, **STOP SOLUTION** 1 N H₂SO₄.
5. 1 vial, 2.0 ml, **0 ng/ml CALIBRATOR**: Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
6. 3 vials, 0.5ml, **CALIBRATORS**: prolactin in equine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. 25, 50 and 100 ng/ml.
7. 1 bottle, 50 ml, **WASH BUFFER CONCENTRATE (20X)**: Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

Additional Materials Required

Disposable tip precision pipets - 0.025, 0.1 and 0.2 ml.
microtiter plate reader.
Distilled or deionized water.

Storage and Stability

Store unopened kits at 2°-8°C. The following components may be stored at ambient temperature: WELLS, SUBSTRATE-CHROMOGEN, WASH BUFFER and STOP SOLUTION. Expiration date printed on the kit indicates limits of stability.



The prolactin ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells, wash buffer, Substrate/Chromogen and Stop can be stored at 2°-30°C.

Chemical or Physical Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

Instruments

Performance of the MICRO-ELISA PROLACTIN test requires use of a precision microtiter plate reader a wavelength of 450 ± 20 nm:

Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA PROLACTIN Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

STORAGE: Specimens may be stored in a tightly sealed tube at 2°-8°C for one day. If the serum is not assayed within 24 hours, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.

Micro-Elisa Prolactin Procedure

Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

Preliminary Comments and Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. **HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS.** Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.

Test Procedure

1. Place sufficient COATED WELLS in a holder to run 0.0 ng/ml, 25 ng/ml, 50 ng/ml and 100 ng/ml CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet 25 µl of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
3. Pipet or dispense 200 µl of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
4. Incubate at room temperature (18°-30°C) for 45 minutes ± 5 minutes.
5. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
6. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.



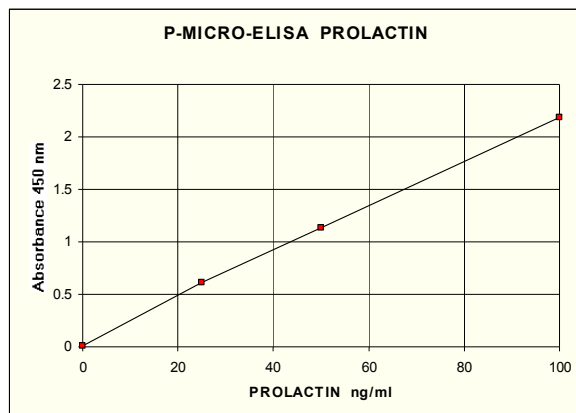
7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
9. Pipet or dispense 100 μ l (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
10. Mix thoroughly and incubate 15 minutes at room temperature (18°-30°C).
11. Pipet or dispense 100 μ l (0.1 ml) of 1 N H₂SO₄ into each well and mix thoroughly.
12. Read the absorbance of each well at 450 \pm 20 nm against water.

Calculation of Results

1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each CALIBRATOR on the vertical (Y) axis versus the corresponding prolactin concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding prolactin concentration in ng/ml from the curve. Multiply the value by the dilution factor if required.

EXAMPLE DATA
DO NOT USE IN PLACE OF CURVE
DETERMINED AT THE TIME OF ASSAY

Specimen I.D.	A ₄₅₀	Mean A ₄₅₀	Prolactin(ng/ml)
CALIBRATOR	0 ng/ml	0.012, 0.013	0.013
CALIBRATOR	25 ng/ml	0.600, 0.626	0.613
CALIBRATOR	50 ng/ml	1.154, 1.115	1.135
CALIBRATOR	100 ng/ml	2.250, 2.114	2.182
SAMPLES			
# 1 (UNKNOWN #1)	0.278, 0.258	0.268	10.7
# 2 (UNKNOWN #2)	0.547, 0.542	0.545	22.2
# 3 (UNKNOWN #3)	1.647, 1.690	1.669	75.5



The range of this assay is 0 – 100 ng/ml. For specimen with prolactin concentrations beyond the standard curve (100 ng/ml), repeat the test by diluting the specimen with the 0 ng/ml CALIBRATOR. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 ng/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

Standardization

The MICRO-ELISA PROLACTIN CALIBRATORS have been standardized against the 1st International Reference Preparation for Prolactin 75/504 provided by the World Health Organization (WHO).

Limitations of the Procedure

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the MICRO-ELISA PROLACTIN test as erroneous results may be obtained. These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

FINAL REACTION STABILITY: The spectrophotometric measurement should be made within 30 minutes after the addition of the H₂SO₄ solution.

Samples with elevated levels of prolactin (up to 7,000 ng/ml) will always assay as >100 ng/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of prolactin greater than the 100 ng/ml CALIBRATOR, the sample should be diluted with the 0 ng/ml CALIBRATOR and re-assayed.



Expected Values

Prolactin values were measured using the MICRO-ELISA PROLACTIN kit in 121 serum samples from apparently normal individuals (MALES AND NON-PREGNANT FEMALES) and the following results were obtained:

	PROLACTIN (ng/ml)		OBSERVED	#
	MEAN	Std. Div.	RANGE	SAMPLES
FEMALE	9.4	3.4	3.2 - 22.7	69
MALE	8.0	2.3	2.5 - 16.2	52

The levels of prolactin increase progressively during pregnancy with levels reaching a mean of >40 ng/ml during the first trimester, >100 ng/ml during the second trimester and >150 ng/ml during the last trimester. In the absence of nursing, levels will return to normal within 2-3 months following delivery.

These values are consistent with those reported in the literature. It is recommended that each laboratory determine its own normal range.

Performance Characteristics of the Test

Assay Specificity

Specificity of this test system was proven by determining a lack of interference of luteinizing hormone (LH), and follicle stimulating hormone (FSH) when added to a serum sample with a known prolactin concentration. The incremental change in prolactin concentration was measured and the relative cross reactivity calculated by dividing the change in prolactin concentration (ng/ml) by the cross reactant concentration (in mIU/ml) times 100. The observed cross reactivity for both FSH and LH was < 0.01%.

Assay Sensitivity

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <1.0 ng/ml.

Assay Reproducibility

Intra-assay reproducibility was determined by measurement of 20 replicates of three serum pools in a single run.

	Mean Prolactin (ng/ml)	SD	%CV
Serum A	7.11	0.40	4.5
Serum B	21.89	1.00	4.6
Serum C	56.12	4.23	7.5

The interassay reproducibility was determined by duplicate measurement of three serum pools in twelve separate runs.

	Mean Prolactin (ng/ml)	SD	% CV
Serum A	7.65	0.44	5.8
Serum B	23.82	1.27	5.3
Serum C	58.82	2.25	3.8

Assay Linearity

A study was performed diluting a serum sample containing an elevated level of prolactin with the 0 ng/ml CALIBRATOR to determine the linearity of the MICRO-ELISA PROLACTIN test.

Dilution Factor	Prolactin ng/ml		
	Expected Value	Observed Value	% of Expected Value
undiluted	-	87.8	-
1:1.33	65.9	66.3	101 %
1:2	43.9	45.1	103 %
1:4	22.0	23.8	108 %
1:8	11.0	12.1	110 %

Assay Recovery

Four aliquots of human serum with a prolactin concentration of 8.45 ng/ml were spiked with 5.0, 15.0, 25.0 and 50.0 ng/ml of prolactin. The samples were assayed in duplicate.

Added Prolactin	Prolactin ng/ml		
	Expected Value	Measured Value	% Recovery
5.0	13.45	13.70	102 %
15.0	23.45	23.43	100 %
25.0	33.45	33.48	100 %
50.0	58.45	57.71	99 %

Comparison to Other Prolactin Tests

Correlation studies on a random group of 92 serum samples with a range of values from <1 – 97 ng/ml, were performed using the quantitative results from the MICRO-ELISA PROLACTIN Test and another ELISA prolactin test. The correlation coefficient of the test results was 0.997

ELISA	n = 92	Correlation		
		Slope	Y-Intercept	Coefficient
		1.072	1.560	0.997

References

1. Daughaday, W. H. *et al.* (1985) *Williams Textbook of Endocrinology*, Philadelphia, W. B. Saunders Co. p.568
2. Davis, J. S: *et al.* (1979) *Rheumatology & Immunology*, New York, Grune & Stratton, Inc., p. 77
3. Highton, J. *et al.* (1984) *J. Immunol. Meth.* **68**:185
4. Masseyeff, R. *et al.* (1973) *J. Immuno. Meth.* **8**:223
5. Santen, R. J. *et al.* (1973) *J. Clin. Invest.* **52**:2616
6. Odell, W. D. *et al.* (1967) *J. Clin. Invest.* **46**:248
7. Primus, F. J. *et al.* (1988) *Clin. Chem.* **34** :261
8. Hansen H. J., *et al.* (1989) *Clin. Chem.* **35**:146
9. Schroff, R. J. *et al.* (1985) *Cancer Res.* **42** : 879