



**AccuDiag™
 Prolactin
 ELISA Kit**


REF 4226

PIC FT4226YR1

IVD  See External Label  8°C  96 Tests

Prolactin ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-200ng/mL
Sample	50 µL
Specificity	97%
Sensitivity	2.0 ng/mL
Incubation Time	65 minutes
Shelf Life	12-14 Months from the manufacturing date

PRODUCT FEATURES

-  Very easy to use with little training
-  Highly specific and consistent Assay
-  Provides accurate results quickly
-  Reading of results both visually and as absorbance data

INTENDED USE

The DAI 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.

SUMMARY AND EXPLANATION

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women¹. 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 chlorpromazine and reserpine, and may be lowered by bromocriptine and L-dopa.⁴

The Diagnostic Automation, Inc. 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.

ASSAY PRINCIPLE

The DAI 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.

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 -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.

MATERIALS AND COMPONENTS

Materials provided with the test kit

- Antibody-Coated Wells (96 wells)
Microtiter wells coated with mouse monoclonal anti-prolactin.
- Enzyme Conjugate Reagent (13 mL)
Contains mouse monoclonal anti-prolactin conjugated to horseradish peroxidase.
- 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.
- TMB Reagent (1 bottle, 11 mL)
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
- Stop Solution - 1N HCl (11 mL)
Contains diluted hydrochloric acid.

Materials required but not provided

- Distilled or deionized water
- Precision pipettes: 0.05, 0.1, 0.2, and 1 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450nm
- Vortex mixer or equivalent
- Absorbent paper
- Linear graph paper
- QC material (e.g., BioRad LyphoCheck Controls)

REAGENT PREPARATION

- All reagents should be allowed to reach room temperature (18-25°C) before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Reconstitute each lyophilized standard with 1.0 mL distilled H₂O. 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

- 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.
- 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.
- All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
- It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

ASSAY PROCEDURE

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

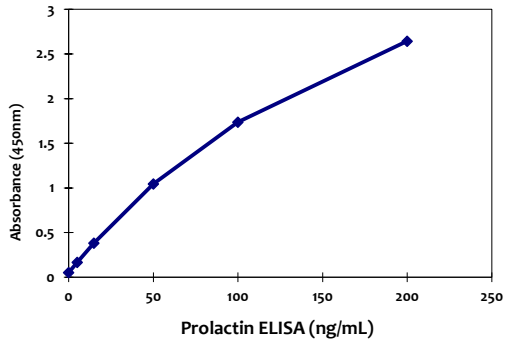
RESULTS

- Calculate the average absorbance value (A_{450}) for each set of reference standards, controls and samples.
- 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.
- 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.
- 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 from Reference #14.

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

PERFORMANCE CHARACTERISTICS

I. 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 the DAI and Serono's Serotype kit provides the following data:

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

II. Sensitivity

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

III. 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%

IV. 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 =			
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%

V. Specificity

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

Hormone Tested	Concentration	Color Intensity Equivalent to Prolactin (ng/mL)
LH (WHO 1 st IRP 68/40)	125 mIU/ml	0
	250 mIU/ml	0
	1,000 mIU/ml	0
TSH	62.5mIU/ml	0



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

VI. 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

- 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.
- 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.
- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
- Pregnancy, estrogen treatment, renal disease, and hypothyroidism may affect prolactin levels.

PRECAUTIONS

- 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

- Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
- Do not use the reagent when it becomes cloudy or contamination is suspected.
- Do not use the reagent if the vial is damaged.
- Replace caps on reagents immediately. Do not switch caps.
- Each well can be used only once.
- Do not pipette reagents by mouth.
- Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
- 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.
- For in vitro diagnostic use.

STORAGE

- 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.
- The opened and used reagents are stable until the expiration date if stored properly at 2-8°C.
- 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.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016



ISO 13485
Quality
Management for
Medical Devices
CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Woodland Hills, California 91367 USA

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Brand Name	AccuDiag™
REF 4226	AccuDiag™ - Prolactin ELISA
PIC	FT4226YR1
EU REP	AR Experts BV, Boeingavenue 209 1119 PD Schiphol-Rijk, The Netherlands info@ar-experts.eu

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