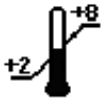
BIOGENIX
INC. PVT. LTD.

Prolactin ELISA

Enzyme Immunoassay for Quantitative Determination of Prolactin in Human Serum

Instructions for use

1. SYMBOL LEGEND

IVD	In vitro diagnostic medical device	CE	EC Declaration of conformity
REF	Catalogue number	LOT	Batch code
	Use by		Manufacturer
	Date of manufacture		Consult operating instructions
	Temperature limitation		Biological risks
MP	Coated microplate (96 wells)	CONJ	Conjugate
WASH P 20X	Wash solution, 20X concentrated	SUB	Substrate
CAL	Calibrators	STOP	Stop solution
CONTROL	Control	H2O	Deionized or distilled water
RCNS	Reconstitute with specified volume of liquid	OD	Optical density
TRIAL 5000X	Trial		Contains sufficient for <n> tests

2. INTENDED USE

Prolactin kit is provided for the **quantitative determination of prolactin in human serum**.

Prolactin is a single-chain protein hormone with a molecular mass of about 23 000 Da. It is synthesized in the frontal lobe of pituitary gland. The determination of prolactin is a valuable tool in diagnostics of testicular and ovarian dysfunctions. Hyperprolactinemia in women may lead to galactorrhea, amenorrhea and other distortions in menstrual cycle. In men it may result in impotence or loss of libido. Measurement of circulating prolactin is used as a primary test for barrenness.

Pathological hyperprolactinemia takes place in the case of hypothyroidism, renal insufficiency and in patients with a pituitary gland tumor - prolactinoma. Physiological increase of prolactin level takes place during the gestation, lactation, in sleep and after physical and emotional stress.

3. PRINCIPLE OF TEST

Prolactin kit is a “sandwich” type of solid-phase enzyme immunoassay, based on two monoclonal antibodies that are specific for different epitopes of prolactin molecule. One of these antibodies is conjugated with horseradish peroxidase (HRP); the other is coated onto the inner surface of microwells. Prolactin molecules from the serum sample bind to both immobilized antibody and anti-prolactin-peroxidase conjugate. Then the wells are washed with wash solution to remove any material not bound on the inner surface of the wells. Quantity of the bound conjugate is directly proportional to prolactin level in sample.

During the incubation with TMB solution the colour is developing. The intensity of the colour is directly proportional to prolactin concentration in specimens. Prolactin concentration in the patient sample is read from a standard curve that is processed in each assay.

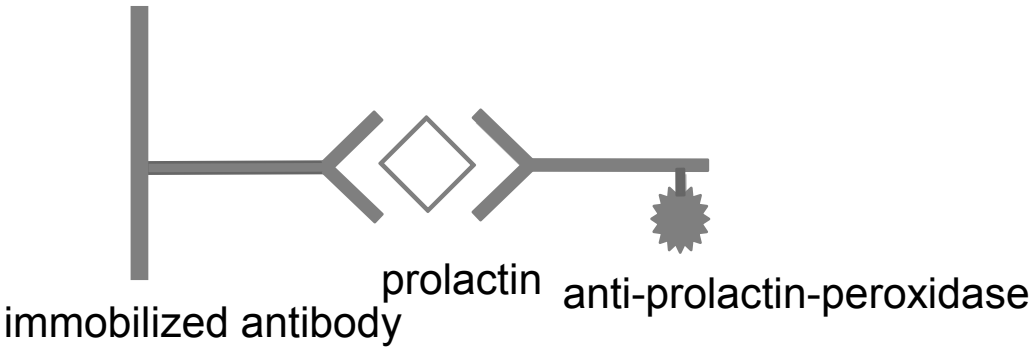


Fig. 1. Assay scheme

4. STORAGE AND STABILITY OF THE KIT

The expiration date of the kit is printed on the box label; the expiration date for each component is printed on the respective label.

Prolactin kit should be stored at +2…+8 °C upon receipt, preferably in the original kit box, until the expiration date. Storage at +25 °C is allowed but for no more than 5 days.

Shelf life of the kit is 18 months.

After initial opening the kit is stable the expiration date if stored at +2…+8 °C.

If used in several separate experiments, after initial opening kit contents should be stored as follows:

- unused strips: in a firmly closed resealable zipper bag at +2…+8 °C until the expiration date;
- vials with conjugate, calibrators and control (ready-to-use): until the expiration date; vials with calibrators and control (reconstituted): at +2…+8 °C for no more than 1 month after opening;

- vial with substrate: at +2…+8 °C until the expiration date, protected from light;
- vials with concentrated Trial, concentrated wash solution and stop solution: at +2…+8 °C until the expiration date;
- wash solution and Trial solution prepared for use: at room temperature (+18…+25 °C) for no more than 5 days or at +2…+8 °C for no more than 4 weeks, in a firmly closed bottle.

Damaged Test Kits

In case of any severe damage of the test kit or components, it has to be informed in writing, during one week after receiving the kit. Usage of severely damaged single components for a test run is not recommended.

5. SAMPLE COLLECTION AND STORAGE

Collect blood by venipuncture. After clotting, the serum is separated by centrifugation.

Do not use plasma, haemolyzed (bright red) or lipemic (milky) serum samples as well as samples containing sodium azide as preservative.

Store serum samples at +2…+8 °C for no more than 5 days. Aliquot and freeze samples for longer storage (–20 °C and lower). Avoid repeated freezing.

6. EXPECTED VALUES

Serum samples of 120 apparently healthy people were assayed with **Prolactin** kit. The results are shown in the table. These limits should be considered as guidelines only.

Group	n	Prolactin concentration range (mIU/L)
Female	120	67-726
Male		105-540

It is highly recommended for each laboratory to determine its own reference range of prolactin concentrations.

7. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

8. REAGENT PREPARATION

Allow all the reagents to reach room temperature, and then thoroughly stir.

MP Keep **microplate** at room temperature (+18…+25 °C) for at least 30 minutes before opening the bag. Place required number of strips onto strip holder. Place unused strips into the resealable zipper bag and reseal duly.

CAL **CONTROL** **Calibrators and Control**

Liquid calibrators and control are ready to use.

Prepare lyophilized calibrators and control as follows. Gently tap on the vial caps to knock off all the dry matter. Open the vials and carefully place the caps upside down on the clean dry surface. Add 0.5 mL of distilled or deionized water to each vial with lyophilized calibrators and control, close vials with the corresponding caps and leave for 10 min at room temperature without stirring. Then stir gently avoiding foaming, until the dry matter is completely dissolved. Leave for another 10 minutes at room temperature stirring gently periodically. Make sure that no dry matter is left on the caps and walls of the vials.

WASH P Prepare required volume of **wash solution** by dilution of the concentrate 20-fold with distilled or deionized water. For example:

5 mL of **WASH P** **20X** + 95 mL of water.

Mix thoroughly, avoid foaming.

SUB Protect **substrate** from direct light.

9. SAMPLE PREPARATION

Allow samples to reach room temperature.
Stir samples gently in order to ensure homogeneity.

10. PERFORMANCE CHARACTERISTICS OF THE ASSAY

10.1. Calibration - Traceability

Prolactin kit was calibrated against the 3rd WHO International Standard 84/500.

10.2. Specificity

No cross-interaction of both anti-prolactin monoclonal antibodies with LH, FSH, TSH, placental lactogen, and hGH was detected.

10.3. Analytical Sensitivity

Analytical sensitivity of **Prolactin kit** i.e. concentration that can be distinguished from zero calibrator, is 50 mIU/L. It is defined as mean OD of 10 replicates of calibrator 0 plus two standard deviations.

10.4. Measurement Range

Prolactin kit was validated for measurement of prolactin concentration within the concentration diapason of 50 - 4500mIU/L.

10.5. Hook Effect

Prolactin kit shows no **high dose hook effect** for prolactin concentrations up to 100 000 mIU/L. **High dose hook effect** was determined by spiking calibrator 0 matrix with antigen.

10.6. Intra- and Inter-Assay Variation

For **intra-assay CV** determination, 7 serum samples were assayed in 9 replicates each. The results are shown below.

Sample	Mean prolactin concentration, mIU/L	Intra-assay CV	
		SD	CV, %
HS 1	155	21.7	14.0
HS 2	432	26.8	6.2
HS 3	513	29.2	5.7
HS 4	660	33.7	5.1
HS 5	1234	55.5	4.5
HS 6	1546	94.3	6.1
HS 7	2567	179.7	7.0

For **inter-assay CV** determination, 8 serum samples were assayed 3 times by different operators with 1-week interval. Each specimen was assayed in 9 replicates. The results are shown below.

Sample	Mean prolactin concentration, mIU/L			Inter-assay CV	
	1 assay	2 assay	3 assay	SD	CV, %
HS 1	154	139	144	7.6	5.4
HS 2	218	202	195	11.8	5.9
HS 3	302	278	262	20.1	7.5
HS 4	324	301	281	21.5	7.4
HS 5	348	311	308	22.3	7.2
HS 6	470	432	431	22.2	5.2
HS 7	958	840	851	65.2	7.7
HS 8	2410	2409	2049	208.1	9.3

11. LIMITATION OF THE METHOD

Any clinical diagnosis should not be based on the results of in vitro diagnostic method alone. For diagnosis establishment, a physician is supposed to consider all available clinical and laboratory findings.

12. SAFETY PRECAUTIONS

- **This kit is for in vitro diagnostic use only.** Operator should follow the manual closely in order to ensure reliable data. The manual is valid for the present kit only, within the listed composition. Any substitution of kit components is not allowed by CE regulations.
- Do not use kit or its components after the expiration date indicated on the label. Take into account stability period for reconstituted reagents.
- Do not mix or use together reagents from different lots of the kit except substrate, stop solution and wash solution.
- Do not use substrate, stop solution and wash solution supplied by other vendors.
- Use only “P”-labeled wash solution.
- Note that stop solution is 1 N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected area thoroughly with plenty of water and seek medical advice.
- Take into account the following common procedural notes:
  - always pipette reagents into wells immediately after washing procedure;
  - avoid contamination of the solutions;
  - in case of partial use of the kit, dispense only required volume of the reagent into the tray;
  - do not pour unused reagents back into the original vials;
  - avoid exposure to direct sunlight during incubations;
  - always pipette reagents in the same order to minimize reaction time differences between wells; the total dispensing time for the calibrators, control and samples must not exceed 15 min;

-the incubation temperature for all the immunological reactions must be kept at +37 °C;

-do not touch the bottom of the wells.

-calibrators should be measured in each separate assay. It is also recommended to measure each time prolactin concentration in the control.

- TMB solution should be colourless. Light colouring of solution is admissible. Avoid direct exposure of substrate to sunlight.

-  Source materials of human origin used for kit components preparation were tested and found negative for HBsAg, anti-HIV and anti-HCV antibodies. However, none of known laboratory test guarantees absence of these viral agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.

-  After usage strips, calibrators, control, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving. Instead of autoclaving pipette tips may be sterilized by disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other components of the kit should be discarded into conventional garbage.

-  During manual washing procedure do not discard the contents of the wells directly to drainage. Use a container with disinfectant solution.

-  As the kit contains potentially hazardous material, the following precautions should be taken:

-do not smoke, eat or drink while performing the assay;

-always use protective gloves;

-never pipette material by mouth;

-in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.

- GLP including all general and individual regulations should be applied for the kit usage.

As the kit contains irritant (CONJ, CAL, CONTROL), the following precautions should be observed:

- P261 - Avoid breathing spray;
- P272 - Contaminated work clothing should not be allowed out of the workplace;
- P280 - Wear protective gloves/protective clothing/eye protection;
- P302+P352 - IF ON SKIN: Wash with plenty of soap and water;
- P333+P313 - If skin irritation or rash occurs: Get medical advice/attention;
- P363 - Wash contaminated clothing before reuse;
- P501 - Dispose of contents/container in accordance with national regulation.

Precautionary statements according to Regulation EC № 1272/2008.

13. MATERIAL PROVIDED

13.1. Material Provided

MP	**Microplate:** 12 breakable 8-well strips (total 96 wells), coated with anti-prolactin monoclonal antibodies	1 pcs
CONJ	**Conjugate:** solution contains anti-prolactin monoclonal antibodies conjugated with HRP	14 mL, ready to use
0–5 **CAL**	**Prolactin calibrators:** protein-based solution or lyophilized preparations containing known prolactin concentrations – 0; 100; 500; 1200; 2500; 4500 mIU/L. The concentrations of calibrators may be different for schemes with or without shaking. For exact prolactin concentration see vial labels.	6 vials, 0.5 mL each, ready to use or lyophilized preparations
CONTROL	**Prolactin control:** protein-based solution or lyophilized preparation containing known prolactin concentration. The range of prolactin concentration may be different for schemes with or without shaking. For exact range of prolactin concentration see vial label.	0.5 mL, ready to use or lyophilized preparation
SUB	**Substrate (TMB solution):** 3,3',5,5'-tetramethylbenzidine solution in citrate solution containing hydrogen peroxide	14 mL, ready to use
WASH P **20X**	**Wash solution P, 20X concentrated:** surfactant in buffered saline, sufficient for preparation of 560 mL of solution	2x14 mL, concentrated
STOP	**Stop solution:** 1 N HCl solution	14 mL, ready to use

13.2. Equipment and Materials Required but not provided

- 1-channel calibrated variable precision pipettes, with disposable tips;
- 8-channel calibrated variable precision pipette, with disposable tips;
- microplate incubator (+37 °C) or ,microplate incubator);
- manual or automatic equipment for rinsing wells;
- calibrated microplate reader (450 nm);
- vortex tube mixer;
- deionized or distilled water;
- graduated beaker and cylinder of appropriate volume;
- latex or plastic gloves;
- trays for pipetting reagents with 8-channel pipette;
- disinfectant;
- absorbent material (for manual wash).

13.3. Test Procedure

Prolactin kit is designed for 96 tests. This is sufficient for 40 unknowns, 6 calibrators, 1 control and 1 blank (OD of TMB solution) in duplicates, provided that all the strips are used simultaneously.

13.3.1. Assay Procedure

13.3.1.1. Protocol with shaking

(See assay scheme, section 13.5)

All samples should be tested in duplicates.

A. Pipette**:**

- **100 µL** of **conjugate** **CONJ** into each well, **except wells A1-A2 (blank);**

B. Pipette**:**

- **20 μL** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates, into the respective wells**;**

except wells A1-A2.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for **60 minutes at +37 °C**

D. Wash 5 times, as described in section 13.3.2.

E. Pipette **100 μL** of **substrate SUB** into each well (including blank); incubate strips **at room temperature** (+18…+25 °C) **in the dark for 15-30 minutes**, depending on the colour intensity,

F. Pipette **100 μL** of **stop solution STOP** into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within** 20 min.

13.3.1.2. Protocol without shaking

(See assay scheme, section 13.6)

All samples should be tested in duplicates.

A. Pipette**:**

- **100 µL** of **conjugate** **CONJ** into each well, **except wells A1-A2 (blank);**

B. Pipette**:**

- **20 µL** of calibrators **CAL**, control **CONTROL** and patient's samples in duplicates, into the respective wells**;**

except wells A1-A2.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation will substantially vary for different samples.

C. Incubate for **180 minutes at +37 °C**

D. Wash 5 times, as described in section 13.3.2.

E. Pipette **100 µL** of **substrate** **SUB** into each well (including blank); incubate strips **at room temperature** (+18…+25 °C) **in the dark for 15-30 minutes**, depending on the colour intensity.

F. Pipette **100 µL** of **stop solution** **STOP** into each well (including blank) in the same sequence and at the same speed as used for dispensing TMB substrate. Shake for 1–2 min at room temperature.

G. Read OD at **450 nm within** 20 min.

13.3.2. Wash Procedure

It is advisable to use an automatic microplate washer set at 5 wash cycles and a volume of 300 µL of wash solution per well per cycle.

If an automatic washer is not available, the wash procedure can be carried out manually as follows:

- remove the contents of the wells into a container with disinfectant;
- dispense 300 µL of wash solution, prepared according to section 8, into each well, shake the plate carefully for 5–10 sec and remove the contents of the wells; repeat 5 times;
- strike the wells sharply on absorbent material to remove any liquid residue.

13.4. Data Processing

If the reader cannot be adjusted to zero using the substrate blank in wells A1-A2, subtract mean OD value of wells A1-A2 from all OD values before further calculations.

Example:

OD (Cal 5) measured = 2.28 and OD (blank) = 0.06;

OD (Cal 5) calculated = 2.28 – 0.06 = 2.22

13.4.1. Data Reliability (for OD Measured at 450 nm)

The data should meet the following criteria:

- average blank OD (in wells A1-A2) ≤0.100;
- average OD of Cal 5 ≥1.0 (after blank subtraction);
- control's concentration must fall within the acceptability range that is shown on the vial label.

If the data obtained do not meet the criteria, the results are considered unreliable and the test should be repeated.

13.4.2. Quantitative Determination

Specialized software for quantitative determination is recommended. Mean OD values of the calibrators at 450 nm versus their respective prolactin concentrations using 4PL or 5PL fit (see typical standard curve, fig. 2). The arithmetical mean OD of the two «Blank» wells is used for zero setting of device.

Extrapolation of the standard curve to prolactin concentration above the nominal value of calibrator 5 is not permitted.

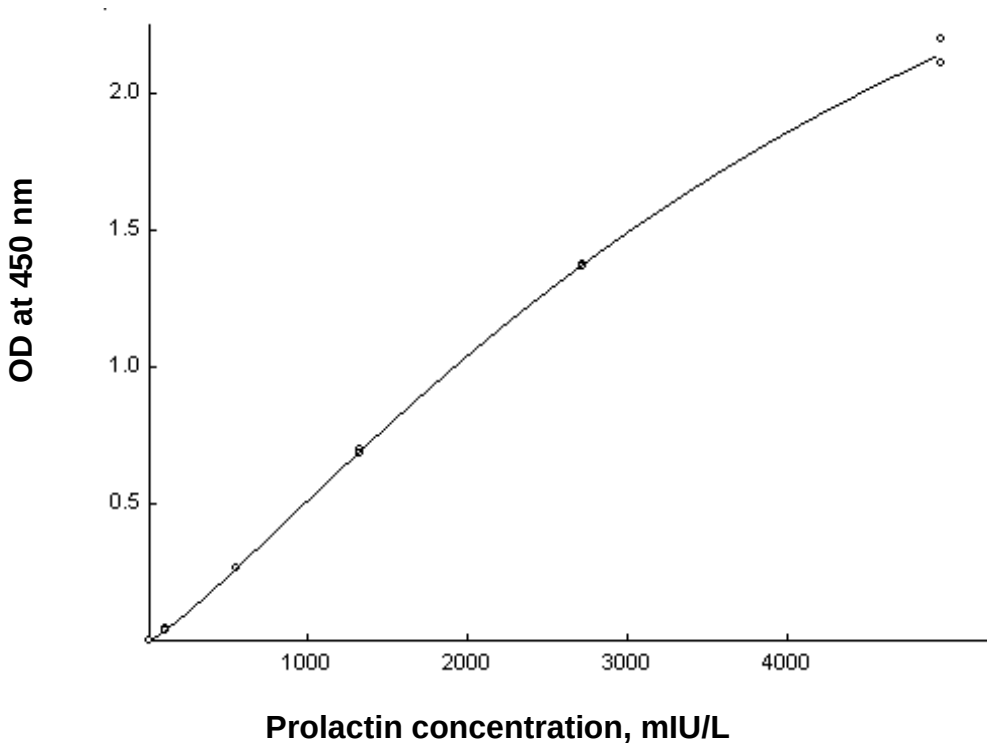


Fig. 2. Example of typical standard curve.
Do not use for evaluation of real assay data!

13.5. Assay scheme

Wells / Reagents	«Blank»	**CAL** **CONTROL**	Samples
CONJ	–	100 µL	100 µL
CAL **CONTROL**	–	20 µL	–
Samples	–	–	20 µL
Incubation No. 1	60 min, +37 °C Incubator		
WASH P (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No. 2	15–30 min, +18…+25 °C, in the dark		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, +18…+25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

13.6.Assay scheme

Wells / Reagents	«Blank»	**CAL** **CONTROL**	Samples
CONJ	–	100 µL	100 µL
CAL **CONTROL**	–	20 µL	–
Samples	–	–	20 µL
Incubation No. 1	180 min, Room Temp.		
WASH P (diluted)	5 x 300 µL		
SUB	100 µL	100 µL	100 µL
Incubation No. 2	15–30 min, +18…+25 °C, in the dark		
STOP	100 µL	100 µL	100 µL
Stirring	1–2 min, +18…+25 °C		
OD measuring	450 nm		
Calculations	Corresponding software		

BIOGENIX INC. PVT. LTD.
Factory: B - 19/A, S.I.L Ancillary Estate, Amausi Industrial Area, Nadarganj, Kanpur Road, Lucknow - 226008 (U.P.), India
Email: biogenix2007@yahoo.com, info@biogenixinc.com
Website: www.biogenixinc.com
Customer care no: +919140971443