



DCM011-11
Ed. 12/2012

PROLACTIN ELISA

for routine analysis

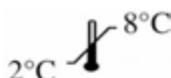
Direct immunoenzymatic determination of Prolactin in human serum.

IVD



LOT

See external label



Σ = 96 tests

REF DKO011

INTENDED USE

The Eagle Biosciences [Prolactin ELISA Assay Kit](#) for quantitative determination of Prolactin concentration in human serum.

Prolactin ELISA Assay Kit is intended for research use only and not to be used in diagnostic procedures.

1. CLINICAL SIGNIFICANCE

Prolactin is a polypeptide hormone synthesized and secreted by the Adenohypophysis (anterior Pituitary gland) and the placenta. It is also produced in other tissues including the breast and the decidua. Pituitary prolactin secretion is regulated by neuroendocrine neurons in the hypothalamus, most importantly by neurosecretory dopamine neurons of the arcuate nucleus, which inhibit prolactin secretion.

Prolactin is present in several body fluids, including blood plasma, amniotic fluid, milk, mucosal secretions and cerebrospinal fluid.

Prolactin has many effects, the most important of which is to stimulate the mammary glands to produce milk (lactation). Other possible functions of prolactin include the surfactant synthesis of the fetal lungs at the end of the pregnancy and immune tolerance of the foetus by the maternal organism during pregnancy.

Prolactin may also have inhibitory effects on gonadal function when present in high concentrations.

There is a diurnal cycle in prolactin secretion.

During pregnancy, high circulating concentrations of estrogen promote prolactin production. The resulting high levels of prolactin secretion cause maturation of the mammary glands, preparing them for lactation. After childbirth, prolactin levels fall as the internal stimulus for them is removed.

High prolactin levels also tend to suppress the ovulatory cycle by inhibiting the secretion of both FSH and GnRH.

Prolactin levels may be checked as part of a sex hormone workup, as elevated prolactin secretion can suppress the secretion of FSH and GnRH, leading to hypogonadism, and sometimes causing erectile dysfunction in men.

Elevations in plasma prolactin concentrations occur during ovulation, pregnancy, nursing and stress. Abnormal elevations in plasma prolactin levels

(hyperprolactinemia) can occur as a result of pituitary adenomas, other anatomic and traumatic abnormalities, in response to certain pharmacologic agents and in hypothyroidism.

Hypoprolactinemia (low prolactin levels) are observed in cases of hypopituitarism.

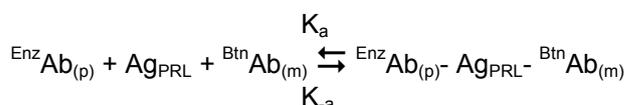
2. PRINCIPLE

The essential reagents required for an immunoenzymatic assay include high affinity and specificity antibodies (enzyme and immobilised) with different and distinct epitope recognition, in excess, and native antigen.

In this Prolactin ELISA Assay Kit, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PRL antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex.

The interaction is illustrated by the following equation:



$\text{B}^{\text{tn}}\text{Ab}_{(m)}$ = Biotinylated Monoclonal Antibody (Excess quantity)

Ag_{PRL} = Native PRL antigen (variable quantity)

$\text{EnzAb}_{(p)}$ = Enzyme labeled polyclonal antibody (Excess quantity)

$\text{EnzAb}_{(p)} - \text{Ag}_{\text{PRL}} - \text{B}^{\text{tn}}\text{Ab}_{(m)}$ = Antigen-Antibodies Sandwich complex

K_a = Rate constant of association

K_d = Rate constant of disassociation

Simultaneously the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody.

This interaction is illustrated below:

$\text{EnzAb}_{(p)}\text{-Ag}_{\text{PRL}}\text{-}^{\text{Bln}}\text{Ab}_{(m)} + \text{Streptavidin}_{\text{CW}} \Rightarrow \text{Immobilized complex}$

$\text{Streptavidin}_{\text{CW}} = \text{Streptavidin immobilized on well}$

Immobilized complex = Antibodies-Antigen sandwich bound.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By using several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Prolactin Calibrators (6 vials, 1 mL each)

CAL0	REF	DCE002/1106-0
CAL1	REF	DCE002/1107-0
CAL2	REF	DCE002/1108-0
CAL3	REF	DCE002/1109-0
CAL4	REF	DCE002/1110-0
CAL5	REF	DCE002/1111-0

2. Prolactin Control (1 vial, 1 mL)

Control Concentration is Lot-specific and is indicated on Quality Control Report

REF **DCE045/1103-0**

3. Conjugate (1 vial, 12 mL)

Antibodies Anti Prolactin conjugated with Horseradish peroxidase (HRP) and Anti Prolactin biotinylated

REF **DCE002/1102-0**

4. Coated Microplate (1 breakable microplate)

Streptavidin adsorbed on microplate

REF **DCE002/1103-0**

5. TMB Substrate (1 vial, 15 mL)

H_2O_2 -TMB 0.26 g/L (avoid any skin contact)

REF **DCE004-0**

6. Stop Solution (1 vial, 15 mL)

Sulphuric acid 0.15 mol/L (avoid any skin contact)

REF **DCE005-0**

7. 10X Conc. Wash Solution (1 vial, 50 mL)

Phosphate buffer 0.2M, Proclin < 0,0015%

REF **DCE054-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.

Microplates reader (450 nm)

Note

Store all reagents at 2-8°C in the dark.

Open the bag of reagent 4 (Coated Microplate) only when it is at room temperature and close it immediately after use.

4. WARNINGS

- This Prolactin ELISA Assay Kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Some reagents of the Prolactin ELISA Assay Kit contain small amounts of Proclin 300^R as preservative. Avoid the contact with skin or mucosa.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/ H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.
- This Prolactin ELISA Assay Kit allows the determination of Prolactin from 5.0 to 100.0 ng/mL.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents of the Prolactin ELISA Assay Kit should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all Prolactin ELISA Assay Kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange Prolactin ELISA Assay Kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added

- in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C₀...C₅)

The Calibrators are ready to use, are calibrated against WHO 3rd IS 84/500 and have the following concentrations:

	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅
ng/mL	0	5	10	25	50	100

The Calibrators are stable until the expiry date printed on the label. Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C. In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.3. Preparation of the Sample

Prolactin determination can be done on human serum. **Do not use plasma for this assay** (plasma samples can lead to false results).

Samples microbiologically contaminated, highly lipemic or haemolysed should not be used in the assay.

To obtain the serum, collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred.

Specimen can be stored at 2-8°C for at short time (max two days). For longer storage the specimen should be frozen at -20°C. Avoid repeated freezing and thawing. Thawed samples should be inverted several times prior to testing.

For sample with concentration over 100 ng/mL dilute the sample 1:2 with Calibrator 0.

The Control is ready for use.

6.4. Procedure

- Allow all reagents to reach room temperature (22-28°C).**

- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C₀-C₅), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/Control	Blank
Sample/Control		50 µL	
Calibrator C ₀ -C ₅	50 µL		
Conjugate	100 µL	100 µL	
Incubate at room temperature (22-28°C) for 1 hours. Remove the contents from each well; wash the wells 3 times with 300 µL of diluted Wash Solution. Important note: gently shake the plate for 5 seconds at each washing step to ensure proper cleaning. <u>After the last wash remove excess solution by tapping the inverted plate on an absorbent paper towel.</u>			
TMB Substrate	100 µL	100 µL	100 µL
Incubate at room temperature (22±28°C) for 15 minutes in the dark.			
Stop Solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank within 5 minutes.			

7. QUALITY CONTROL

Each laboratory should assay controls at normal, high and low levels range of Prolactin for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the calibration curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. RESULTS

8.1. Mean Absorbance

Calculate the mean of the absorbance (Em) for each point of the calibration curve (C₀-C₅) and of each sample.

8.2. Calibration curve

Plot the values of absorbance (Em) of the calibrators (C₀-C₅) against concentration. Draw the best-fit curve through the plotted points (Es: Four Parameter Logistic or Sigmoide).

8.3. Calculation of Results

Interpolate the values of the samples on the calibration curve to obtain the corresponding values of the concentrations expressed in ng/mL.

9. REFERENCE VALUES

Each laboratory must establish its own normal ranges based on patient population.

The serum Prolactin values are comprised in the following intervals:

Sample	Range ng/mL	
Male	1.8 - 17.0	
Female:	menstrual cycle	1.2 - 19.5
	menopause	1.5 - 18.5

Some of the female population tested in this group were probably using oral contraceptives, which may affect results.

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.

10. PERFORMANCE AND CHARACTERISTICS

10.1. Precision

10.1.1. Intra Assay Variation

Within run variation was determined by replicate (20x) the measurement of three different control sera in one assay.

Sample	N	X	σ	C.V.
Level 1	20	5.33	0.15	2.78%
Level 2	20	18.21	0.73	4.03%
Level 3	20	37.20	1.38	3.71%

10.1.2. Inter Assay Variation

Between run variation was determined by replicate (10x) the measurement of three different control sera with kits of different lots.

Sample	N	X	σ	C.V.
Level 1	10	5.46	0.30	5.49%
Level 2	10	17.72	0.91	5.16%
Level 3	10	36.29	1.67	4.60%

10.2. Sensitivity

The lowest detectable concentration of Prolactin that can be distinguished from the Calibrator 0 is 0.12 ng/mL at the 95 % confidence limit.

10.3. Accuracy

The recovery of 3,13 - 6,25 - 12,50 - 25,00 - 50,00 ng/mL of Prolactin added to sample gave an average value (\pm SD) of 102.52% \pm 9.75% with reference to the original concentrations.

The dilution test performed on three sera diluted 2 - 4 - 8 - 16 times gave an average value (\pm SD) of 102.19% \pm 9.80%.

10.4. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham are shown in the table:

hProlactin	100%
LH	N.D.
FSH	N.D.
hCG	N.D.
TSH	N.D.
hGH	N.D.

10.5. Correlation

Diametra Prolactin kit (y) was compared to another commercially available Prolactin assay (x). Serum samples of 37 subjects were analysed.

The linear regression curve was calculated:

$$y = 1.01 x + 1.94$$
$$r^2 = 0.957$$

The new Diametra Prolactin kit (y) was compared to the old Diametra Prolactin kit (x). Serum samples of 37 subjects were analysed.

The linear regression curve was calculated:

$$y = 0.85 x + 2.58$$
$$r^2 = 0.937$$

10.6. Hook Effect

Diametra Prolactin assay shows no Hook effect up to 200 ng/mL.

11. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers
- insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)
- too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation