DRG

DRG® Prolactin ELISA   (EIA-1291)

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1   INTRODUCTION

1.1   Intended Use

The **DRG Prolactin ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of Prolactin in serum.

1.2   Summary and Explanation

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 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.

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 (11). 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 (12).

The DRG Prolactin ELISA provides a rapid, sensitive, and a reliable assay. The antibodies developed for the test will determine a minimal concentration of human prolactin of 0.35 ng/mL. There is no cross-reactivity with hCG, TSH, LH, FSH, or hGH.

2   PRINCIPLE OF THE TEST

The DRG Prolactin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on a Prolactin molecule. An aliquot of patient sample containing endogenous Prolactin is incubated in the coated well with enzyme conjugate, which is an anti- Prolactin antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of Prolactin in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Prolactin in the patient sample.

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3   WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets. Material Safety Data Sheets for this product are available upon request directly from DRG.

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4   REAGENTS

4.1   Reagents provided

1. *Microtiterwells*, 12x8 (break apart) strips, 96 wells;
   Wells coated with anti-Prolactin antibody (monoclonal).

2. *Standard (Standard 0-5)*, 6 vials (lyophilized), 1 mL;
   Concentrations: 0; 5; 20; 50; 100; 200 ng/mL
   Conversion: 1 ng/mL = 21.1 mIU/L
   The standards are calibrated against WHO 3rd International Standard for Prolactin IRP (84/500.)
   See „*Preparation of Reagents*";
   * contain 0.03% Proclin 300, 0.01% MIT and 0.015% BND as preservatives.

3. *Enzyme Conjugate*, 1 vial, 11 mL, ready to use,
   Anti-Prolactin antibody conjugated to horseradish peroxidase;
   * contains 0.03% Proclin 300, 0.01% MIT and 0.015% BND as preservatives.

4. *Substrate Solution*, 1 vial, 14 mL, ready to use,
   Tetramethylbenzidine (TMB).

5. *Stop Solution*, 1 vial, 14 mL, ready to use,
   contains 0.5M H_2SO_4,
   Avoid contact with the stop solution. It may cause skin irritations and burns.

   *   BND   = 5-bromo-5-nitro-1,3-dioxane
        MIT   = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available on request.

4.2   Materials required but not provided

− A microtiter plate calibrated reader (450±10 nm) (e.g. the DRG Instruments Microtiter Plate Reader).
− Calibrated variable precision micropipettes.
− Absorbent paper.
− Aqua dest.
− Timer
− Semi logarithmic graph paper or software for data reduction

4.3   Storage Conditions

When stored at 2 °C - 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C - 8 °C. Microtiter wells must be stored at 2 °C - 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

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4.4   Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use

Standards
Reconstitute the lyophilized contents of the standard vial with 1 mL Aqua dest.
Note: The reconstituted standards are stable for 2 months at 2 °C - 8 °C. For longer storage freeze at -20°C.

4.5   Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets.

4.6   Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5   SPECIMEN COLLECTION AND PREPARATION

Only serum should be used in this assay.
(The use of EDTA- or Heparin samples may lead to increased values while the use of citrate plasma may lead to decreased values.)
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1   Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

5.2   Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2 °C - 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.

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5.3   Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10:   10 µL Serum + 90 µL *Standard 0* (mix thoroughly)

b) dilution 1:100:   10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6   ASSAY PROCEDURE

6.1   General Remarks

– All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.

– Once the test has been started, all steps should be completed without interruption.

– Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination

– Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.

– As a general rule the enzymatic reaction is linearly proportional to time and temperature.

– Pipetting of all standards, samples, and controls should be completed within 6 minutes. (Note this especially for manual pipetting.)

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6.2   Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the holder.
2. Dispense **25 µL** of each *Standard, Control* and samples <u>with new disposable tips</u> into appropriate wells.
3. Dispense **100 µL** *Enzyme Conjugate* into each well.
    Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
    Rinse the wells 5 times with distilled water (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
    Important note:
    The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **100 µL** of *Substrate Solution* to each well.
7. Incubate for **10 minutes** at room temperature.
8. Stop the enzymatic reaction by adding **50 µL** of *Stop Solution* to each well.
9. Determine the absorbance (OD) of each well at **450 ± 10 nm** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3   Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value 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 from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 200 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1   Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

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Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	0.04
Standard 1 (5 ng/mL)	0.13
Standard 2 (20 ng/mL)	0.40
Standard 3 (50 ng/mL)	0.80
Standard 4 (100 ng/mL)	1.34
Standard 5 (200 ng/mL)	1.92

7   EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG Prolactin ELISA the following values are observed:

Population	Mean (ng/mL)	S.D. (ng/mL)	5% Percentile (ng/mL)	95% Percentile (ng/mL)
Males	6.44	5.50	0.94	20.94
Females	14.27	5.88	2.39	25.15

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8   QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
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. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

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9   PERFORMANCE CHARACTERISTICS

9.1   Assay Dynamic Range

The range of the assay is between 0.35 – 200 ng/mL.

9.2   Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Hormone Tested	Concentration	Produced Color Intensity Equivalent to Prolactin in Serum(ng/mL)
hCG (WHO 1st IRP 75/537)	62,500 mlU/mL 125,000 mlU/mL 250,000 mlU/mL 500,000 mlU/mL	0 0 0 0
TSH (WHO 2nd IRP 80/558)	250 µlU/mL 500 µlU/mL	0 0
LH (WHO 1st IRP 68/40)	500 mlU/mL 1000 mlU/mL	0 0
FSH (WHO 2nd IRP-HMG)	250 mlU/mL 500 mlU/mL	0 0
hGH (WHO 1st IRP 66/217)	1000 µg/mL	2.5

9.3   Sensitivity

The analytical sensitivity of the DRG ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of *Standard 0* and was found to be 0.35 ng/mL.

9.4   Reproducibility

9.4.1   Intra Assay

The within assay variability is shown below:

Sample	1	2	3
Mean (ng/mL)	6.16	14.10	32.48
SD (ng/mL)	0.28	0.41	1.91
CV (%)	4.58	2.91	5.87
n =	10	10	10

9.4.2   Inter Assay

The between assay variability is shown below:

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Sample	1	2	3
Mean (ng/mL)	5.96	12.64	25.99
SD (ng/mL)	0.37	0.71	1.53
CV (%)	6.22	5.64	5.90
n =	12	12	12

9.5   Recovery

Samples have been spiked by adding Prolactin solutions with known concentrations in a 1:1 ratio.
The % Recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100.

Sample	Endogenous Prolactin ng/mL	Added Prolactin ng/mL	Measured Conc. Prolactin ng/mL	Expected * Prolactin ng/mL	Recovery (%)
1 Serum	8.4	0.0	8.4		
		10.0	13.8	14.2	97.4
		25.0	29.9	29.2	102.6
		50.0	51.5	54.2	95.1
		100.0	90.0	104.2	86.4
2 Serum	20.0	0.0	20.0		
		10.0	22.0	20.0	110.2
		25.0	34.3	35.0	98.1
		50.0	52.2	60.0	87.0
		100.0	94.9	110.0	86.3
3 Serum	31.8	0.0	31.8		
		10.0	26.2	25.9	101.3
		25.0	40.4	40.9	98.7
		50.0	58.3	65.9	88.4
		100.0	103.7	115.9	89.4

(* Endogenous Prolaktin / 2 + added Prolactin because of a 1:1 dilution of serum with spike material.)

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9.6   Linearity

Sample	Dilution	Measured Conc. (ng/mL)	Expected Conc. (ng/mL)	Recovery (%)
1	None	8.40	8.38	
	1:2	4.22	4.19	100.8
	1:4	1.98	2.09	94.4
	1:8	1.15	1.05	109.9
	1:16	0.58	0.52	111.0
2	None	20.0	19.96	
	1:2	10.74	9.98	107.6
	1:4	5.56	4.99	111.4
	1:8	2.75	2.49	110.2
	1:16	1.28	1.25	102.2
3	None	31.80	31.81	
	1:2	14.35	15.91	90.2
	1:4	6.95	7.95	87.4
	1:8	3.55	3.98	89.2
	1:16	1.76	1.99	88.7

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1   Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (**up to 0.9 mg/mL**) have no influence on the assay results.

10.2   Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Prolactin in a sample.

10.3   High-Dose-Hook Effect

No hook effect was observed in this test up to 2000 ng/mL of Prolactin.

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11 LEGAL ASPECTS

11.1   Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2   Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3   Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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12 REFERENCES
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