in vitro diagnostic Reagent, not for medicinal use

J. Mitra & Co. Pvt. Ltd.
A 180-181, Okhla Ind. Area, Ph-1, New Delhi-110 020, INDIA
Ph: +91-11-47130300, 47130500
e-mail: jmitra@jmitra.co.in Internet: www.jmitra.co.in

PROLACTIN *i*Clia

Chemiluminesence Immunoassay for the quantitative detection of PROLACTIN (PRL) in human serum and plasma

1. INTRODUCTION

Prolactin (PRL) is a protein hormone of the anterior pituitary gland that was originally named for its ability to promote lactation in response to the suckling stimulus of hungry young mammals. It is one of a family of related hormones including growth hormone (GH) and placental lactogen (PL) that a single-chain polypeptide hormone with molecular weight of approximately 22,000 Daltons, secreted by the hypothalamus. The increased level of prolactin hormone is mainly seen during pregnancy, stress, physical activities, breast stimulation and in nursing mothers. The decreased level of prolactin hormone is mainly because of hypopituitarism. The main disorders caused by the prolactin hormone are hyperprolactinaemia and hypoprolactinaemia. Determination of PRL is used for diagnosis and monitoring of pituitary adenoma, hypothalamic disease, hyperprolactinemia disease, monitor the prolactin-producing tumours, and causes of infertility in both males and females.

2. INTENDED USE

PROLACTIN iClia is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative deterction of PROLACTIN (PRL) in human serum and plasma. The assay kit is intended for in vitro diagnostic use. This kit is only operational in conjuction with CLIA-181 Analyzer.

3. PRINCIPLE

PROLACTIN iClia is chemiluminescent immunoassay based on the "Sandwich" principle. The magnetic microspheres are coated with Anti- PROLACTIN antibodies .

The samples are added in the assay cup containing Assay Buffer and anti-PRL IgG coated microspheres followed by addition of AE conjugate (Anti- PROLACTIN antibodies linked to acridinium ester) to assay cup . A sandwich complex is formed wherein PROLACTIN (from serum sample) is "trapped" or "sandwiched" between the microspheres coated antibody and antibody labelled with AE conjugate. Unbound conjugate is then washed off with wash buffer. The amount of bound AE conjugate is proportional to the concentration of PROLACTIN present in the sample. Finally pre-trigger and trigger solution containing hydrogen peroxide and sodium hydroxide solution is added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative Light units (RLUs). There is a direct relationship between the amount of PROLACTIN present in the sample and the RLUs detected by the optical system. Results are calculated automatically based on the established calibration curve.

4. DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.

	Manufactured By	IVD	*In vitro* diagnostic medical device
Σ	No. of tests		See Instruction for use
LOT	Lot Number	2°C 8°C	Temperature Limitation
	Batch Number		Caution -
	Manufacturing Date	REF	See instruction for use
	Expiry Date		Catalogue Number
	Do not use if package is damaged		Keep away from sunlight
BIO	Contains biological Material of Human Origin	BIO	Contains biological Material of Animal Origin
IN	Country of Manufacture		Keep Dry

5. KIT PRESENTATION

- 25 Test Pack
- 50 Test Pack

6. KIT & ITS COMPONENTS

COMPONENT	DESCRIPTION
Microparticles Buffer	Magnetic microparticle buffer coated with anti-Prolactin antibodies with preservatives.
AE Conjugate	anti-PROLACTIN antibodies linked to acridinium ester with protein stabilizers.
Assay Buffer	Tris buffer containing BSA with stabilizer.
Calibrator-1 (C0)	Low concentration of Prolactin in Human Serum containing preservatives.
Calibrator-2 (C1)	High concentration of Prolactin in Human Serum containing preservatives.
Control-1 (Q1)	Low concentration of Prolactin in Human Serum containing preservatives.
Control-2 (Q2)	High concentration of Prolactin in Human Serum containing preservatives.
Reagent Sealers	Adhesive sheets to cover the opened reagents.

7. STORAGE AND STABILITY

The shelf-life of the kit is 12 months from the date of manufacturing, when stored at 2-8°C. **Once the kit is opened, onboard stability of reagents, calibrator and control is 30 days at 2-8°C.**

8. ADDITIONAL MATERIAL AND INSTRUMENTS REQUIRED

- **Pre-Trigger Solution:** Hydrogen peroxide solution.
- **Trigger Solution:** Sodium hydroxide solution.
- **Wash Buffer:** Phosphate buffered saline solution with surfactant.
- **Assay Cup**
- **CLIA 181 Analyzer**

All materials and analyzer to be used for running the PROLACTIN Clia shall be from J. Mitra & Co. Pvt. Ltd.

9. SPECIMEN COLLECTION & HANDLING

1. Only human serum or plasma samples should be used for the test.
2. For serum collection use serum vacutainer. While preparing serum samples, remove the serum from the clot as soon as possible to avoid hemolysis. Fresh serum/plasma samples are preferred.
3. For plasma collection: use Dipotassium EDTA, Tripotassium EDTA, Sodium heparin and lithium heparin gel vacutainer.
4. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
5. Do not use heat inactivated samples as their use may give false results. Hemolyzed and Icteric hyperlipemic samples may give erroneous results.
6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
7. Always use clear specimens. Centrifuge viscus/ thick or turbid specimen at 10,000 RPM for 15 minutes prior to use to avoid inconsistent result.
8. Use of disposable pipettes or pipette tips is recommended to prevent cross contamination.

10. SPECIMEN PROCESSING

(A) FROZEN SAMPLE

PROLACTIN Clia test is best used with fresh samples that have not been frozen and thawed. However most frozen samples will perform well if the procedure suggested below is followed.

Allow the frozen sample to thaw in a vertical position in the rack. Do not shake the sample. This allows particles to settle to the bottom. Centrifuge the sample at 10,000 rpm for 15 minutes.

(B) TRANSPORTATION

If the specimen is to be transported, it should be packed in compliance with the current Government regulations regarding transport of aetiologic agents.

11. WARNING & PRECAUTION

CAUTION: THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8ºC.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microspheres that may have settled during transport or storage.
5 Once reagents are opened, reagent Sealer must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent sealers are not used according to the instructions given.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7 To avoid contamination, wear clean gloves when placing a reagent sealer on an uncapped reagent bottle.
8. Once a reagent sealer has been placed on an open reagent bottle,do not invert the bottle as this will result in reagent leakage and may compromise assay results.
9. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8ºC (with Reagent Sealers) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent Sealer placed) while in refrigerated storage off the system, the reagent kit must be discarded.
10. Run control-1 & control-2 in each assay to evaluate validity of the kit.
11. Distilled or deionised water must be used for wash buffer preparation.
12. Avoid strong light exposure during the assay.
13. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia-181 user manual for detailed information on preparing the analyzer.
2. Before loading the PROLACTIN iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microspheres that may have settled during transporation/ storage. Once the microspheres have been loaded, no further mixing is required.

Note: Swirl the microparticle bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. Once the microspheres have been resuspended, place a reagent sealer on the bottle.

3. Load the PROLACTIN iClia reagent kit on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 µL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 20 µL.
7. Ensure sample positons are properly define at the time of loading in the analyzer.
8. The Prolactin iClia parameters are stored in barcode placed on the reagent tray and read through barcode reader. In cases, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
9. Mix PROLACTIN iClia calibrator and controls by gentle inversion before use. Open the cap and place the calibrators and control-1 & control-2 vials into each respective sample positions. Read the barcode for calibrator and controls provided with the kit.
10. Run calibration as mentioned in heading **calibration** below.
11. Press Run. The test result for first sample will be obtained at 30 minutes.
12. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Sample Dilution Procedures

- Samples with a PROLACTIN value exceeding 200 ng/ml shall be diluted using the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:10

- Add 100 µL of the sample to 900 µL of normal saline.
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.

Calibration

1. Traceability: PROLACTIN iClia has been standardized against the WHO International Standard NIBSC 84/500.
2. Every PROLACTIN iClia kit has a two-dimension code label containing the predefined master curve of the particular reagent lot.
3. Test all 2 Calibrators in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that controls values are within the validity range specified in the PROLACTIN iClia QC data sheet.
4. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless:
5. Recalibrate the analyzer in following conditions:

a) After each exchange/use of new lot (Test reagent and pritrigger/ Trigger solution/ wash buffer).
b) Every week and/or at the time of any component to be changed.
c) Controls are out of validation range.
d) Required by pertinent regulations.
e) After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the PROLACTIN iClia.

RESULT CALCULATION:

The analyzer automatically calculates the concentrations of each sample. The results are given in ng/ml.

RESULT INTERPRETATION

If sample concentration is lower than the lower limit of the linear range, report the result <0.2 ng/ml, while > 200.00 ng/ml when it is higher than the upper limit of linear range.

14. PERFORMANCE CHARACTERISTICS

- Assay results obtained in individual laboratories may vary from data presented in this product insert.
- The observed limit of blank valve was ≤0.1 ng/ml.

Accuracy: The accuracy of PROLACTIN iClia was detected with 60 clinical specimen and compared with Roche CLIA. The co-relation co-efficient is >0.990.

Precision

Intra Assay Variation

Within run variation was determined by 10 replicate measurements of two different PROLACTIN control sera(Low) and (High) in one assay in 3 different lots. The within assay variability is <8.0 %.

Inter Assay Variation

Between run variation was determined by 10 replicate measurements in 10 sequential days of two different control sera(Low) and (High)in 3 different lots.The between assay variability is <15%.

Intra-Assay, n=10			Inter-Assay, n=10×3		
Control	Mean (ng/ml.)	CV	Sample	Mean (ng/ml.)	CV
1	4.6	4.64%	1	4.61	5.84%
2	176.71	5.08%	2	176.74	5.92%

Inter machine(CLIA-181 Analyzer) Variation

Between machine variation was determined by 3 replicate measurements of two different PROLACTIN control sera(Low) and (High)in 3 different lots in 3 different CLIA-181 Analyzer. The between machine variability is < 10.0 %.

Linearity

The linearity was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP6-A requirements.

The linearity range was verified by more than 6 concentration levels which encompass or be equal to the minimum and the maximum values of linearity range and duplicate assays in triplicate in single run for each lot at all 6 levels.

The PROLACTIN iClia kit has been demonstrated to be linear from 0.2 ng/ml to 200 ng/ml, regression (R^2) of more than >0.990.

Specificity

Cross-Reactivity

A study was performed based on guidance from CLSI EP7-A2.

The cross-reactants listed below (Using PROLACTIN-free samples) were evaluated to determine whether PROLACTIN concentrations were affected when using the Thyroid Stimulating Hormone assay.

Cross-Reactant	Cross-ReactantConcentration	Results
GH2	200 ng/mL	≤ 20.0 µIU/mL

Interference

A study was performed based on guidance from CLSI EP7-A2.

Potentially interfering substances were evaluated to determine whether Prolactin concentrations were affected when using the Prolactin iClia (Prolactin Hormone assay) kit. Samples containing the potential interferents were prepared at two Prolactin concentrations. The samples were assayed, and the Prolactin concentrations of the spiked samples were compared to the reference samples.

Potential Interferent	Interferent Concentration	% Interferent Bias
Bilirubin	20 mg/dL	<10%
Hb	500 mg/dL	<10%
Intralipid	1000 mg/dL	<10%
Total protein	10 g/dL	<10%
RF	1000IU/mL	< 10%
ANA	400AU/mL	< 10%
HAMA	600ng/mL	< 10%

15. LIMITATION OF THE TEST

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the PROLACTIN results are inconsistent with clinical evidence, additional testing is recommended.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.
- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Additional information may be required for diagnosis.
- There is no high-dose HOOK effect at PROLACTIN concentrations up to 200 ng/ml.

16. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

17. REFERENCES

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18. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls/ calibrator deterioration due to improper storage or used after expiry.	Use controls/ calibrator within 30 days once opened and Check storage temp. It should be 2-8ºC.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration due to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8ºC.
2) High prolactin test results	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
3) Low prolactin results	a)Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microspheres by gentle shaking/ inversion before use.