Instructions for Use

Prolactin (CLIA)

[Product Name]

Prolactin (CLIA)

[Packing Size]

24×1 Tests/Pkg (Calibrators included); 60×1 Tests/Pkg (Calibrators included); 60×1 Tests/Pkg

[Intended Use]

Prolactin (PRL) assay is a chemiluminescent immunoassay (CLIA) used for the quantitative determination of prolactin (PRL) in human serum, plasma, or whole blood.

Prolactin is a hormone synthesized and secreted by pituitary lactotroph cells. It has a molecular weight of approximately 23 kDa and is made up of 198 amino acids [1, 2]. Serum prolactin comes in three main forms: the major biologically and immunologically active monomeric ("little") form, the dimeric ("big") form and the tetrameric ("big-big") form. The monomeric form accounts for about 80% of the serum prolactin [3-5]. The main roles of prolactin in women are to promote and maintain lactation and to inhibit menstruation. The hormone is secreted in episodes and regulated by a variety of endocrine factors, including estrogen, dopamine, and thyrotropin releasing hormone (TRH) [6, 7]. Excluding those physiological factors (like coitus, exercise, stress, pregnancy, and lactation), medication use (like dopamine antagonists or antipsychotic agents) [3, 8], renal failure, hypothyroidism, and parasellar tumors, hyperprolactinemia (raised serum prolactin level) is usually associated with disorders of the reproductive system [7, 9]. In clinic, prolactin level is utilized in the diagnosis of hyperprolactinemia with different causes.

[Principle of the Assay]

This prolactin assay takes the double-antibody sandwich format. The detection principle is described below:

(1) Mix the sample with magnetic microparticle coated with an anti-PRL antibody and reaction diluent, add another acridinium labeled anti-PRL antibody. After mixing and incubation, PRL in the sample will react with anti-PRL antibody coated on the magnetic microparticle, acridinium labeled anti-PRL antibody will react with another site on PRL, forming antigen-antibody complex;

(2) A magnet captures microparticle, and then unbound substance is washed off. Add pre-trigger and trigger solution to the reaction mixture sequentially to initiate chemiluminescence reaction;

(3) A photomultiplier tube is used to measure photons generated from the reaction. Signal is amplified exponentially. The count is proportional to PRL concentration in the sample. PRL concentration is determined by an internal calibration curve.

[Main Components]

Packing Size

Component \ Packing Size		Fill Volume		
		24×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg (Calibrators included)	60×1 Tests/Pkg
PRL Reagent Cartridge	Microparticle (R1)	24×50 μL	60×50 μL	60×50 μL
	Conjugate (R2)	24×100 μL	60×100 μL	60×100 μL
	Reaction diluent (R3)	24×100 μL	60×100 μL	60×100 μL
PRL Calibrator	PRL Calibrator C1	1×1.0 mL	1×1.0 mL	/
	PRL Calibrator C2	1×1.0 mL	1×1.0 mL	/
Calibration Card		1 pcs	1 pcs	/

Main Composition

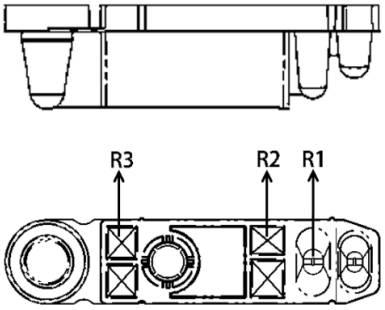
Component	Main Composition	
PRL Reagent Cartridge	Microparticle (R1)	Mouse anti-PRL antibody coated microparticle, ~0.3 g/L; Tris buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Conjugate (R2)	Acridinium labeled mouse anti-PRL antibody, ~200 μg/L; MES buffer, 50 mmol/L; ProClin 300, 0.5 g/L
	Reaction diluent (R3)	Tris buffer, 50 mmol/L;ProClin 300, 0.5g/L
PRL Calibrator C1	PRL(recombinant); MOPS buffer, 50 mmol/L; ProClin 300, 0.5 g/L	
PRL Calibrator C2	PRL (recombinant); MOPS buffer, 50 mmol/L; ProClin 300, 0.5 g/L	
Calibration Card	Calibration curve and calibrator information	

Note: (1) Components in different lots of reagent cannot be mixed or exchanged for use.

(2) Traceability: This quantification method can be traced back to the 4th International Standard (IS), coded 83/573.

(3) Information about the calibrators (such as lot number and concentration etc.) can be found on the instrument interface after scanning the Calibrator Card.

The position of each component in the reagent pack is shown in the front view (Upper) and vertical view (Down) of the reagent cartridge.



Instruments and accessories needed but not supplied (Medcaptain has the supplies)

(1) Medcaptain Immu F6/F6S Automatic Chemiluminescent Immunoassay Analyzers;

(2) Pre-trigger solution;

(3) Trigger solution;

(4) Washing solution;

(5) 500 μL pipette tips;

(6) Sexual Hormone Controls.

[Storage and Shelf-life]

Storage: Store sealed reagent cartridges and calibrators at 2~8℃ in upright position, and avoid freezing.

Shelf life: 14 months.

Stability of Calibrators: Sealed vial of calibrators can be kept at 2~8℃ in dark for 14 months. After calibrator C1 and C2 is uncapped and sealed again, it can be stored at 10~30℃ for 5 days, and at 2~8℃ for 60 days.

The dates of manufacturing and expiration can be found on the labels.

[Matched Instruments]

Medcaptain Immu F6/F6S Automatic Chemiluminescent Immunoassay Analyzers

[Specimen Types]

Serum, plasma, and whole blood (collected with EDTA-K_2, EDTA-K_3, lithium heparin, and sodium heparin as the anti-coagulants) can be used in the testing.

Sample volume for each test: 20 μL.

The collected sample should be tested as soon as possible. Whole blood should be tested within 4 hours after sample collection. Serum and plasma can be kept at 10~30℃ for 24 hours, at 2~8℃ for 7 days, and at -20℃ or below for 6 months. Frequent freeze-thaw cycles should be avoided, and only one freeze-thaw cycle is allowed. If the sample contains precipitate or frozen floccule, centrifugation is needed to clear the sample before testing.

Sample collection tubes from different manufacturers may lead to variation in the test results, due to the difference in tube material and additives in the tubes. Medcaptain has not evaluated all types of sample collection tubes from different manufacturers. Each laboratory should make its own judgment about the usability of sample collection tubes.

[Test Procedure]

Reagent Preparation

Reagent: PRL reagent cartridge (containing magnetic microparticle R1, acridinium labeled conjugate R2, and reaction diluent R3) is ready for use. It can be loaded directly into the instrument after opening the package.

Calibrators: Calibrator C1 and C2 are ready for use. Each calibrator can be added to a sample cup, and the cups are loaded to a sample rack. The sample rack can be pushed directly into the instrument for calibrator testing.

Calibration

Refer to the relevant chapter about system calibration in the operation manual of each chemiluminescent immunoassay analyzer.

Calibration tests should be ordered before the first time use of PRL assay. Medcaptain provides PRL reagent pack and matched calibrators to calibrate the instrument.

Before calibration, scan the calibration card provided in the kit, and the calibration curve and calibrator information will be scanned into the system. Take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.); Put calibrators on a sample rack, load the sample rack into the instrument. On the screen interface of "Reagent > Request Calibration", select test name and lot number to request a calibration. Select the position of each calibrator on the sample rack, set the number of repeat tests, start calibration.

Automatic immunoassay analyzer makes use of calibration data to validate the calibration, and adjust the calibration curve automatically.

Instrument calibration is effective for 60 days. A new calibration is needed in the following situation:

(1) Change into a new lot of reagent;

(2) Control test results are out of the target range;

(3) The lot of reagent has been used on the same instrument for more than 60 days.

Refer to the Chapter of "Calibration" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about calibration.

Control Testing

Sexual Hormone Controls are matched with PRL reagent pack. There are two levels of controls: Low Control (L) and High Control (H).

Test two levels of controls with PRL reagent on the instrument in accordance with any local applicable regulations. Control testing is highly recommended every time the lot of reagent has been changed, the instrument has been re-calibrated, or after trouble shooting/ maintenance service.

Before the control testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

Put controls on a sample rack, and load the sample rack into the instrument; Select "Control" on the interface of test menu, select test name and control lot; Click on "Start" and begin the testing. Check the results after the control test is finished.

Control test results should fall into a specific range. If it is out of the target range, the user should check the system, such as expiration date of the controls, storage condition, instrument performance and status. After root cause analysis and correction, the user should test controls again. If the same problem exists, please contact customer service of Medcaptain.

Each laboratory should set up its own control range and frequency of control testing, based on its own practice.

Refer to the Chapter of "Control Testing" in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about control testing.

Sample Testing

Before sample testing, take out reagent cartridges from the package, and load them into the instrument. The instrument scans two-dimensional barcode on the reagent pack automatically to obtain information of the reagent (reagent name, Lot No., and expiration date etc.).

If a sample collection tube is directly loaded to the instrument for testing, the sample volume should be at least 1.0 mL.

Un-cap sample collection tubes, put samples on a sample rack, and push the sample rack into the instrument; Select "Sample" on the interface of test menu, enter information of samples, select test name; Click on "Start" and begin the testing. Check the results after sample test is finished.

The reagent usage for each test is: R1 50 μL, R2 100 μL, and R3 100 μL; The instrument aspirates and mixes each component in the reagent cartridge and incubates at 37℃. Time duration from sampling to result is about 15 min.

Refer to the Chapter of "Sample Testing" in instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about sample testing.

Result Calculation

Based on the built-in calibration curve, the instrument automatically calculate PRL concentration of each sample, either in a unit of μIU/mL or ng/mL. Unit conversion factors: 1 μIU/mL = 0.047 ng/mL.

[Reference Intervals]

Samples for the study of reference intervals come from local area in Guangdong Province. A total of 246 healthy and normal people have been recruited (Male: 122; Female: 124), age ranges from 16 to 89 years old. Serum tests give a reference interval correspond to the 2.5th and 97.5th percentiles.

Men: 3.9~15.1 ng/mL (82-321 μIU/mL);

Women: 4.9~23.6 ng/mL (104-502 μIU/mL).

Due to the differences in geography, race, sex, and age of tested population, the reference interval may vary in different laboratories. It is highly recommended for each clinical lab to establish its own reference intervals.

[Interpretation of Test Results]

The test data is for clinical reference only. It cannot be used as the only confirmatory evidence nor to eliminate the possibility of diseases. Clinical diagnosis of patients should take clinical symptoms, body sign, disease history, other lab test results, and

treatment response into comprehensive consideration.

The measurement range of this assay is: 0.047~470 ng/mL (1.00~10000 μIU/mL). If PRL concentration is lower than LoD, it will be reported as <0.047 ng/mL (1.00 μIU/mL); If PRL concentration is over the upper limit, it will be reported as >470 ng/mL (10000 μIU/mL). For a sample with PRL concentration of >470 ng/mL (10000 μIU/mL), sample diluent can be used to dilute the sample manually (a dilution factor of 1:10 is recommended). Test the diluted sample in duplicate if more accurate result is needed.

When the instrument shows a warning sign of "SMPL", it means there is insufficient sample volume. Make sure enough sample is added for repeating the test. When the instrument shows a warning sign of "SMPJ", it means the sample probe has been blocked. Clean sample clot in the probe before repeating the test.

Some results are tagged with other signs. Refer to the Chapter of "Result Signs" in the instruction manual of Automatic Chemiluminescent Immunoassay Analyzer for detailed information about results tagged with signs.

[Limitation of the Test Method]

The test data is for clinical reference only. It cannot be used individually as the evidence to confirm or eliminate the possibility of diseases.

There is no Hook effect for a sample with PRL concentration of ≤12690 ng/mL. For each endogenous interference substance with concentration less than the value shown in the table below, measurement error caused by the interference is within ±10%.

Endogenous interference substance	Concentration of interference substance
Total Protein	12 g/dL
Bilirubin	30 mg/dL
Hemoglobin	500 mg/dL
Triglyceride	1500 mg/dL

For each potential cross-reactant with concentration less than the value shown in the table below, the test result of PRL is less than 0.047 ng/mL (1.00 μIU/mL).

Cross-reactant	Concentration
hGH	1000 ng/mL
hCG	100000 mIU/mL
hPL	100000 ng/mL
TSH	20000 μIU/mL
FSH	1000 mIU/mL
LH	5000 mIU/mL

For prolactin assay, it should be aware that the concentration measured depends on when the blood sample is taken, since the secretion of PRL occurs in episodes, and is also subject to a 24-hour cycle. The secretion of PRL is also inhibited by dopamine, L-dopa and ergotamine derivatives etc.

Heterophilic antibodies in human serum may react with immunoglobulin in the reagent or sample, and interfere with immunoassay *in vitro*. More clinical or diagnostic information is needed to confirm disease diagnosis of patients. Some patients have frequent contact with animals, or have been treated or diagnosed with mouse monoclonal antibodies. They may have generated heterophilic antibodies. For example, some patients under monoclonal antibody treatment may have human anti-mouse antibodies (HAMA) in blood circulation, leading to false positive or false negative results. Anti-interference components are added to this reagent formulation to minimize the impact of HAMA and ANA, but the problem may not be totally eliminated, and some sample testing may still be impacted. More clinical and diagnostic information is needed to make a solid conclusion.

Samples with a titer of no less than 1:1000 by anti-nuclear IgG test kit (indirect immune-fluorescence method) were studied in interference tests. It has shown less than ±10% error in testing results. For RF at a concentration of less than 1500 IU/mL, and for multiple representative human HAMA samples, the measurement error caused by the interference is within ±10%.

[Property and Performance]

1 Limit of Blank

LoB ≤0.024 ng/mL (0.50 μIU/mL).

2 Limit of Detection

LoD ≤0.047 ng/mL (1.00 μIU/mL).

3 Accuracy

Accuracy should meet at least one of the following criteria:

a) Take PRL International Reference Material which can be used as reference samples for routine evaluation. Relative deviation between the measurement result and the target value must not exceed 10.0%.

b) Test the PRL accuracy reference samples at two concentration levels multiple times respectively. Relative deviation between the measurement result and the target value must not exceed 10.0%.

c) Spike PRL of a known concentration into real samples at different PRL levels. Spiked recovery should be 100.0±15.0%.

4 Linearity

Test PRL samples with concentration in the range of 0.047~470 ng/mL (1.00~10000 μIU/mL), the linearity correlation coefficient r≥0.9900.

5 Repeatability

Coefficient of variation (CV) for the test results of low (25±5 ng/mL) or high (100±20 ng/mL) PRL corporate reference sample is less than 8.0%.

6 Lot-to-lot Variation

Coefficient of variation (CV) for the test results of low (25±5 ng/mL) or high (100±20 ng/mL) PRL corporate reference sample with three batches of reagent is less than 10.0%.

7 Accuracy of Calibrator Value Assignment

Use primary calibrators with assigned values from higher level measurement procedure, calibrate the immunoassay analyzer, and use the same lot of reagent to measure the value of each product calibrator. The measured value of Calibrator C1 or C2 has a relative deviation within ±10.0% from its assigned value.

8 Homogeneity of Calibrators

8.1 Within-vial Homogeneity

Within-vial homogeneity of Calibrator C1 or C2 is represented with coefficient of variation, and CV≤8.0%.

8.2 Between-vial Homogeneity

Between-vial homogeneity of calibrator C1 or C2 is represented with coefficient of variation, and CV≤5.0%.

[Attention Notes]

1 It is for in-vitro diagnosis only.
2 It can only be used by professionals.
3 Never use expired reagent kit.
4 Never mix components from different kits, or from different reagent lots.
5 Do not put the reagent cartridges upside down.
6 Measurement of PRL in a sample using different detection systems may yield different results, due to the difference in test methods, assay specificity, and factors of interference. The measured values from different systems should not be directly compared to avoid inappropriate clinical interpretation.
7 Strictly follow the protocol in the package insert, and operate according to the lab guidelines.
8 The test results can only be used for clinical reference. Clinical diagnosis of patients should take symptoms, body sign, disease history, other laboratory test results, and response to treatment for comprehensive assessment.
9 User should wear gloves and lab coat. Rinse with water if the skin is in contact

with the reagent. Flush eyes with copious of water if eyes are in touch with the reagent, and see a doctor immediately.

10 Take all samples and reaction waste as potential biohazard. All waste must be handled following the local government regulation.

11 This product is a single-use cartridge. Reagent cartridge should be put back into refrigerator and stored at 2~8℃ if they have been placed at room temperature but not opened yet.

[Interpretation of Signs]

	Temperature limit.		Date of manufacturing
	*In vitro* diagnostic medical device		Catalogue number
	Batch Code		Consult instruction for use
	Use-by Date		Authorized representative in the European Community
	This way up		CE marking of conformity
	Manufacturer		Unique device identifier
	Biological risks		

[References]

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[Basic Information]

MEDCAPTAIN MEDICAL TECHNOLOGY CO., LTD.

12th Floor, Baiwang Research Building, No. 5158 Shahe West Road, Xili, Nanshan, 518055 Shenzhen, Guangdong, PEOPLE'S REPUBLIC OF CHINA

Telephone: +86-755-26953369

Website: http://www.medcaptain.com

Post-sales service: MEDCAPTAIN MEDICAL TECHNOLOGY CO., LTD.

Telephone: +86-755-26953369

Postal code: 518055

Manufacture Location: Building C, Jiale Science and Technology Industrial Park, Matian Street, Guangming, 518106 Shenzhen, Guangdong, PEOPLE'S REPUBLIC OF CHINA.

R Sight B.V.

Roald Dahllaan 47, 5629 MC, Eindhoven. The Netherlands

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