Snibe Diagnostic

CE

REF 130202006M: 100 tests
130602006M: 50 tests

MAGLUMI® PRL (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of PRL in human serum with the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 1000 Plus, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus, MAGLUMI X8, MAGLUMI X3 and MAGLUMI X6) and Biolumi series Integrated System (including Biolumi CX8)..

SUMMARY AND EXPLANATION OF THE TEST

Prolactin (PRL), also known as luteotropic hormone or luteotropin, is a protein composed of 198 amino acids with three inter-chain disulfide bonds and a molecular weight of approximately 23 kDa that is best known for its role in enabling mammals, usually females, to produce milk. It is influential in over 300 separate processes in various vertebrates, including humans[1]. Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. Prolactin is secreted in pulses in between these events. Secretion is controlled via a negative feedback loop whereby circulating prolactin stimulates hypothalamic dopamine secretion which inhibits prolactin release by the pituitary gland. The target organ for prolactin is the mammary gland and the principal functions of prolactin are exerted as part of the hormonal changes of pregnancy. During pregnancy, the pituitary gland enlarges, the number of lactotrophs increase and serum prolactin levels rise approximately ten-fold thereby contributing to breast development and lactation[2-3].

Prolactin plays an essential role in metabolism, regulation of the immune system and pancreatic development. In mammals, prolactin is associated with milk production; in fish it is thought to be related to control of water and salt balance. Prolactin also acts in a cytokine-like manner and as an important regulator of the immune system. It has important cell cycle-related functions as a growth-, differentiating- and anti-apoptotic factor. As a growth factor, binding to cytokine-like receptors, it influences hematopoiesis, angiogenesis and is involved in the regulation of blood clotting through several pathways. The hormone acts in endocrine, autocrine and paracrine manner through the prolactin receptor and a large number of cytokine receptors[1].

Three fractions of immunoreactive PRL of differring molecular mass were found in early studies using gel filtration chromatography (GFC). The terms used to identify the fractions varied initially, but a common terminology evolved to describe the most common pattern: approximately 60--90% 23 kDa monomeric PRL (also referred to as free or little PRL); 15--30% 40--60 kDa big PRL; and 0--10% >100 kDa big—big PRL (also named marcroprolactin)[4]. Macroprolactin is a high molecular mass complex of prolactin (PRL) which remains reactive in immunoassays and, because of its prolonged half-life in plasma, may be a cause of elevated total serum PRL in some immunoassays. Macroprolactin has minimal bioactivity in vivo, but if the cause is not recognized, hyperprolactinaemia due to macroprolactin can lead to misdiagnosis and inappropriate treatment[5-8].

Excess serum prolactin could result in hyperprolactinaemia, which is associated with hypoestrogenism, anovulatory infertility, oligomenorrhoea, amenorrhoea, unexpected lactation and loss of libido in women and erectile dysfunction and loss of libido in men. Serum prolactin deficiency could result in hypoprolactinemia, which is associated with ovarian dysfunction in women, and arteriogenic erectile dysfunction, premature ejaculation oligozoospermia, asthenospermia, hypofunction of seminal vesicles and hypoandrogenism in men[9-10].

PRINCIPLE OF THE TEST

The PRL assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-PRL monoclonal antibody, ABEI labeled with another monoclonal antibody are mixed thoroughly and incubated, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then perform a wash cycle. Subsequently, the Starter 1+2 are added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLUs), which is proportional to the concentration of PRL present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130202006M)	50 tests (REF: 130602006M)
Magnetic Microbeads	Coated with anti-PRL monoclonal antibody, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing bovine serum and PRL antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing bovine serum and PRL antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
ABEI Label	Anti-PRL monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	10.5 mL	7.0 mL
Diluent	0.9% NaCl.	25.0 mL	15.0 mL
Internal Quality Control	Containing bovine serum and PRL antigen, NaN_3 (<0.1%).	2.0 mL	2.0 mL
All reagents are provided ready-to-use.			

Accessories Required But Not Provided

MAGLUMI and Biolumi Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299027M
Wash Concentrate	REF: 130299005M
Light Check	REF: 130299006M
Reaction Cup	REF: 130105000101

Please order accessories from Shenzhen New Industries Biomedical Engineering Co., Ltd. (SNIBE) or our authorized representative.

CALIBRATION

Traceability: This method has been standardized against WHO 3rd International Standard 84/500.
Test of assay specific calibrators allows the RLU values to adjust the assigned master curve. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve(10 calibrations) provided via the reagent Radio Frequency Identification (RFID) CHIP.
Recalibration is recommended if any of the following conditions occurs:

- After each change of lots (Reagent or Starter 1+2).
- Every 4 weeks and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If control results lie outside the expected range.

QUALITY CONTROL

Follow government regulations or accreditation requirements for quality control frequency.
Internal quality control is only applicable with MAGLUMI and Biolumi systems. For instructions for use and target value refer to ***PRL (CLIA) Quality Control Information***. User needs to judge results with their own standards and knowledge.
For detailed information about entering quality control values, refer to the corresponding Analyzer Operating Instructions.
To monitor system performance and chart trends, commercially available quality control materials are required. Treat all quality control samples the same as patient samples. A satisfactory level of performance is achieved when analyte values obtained are within the acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme. If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- If necessary, contact your local technical supporters or distributors for assistance.

SPECIMEN COLLECTION AND PREPARATION

- Use standard sampling tubes or tubes containing separating gel. Collect blood aseptically following the universal precautions for venipuncture.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time.
- If the specimen is centrifuged before a complete clotting, the presence of fibrin may cause erroneous results. Samples must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate substance or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeating freeze-thaw cycles. The serum sample can be only frozen and thawed one time. Specimens must be mixed thoroughly after thawing.
- Centrifuged specimens with a lipid layer on the top must be transferred to a sample cup or a secondary tube. Care should be taken to transfer only the clarified specimen without the lipemic material.
- All samples (patient specimens and controls) should be tested within 3 hours when placed on board the MAGLUMI and Biolumi Systems. Refer to the SNIBE service for more details of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 48 hours at 2-8°C. Freeze samples at or below -20°C if the sample is not assayed within 48 hours.
- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens should be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens should be shipped frozen.
- The sample volume required for a single determination of PRL is 30 µL.

WARNING AND PRECAUTIONS FOR USERS

- **IVD**
- For *In Vitro* Diagnostic Use.
- Follow the package insert carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

- **CAUTION**: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the 29 CFR 1910.1030 Occupational exposure to bloodborne pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- All samples, biological reagents and materials used in the assay should be considered potentially able to transmit infectious agents. They should therefore be disposed in accordance with the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.
- This product contains Sodium Azide. Dispose of contents and containers must be in accordance with all local, regional and national regulations.
- Refer to safety data sheets which are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not interchange reagent components from different reagents or lots.
- Prior to loading the reagent kit on the system for the first time, the reagent kit requires mixing to re-suspend magnetic microbeads that have settled during shipment.
- For magnetic microbeads mixing instructions, refer to the Preparation of the Reagent section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and samples.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

STORAGE AND STABILITY

- Sealed: Stored at 2-8°C until the expiration date.
- Opened at 2-8°C: Minimum stability is 4 weeks.
- On-board: Minimum stability is 4 weeks.
- To ensure the best kit performance, it is recommended to place opened kits in the refrigerator after the end of the intraday test work. It is still possible to keep on using the kit beyond the opened or on-board period if the controls are found within the expected ranges.
- Keep upright for storage to facilitate later proper resuspension of magnetic microbeads.
- Keep away from sunlight.

TEST PROCEDURE

Preparation of the Reagent

- Resuspension of the magnetic microbeads takes place automatically when the kit is loaded successfully, ensuring the magnetic microbeads are totally resuspended homogenous prior to use.
- To ensure proper test performance, strictly adhere to the corresponding Analyzer Operating Instructions. Each test parameter is identified via a RFID CHIP on the Reagent kit. For further information please refer to the corresponding Analyzer Operating Instructions.

DILUTION

Samples with concentrations above the measuring range can be diluted.
After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the analyzer software automatically takes the dilution into account when calculating the sample concentration.
The automatic sample dilution is available after dilution settings are done in the MAGLUMI and Biolumi series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the corresponding Analyzer Operating Instructions.

High-Dose Hook

For the PRL assay, no high dose hook effect was observed when samples containing PRL up to 100,000 µIU/mL.

LIMITATIONS

- Whenever prolactin is determined, it should always be taken into consideration that women's reactions to interfering factors are much more pronounced:
  Physical stress (especially surgery or gynaecological examinations).
  Psychological stress.
  Drugs (e.g. TRH, estrogens, dopamine, partly insulin).
  Diet (influence of essential amino acids).
  Breast stimulation (e.g. irritation from infant sucking).
- Falsely elevated prolactin values may be found in the presence of macro prolactinaemia (syn: pseudo hyperprolactinaemia). It is reported that macroprolactin, which may present in the serum of female patients with various endocrinological diseases or during pregnancy, may interfere in immunoassays, and result in falsely elevated prolactin level, therefore additional tests or information should be required for accurate diagnosis[11-13].
- Elevated serum prolactin alone cannot be taken as evidence for the presence of a pituitary tumor but may only be interpreted in context with the clinical picture and other diagnostic procedures.
- Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

RESULTS

Calculation of Results

The analyzer automatically calculates the PRL concentration of each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are reported in the unit of µIU/mL. For further information please refer to the corresponding Analyzer Operating Instructions.

Interpretation of Results

The expected ranges for the PRL assay were obtained by testing 330 apparently healthy individuals (115 males and 215 females) in China, and gave the following expected values:
Males: 54-340 µIU/mL (2.5^{th}-97.5^{th} percentiles).
Females:

Phase	N	2.5^{th}-97.5^{th} percentiles (µIU/mL)
Normal	115	66-490
Postmenopausal	100	62-410

Results may differ between laboratories due to variations in population and test method. It is recommended that each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision

Precision for the PRL assay was determined as described in the CLSI EP5-A2. 3 human serum pools and 2 controls containing different concentration of analyte were assayed in duplicate at two independent runs per day for 20 testing days. The results are summarized in the following table:

Sample	Mean (µIU /mL) (N=80)	Within-Run		Between-Run		Total	
		SD(µIU /mL)	%CV	SD(µIU /mL)	%CV	SD(µIU /mL)	%CV
Serum Pool 1	62.514	4.309	6.89	1.295	2.07	4.624	7.40
Serum Pool 2	408.868	20.179	4.94	2.585	0.63	20.344	4.98
Serum Pool 3	2904.280	63.466	2.19	18.405	0.63	66.081	2.28
Control 1	349.347	14.804	4.24	7.957	2.28	17.743	5.08
Control 2	1802.157	44.626	2.48	6.552	0.36	53.980	3.00

Limit of Blank (LoB)

The LoB for the PRL assay is 5 µIU/mL.

Limit of Detection (LoD)

The LoD for the PRL assay is 7.5 µIU/mL.

Measuring Range

5-5000 µIU /mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <5 µIU/mL. Values above the measuring range are reported as >5000 µIU/mL.

Linearity

The assay is linear between 7.5 µIU/mL and 5000 µIU/mL based on a study performed with guidance from CLSI EP6-A. Nine equally distributed levels of samples were prepared by blending a serum sample containing PRL 5400 µIU/mL with a serum sample depleted of PRL (0.0 µIU/mL). The mean sample recovery ranged from 90% to 110%.

Method Comparison
A total of 120 samples in the range of 6.791 and 4802.048 µIU/mL were tested by the PRL assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.014x-8.8209, r^2=0.9884

Analytical Specificity
The specificity of the assay was obtained by adding LH (5000 mIU/mL), hGH (1000 ng/mL), hCG (100 IU/mL), hPL (10 µg/mL), TSH (200 mIU/mL), and FSH (1000 mIU/mL) to serum samples at the indicated concentrations. No interference was found.

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 30 mg/dL
- Hemoglobin 1500 mg/dL
- Triglyceride 1500 mg/dL

REFERENCES

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SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
2°C 8°C	Temperature limit (Store at 2-8 °C)		Use-by date
Σ	Contains sufficient for		Keep away from sunlight
	This way up	EC REP	Authorized representative in the European Community
IVD	*In vitro* diagnostic medical device	CONTENTS	Kit components
REF	Catalogue number	LOT	Batch code