Snibe Diagnostic

CE REF 130202009M: 100 tests
130602009M: 50 tests

MAGLUMI® PRG (CLIA)

INTENDED USE
The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of progesterone (PRG) in human serum using 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
Progesterone is an endogenous steroid and progestogen sex hormone involved in the menstrual cycle, pregnancy, and embryogenesis of humans and other species. It belongs to a group of steroid hormones called the progestogens, and is the major progestogen in the body. Progesterone is also a crucial metabolic intermediate in the production of other endogenous steroids, including the sex hormones and the corticosteroids, and plays an important role in brain function as a neurosteroid[1-2].
Progesterone is the most important progestogen in the body, the result of its action as a potent agonist of the nuclear progesterone receptor (nPR)[1]. In addition, progesterone is an agonist of the more recently discovered membrane progesterone receptors (mPRs), as well as a ligand of the PGRMC1 (progesterone receptor membrane component 1; formerly known as the σ_2 receptor). Moreover, progesterone is also known to be an antagonist of the σ_1 receptor, a negative allosteric modulator of the nACh receptors, and a potent antagonist of the mineralocorticoid receptor (MR)[2-6]. Progesterone prevents MR activation by binding to this receptor with an affinity exceeding even those of aldosterone and glucocorticoids such as cortisol and corticosterone, and produces antimineralocorticoid effects, such as natriuresis, at physiological concentrations. In addition, progesterone binds to and behaves as a partial agonist of the glucocorticoid receptor (GR), albeit with very low potency[6-9].
In terms of hormone interactions, progesterone has a number of physiological effects that are amplified in the presence of estrogens. Estrogens through estrogen receptors (ERs) induce or upregulate the expression of the PR[10]. Elevated levels of progesterone potently reduce the sodium-retaining activity of aldosterone, resulting in natriuresis and a reduction in extracellular fluid volume. Progesterone withdrawal, on the other hand, is associated with a temporary increase in sodium retention (reduced natriuresis, with an increase in extracellular fluid volume) due to the compensatory increase in aldosterone production, which combats the blockade of the mineralocorticoid receptor by the previously elevated level of progesterone[11]. As for the function in reproductive system, progesterone has key effects via non-genomic signalling on human sperm as they migrate through the female tract before fertilization occurs, though the receptor(s) as yet remain unidentified[12]. In addition, progesterone also has biological effects in other respects such as breast development, sexuality, nervous system, aging, brain damage and so on.

PRINCIPLE OF THE TEST
The PRG assay is a competitive chemiluminescence immunoassay.
The sample (or calibrator/control, if applicable), buffer, magnetic microbeads coated with PRG antigen, ABEI labeled with anti-PRG monoclonal antibody are mixed thoroughly and incubated. PRG present in the serum sample (or calibrator/control, if applicable) competes with PRG antigen immobilized on the magnetic microbeads for a limited number of binding sites on the ABEI-labeled anti-PRG antibody. 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 inversely proportional to the concentration of PRG present in the sample (or calibrator/control, if applicable).

KIT COMPONENTS
Material Provided

Components	Contents	100 tests (REF: 130202009M)	50 tests (REF: 130602009M)
Magnetic Microbeads	Magnetic microbeads coated with PRG antigen, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing BSA and PRG antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator High	Containing BSA and PRG antigen, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 mL
ABEI Label	Anti-PRG monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	6.5 mL	4.0 mL
Internal Quality Control	Containing BSA and PRG 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 representatives.

CALIBRATION
Traceability: This method has been standardized against the USP Progesterone Reference Material.
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 2 weeks and/or each time a new reagent kit is used (recommended).
- After instrument service is required.
- If controls 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 ***PRG (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 24 hours at 2-8°C. Freeze samples at or below -20°C if the sample is not assayed within 24 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 progesterone is 40 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

Sample dilution by analyzer is not available in this reagent kit.
Samples with concentrations above the measuring range can be diluted manually. After manual dilution, multiply the result by the dilution factor. Please choose applicable diluents or ask SNIBE for advice before manual dilution.

LIMITATIONS

- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with the patient's clinical picture and other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgments.
- Any therapeutical decision should also be taken on a case-by-case basis.
- 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 PRG concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in ng/mL. For further information, please refer to the corresponding Analyzer Operating Instructions.

Conversion factor: ng/mL×3.18=nmol/L.

Interpretation of Results

The expected ranges for the PRG assay were obtained by testing 49 males,158 non-pregnant females and 167 pregnant females from healthy individuals in China, and gave the following expected values:

Males:

N	Median (ng/mL)	2.5^{th}-97.5^{th} percentiles (ng/mL)
49	0.57	0.23-1.50

Non-pregnant females:

Phase	N	Median (ng/mL)	2.5^{th}-97.5^{th} percentiles (ng/mL)
Follicular phase	46	0.53	0.36-1.21
Ovulation phase	42	1.24	0.39-22.87
Luteal phase	44	12.86	2.12-26.44
Postmenopausal	26	0.36	0-0.89

Pregnant females:

Week	N	Median (ng/mL)	2.5^{th}-97.5^{th} percentiles (ng/mL)
0-12	63	24.23	1.17-49.9
13-28	72	37.77	15.4-68.9
29-40	32		59.8->80

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

PERFORMANCE CHARACTERISTICS

Precision

Precision for the PRG 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(ng/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(ng/mL)	%CV	SD(ng/mL)	%CV	SD(ng/mL)	%CV
Serum Pool 1	0.416	0.018	4.33	0.030	7.21	0.035	8.41
Serum Pool 2	30.995	1.315	4.24	1.133	3.66	1.736	5.60
Serum Pool 3	70.789	1.427	2.02	1.295	1.83	1.927	2.72
Control 1	22.884	1.114	4.87	0.570	2.49	1.251	5.47
Control 2	48.540	1.824	3.76	1.297	2.67	2.238	4.61

Limit of Blank (LoB)

The LoB for the PRG assay is 0.1 ng/mL.

Limit of Detection (LoD)

The LoD for the PRG assay is 0.2 ng/mL.

Measuring Range

0.1-80 ng/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <0.1 ng/mL. Values above the measuring range are reported as >80 ng/mL.

Recovery

Known concentrations of PRG were added to normal human serum samples. The concentration of PRG was determined using the PRG assay, and the resulting percent recovery was calculated. The recovery should be within 90% -110%.

Sample	Amount Added (ng/mL)	Observed (ng/mL)	%Recovery
S1	-	0.448	
	0.67	1.119	100.15
	14.89	15.100	98.40
S2	-	22.214	
	0.67	22.917	104.95
	14.89	36.240	94.20
S3	-	56.798	
	0.67	57.464	99.35
	14.89	71.115	96.15

Method Comparison

A total of 100 samples in the range of 0.53 to 79.84 ng/mL were tested using the PRG assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: $y=1.013x-0.613$, $r^2= 0.979$.

Analytical Specificity

The specificity data of the assay was obtained by adding these substances to serum samples at the indicated concentrations. No interference was

found when the cross reactant up to the concentrations listed in the table below.

Cross Reactant	Cross Reactant Concentration (ng/mL)
Testosterone	1000
Aldosterone	1000
Cortisol	1000
DHEA-S	100000
Estriol	400
Estradiol	1000

Endogenous Interference
Substances up to the following concentrations did not interfere with the assay:
- Bilirubin 54 mg/dL
- Hemoglobin 1000 mg/dL
- Triglyceride 720 mg/dL

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Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax: +86-755-28292740

EC REP **Shanghai International Holding Corp. GmbH (Europe)**
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726

SYMBOLS EXPLANATIONS

	Consult instructions for use		Manufacturer
8°C 2°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