Snibe Diagnostic

CE

REF 130201023M: 100 tests
130601023M: 50 tests

MAGLUMI™ ProGRP (CLIA)

INTENDED USE

The kit is an *in vitro* chemiluminescence immunoassay for the quantitative determination of ProGRP in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer (including Maglumi 600, Maglumi 800, Maglumi 1000, Maglumi 2000, Maglumi 2000 Plus, Maglumi 4000, Maglumi 4000 Plus and MAGLUMI X8).

SUMMARY AND EXPLANATION OF THE TEST

Gastrin-releasing peptide (GRP) is an important regulatory molecule that is implicated in a number of physiological and pathophysiological processes in humans, which was discovered as an analogue of amphibian bombesin, originally isolated from non-sinus gastric epithelium of porcine in 1978 by McDonald and widely distributed throughout the normal human brain, gastrointestinal nerve fibers and fetal lung neuroendocrine tissue[1,2]. The encoding product of Human GRP gene is a 148 amino acid Gastrin-releasing peptide precursor (preproGRP), composed of signal peptide, GRP(1-27) and C-terminal of GRP(31-125). Followed by preproGRP conversion to Pro-GRP(1-125), production of mature GRP(1-27), GRP(18-27) and C-terminal extension peptides by endogenous proteolysis and amidation, and the C-terminal extension peptide continues to produce a C-terminal ProGRP molecule[3,4,5]. The study found that tumor cells in patients with small cell lung cancer (SCLC) can synthesize and release GRP. GRP is involved in tumor growth and metastasis through autocrine or intercellular interaction, so the detection of GRP can reflect the occurrence of SCLC. Due to its short half-life of 2 minutes it is not possible to measure GRP in blood. Studies have confirmed that SCLC patients with tumor cells produced GRP and ProGRP was positively correlated, therefore, the detection of ProGRP in serum is a general method.

ProGRP is one of several molecules (such as neuron-specific enolase (NSE)), which are associated with neuroendocrine derived tissues and tumors. The increased levels of serum ProGRP have been reported in several neuroendocrine derived tumor types, including small cell lung cancer, carcinoids, undifferentiated large cell carcinomas of the lung with neuroendocrine features, medullary thyroid carcinoma, other neuroendocrine malignancies, and in a subset of androgen-independent prostate cancer with neuroendocrine features[6,7]. ProGRP is useful for the differential diagnosis of SCLC and non-small cell lung cancer (NSCLC). ProGRP has been reported as a specific biomarker for SCLC, but abnormal levels may be found in a small subset of NSCLC patients[8]. These concentrations are significantly lower than the ProGRP serum levels found in SCLC patients. The serum concentration of ProGRP is associated with the degree of tumor infiltration, and the possibility of SCLC is as high as 93% when the serum concentration of ProGRP was greater than 150 pg / mL. Using a cutoff of 150 pg/mL as one of the criteria, ProGRP predicted a diagnosis of SCLC with a sensitivity of 72.5 %[9,10].

Several investigators have reported that ProGRP is helpful in therapy monitoring of SCLC patients and for the detection of recurrent disease[11]. NSE can be a complementary biomarker in SCLC and combining NSE and ProGRP results in enhanced precision in the histological diagnosis, prognosis, and follow-up[12,13].

The use of ProGRP assay values is an aid in monitoring progressive disease or therapy in patients with SCLC, can not be used as a basis for early diagnosis or a definite diagnosis of malignant tumors, should not be used for the general population of cancer screening.

PRINCIPLE OF THE TEST

The ProGRP assay is a sandwich chemiluminescence immunoassay.

The sample (or calibrator/control, if applicable), magnetic microbeads coated with anti-ProGRP monoclonal antibody, and buffer are mixed thoroughly and incubated, the ProGRP antigen present in the sample binds to the anti-ProGRP coated magnetic microbeads. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. After that, ABEI labeled with another anti-ProGRP monoclonal antibody is added and incubated, forming sandwich of immuno-complexes. After precipitation in a magnetic field, the supernatant is decanted and then a another wash cycle is performed. 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 ProGRP present in the sample (or calibrator/control, if applicable) .

KIT COMPONENTS

Material Provided

Components	Contents	100 tests (REF: 130201023M)	50 tests (REF: 130601023M)
Magnetic Microbeads	Magnetic Microbeads coated with anti-ProGRP, containing BSA, NaN_3 (<0.1%).	2.5 mL	2.0 mL
Calibrator Low	Containing ProGRP antigen (recombinant) and BSA, NaN_3 (<0.1%).	2.0 mL	1.0 mL
Calibrator High	Containing ProGRP antigen (recombinant) and BSA, NaN_3 (<0.1%).	2.0 mL	1.0 mL
Buffer	Containing BSA, NaN_3 (<0.1%).	8.5 mL	5.5 mL
ABEI Label	Anti-ProGRP monoclonal antibody labeled with ABEI, containing BSA, NaN_3 (<0.1%).	23.5 mL	13.0 mL
Diluent	0.9%NaCl.	15.0 mL	10.0 mL
Control 1	Containing ProGRP antigen (recombinant) and BSA, NaN_3 (<0.1%).	2.0 mL	1.0 mL
Control 2	Containing ProGRP antigen (recombinant) and BSA, NaN_3 (<0.1%).	2.0 mL	1.0 mL

Accessories Required But Not Provided

MAGLUMI Series:

Reaction Module	REF: 630003
Starter 1+2	REF: 130299004M, 130299012M, 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 SNIBE internal reference substance.

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 exchange of lots (Reagent or Starter 1+2).
- Every week 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 system. For instructions for use and target value refer to ***ProGRP (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 operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.
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

- Serum collected using standard sampling tubes or tubes containing separating gel. For plasma specimens, the anticoagulant EDTA-2K has been verified and could be used with the assay. Heparin plasma is not suitable for this assay. Collect blood aseptically followed the universal precautions for venipuncture.
- Ensure that complete clot formation in specimens have taken place prior to centrifugation. Some serum 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. Specimens must be free of fibrin and other particulate matter.
- Do not use hemolyzed or grossly lipemic specimens as well as specimens containing particulate matter or exhibiting obvious microbial contamination. Inspect all specimens for bubbles, and remove bubbles before analysis for optimal results.
- Avoid repeated freezing and thawing. The specimens can be frozen and thawed for only two times. Stored specimens should be thoroughly mixed prior to use (Vortex mixer). Frozen specimens must be mixed THOROUGHLY after thawing by LOW speed vortexing.
- 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 2 hours when being placed on board the MAGLUMI System. Refer to the SNIBE service for more detailed discussion of onboard sample storage constraints.
- Specimens removed from the separator, cells or clot may be stored up to 72 hours at 2-8°C, and stored up to 12 weeks frozen at -20°C or colder.
- Before shipping specimens, it is recommended that specimens be removed from the 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 ProGRP is 100 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 should 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 of 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 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 6 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 operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer. Each test parameter is identified via a RFID CHIP on the Reagent. For further information please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

DILUTION

Samples with ProGRP concentrations above the measuring range may be diluted automatically by analyzers or manually. The recommended dilution is 1:9.
After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the 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 series Fully-auto chemiluminescence immunoassay analyzer user software. Please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

High-Dose Hook

For the ProGRP assay, no high dose hook effect was observed when samples containing ProGRP up to 200,000 pg/mL.

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 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 judgement.
- Any therapeutical decision should also be taken on a case-by-case basis state.
- 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 ProGRP 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 pg/mL. For further information please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Interpretation of Results

The expected range for the ProGRP assay was obtained by testing 256 healthy individuals in China, and gave the following expect value:≤ 69.2 pg/mL(95th percentile).
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 ProGRP 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 result is summarized in the following table:

Sample	Mean(pg/mL) (N=80)	Within-Run		Between-Run		Total	
		SD(pg/mL)	%CV	SD(pg/mL)	%CV	SD(pg/mL)	%CV
Serum Pool 1	20.4	1.11	5.43	0.70	3.44	1.32	6.43
Serum Pool 2	204	8.9	4.37	5.56	2.72	10.5	5.15
Serum Pool 3	2054	32.1	1.56	76.1	3.70	82.6	4.02
Control 1	71.2	3.96	5.57	0.66	0.93	4.02	5.64
Control 2	406	16.5	4.06	3.45	0.85	16.9	4.15

Limit of Blank (LoB)

The LoB for ProGRP assay is 2.00 pg/mL.

Limit of Detection (LoD)

The LoD for ProGRP assay is 3.00 pg/mL.

Limit of Quantitation (LoQ)

It is defined as the concentration of ProGRP that can be measured with an inter assay CV of 20%. The LoQ for ProGRP assay is 7.00 pg/mL.

Measuring Range

2.00-5000 pg/mL (defined by the limit of blank and the maximum of the master curve). Values below the limit of blank are reported as <2.00 pg/mL. Values above the measuring range are reported as >5000 pg/mL.

Linearity

The assay is linear between 3.00 pg/mL and 5000 pg/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 ProGRP 5500 pg/mL with a serum sample containing ProGRP 3.00 pg/mL. The mean sample recovery ranged between 90% to 110%.

Method Comparison

A total of 131 samples in the range of 11.498 to 4804.721 pg/mL were tested by ProGRP assay (y) and a commercially available immunoassay (x). The data from the resulting linear regressions are summarized as: y=1.0054x+0.6316, r^2=0.9989.

Analytical Specificity

The substances up to the following concentrations did not interfere with the assay:

Compound	Concentrations
GRP	100 ng/mL

Drugs Interference

The drugs up to the following concentrations did not interfere with the assay:

Compound	Concentrations

Compound	Concentrations
Carboplatin	500 µg/mL
Cisplatin	165 µg/mL
Cyclophosphamide	500 µg/mL
Doxorubicin	1.16 µg/mL
Methotrexat	45 µg/mL
Bleomycin	100 µg/mL
Cytarabine	30 µg/mL
Tamoxifen	60 µg/mL
MitomycinC	75 µg/mL
Vinblastine	1.5 µg/mL
Paclitaxel	3.5 ng/mL
Fluorouracil	500 µg/mL

Endogenous Interference

Substances up to the following concentrations did not interfere with the assay:

- Bilirubin 40 mg/dL
- Hemoglobin 2000 mg/dL
- Triglyceride 1000 mg/dL
- ANA 5 (S/CO)
- RF 1500 IU/mL
- HAMA 40 ng/mL

Note: ANA concentration is measured with ANA screen test kit (ELISA) from EUROIMMUN.

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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