# Instruction for Use

**【Product Name】**

Pro-Gastrin-Releasing Peptide (eCLIA)

**【Order Information】**

| REF NO. | Package Size |
|---|---|
| 0320501301 | 50T |
| 0320501302 | 2×50T |
| 0320501303 | 100T |
| 0320501304 | 2×100T |

**【Intended Use】**

Pro-Gastrin-Releasing Peptide (eCLIA) is an immunoassay reagent for in vitro quantitative determination of pro-Gastrin-Releasing Peptide in human serum and plasma by fully automated immunassay analyzer to aid clinical monitoring of small cell lung cancer (SCLC).

Gastrin-releasing peptide (GRP) is a functional analogue of amphibian cerulein in mammals and widely distributed in the gastrointestinal tract, nervous system and pulmonary respiratory tract of mammals.[1] Lung cancer cells themselves can also produce and secrete GRP, which promotes the growth of tumor cells in an autocrine or diffuse manner. But GRP is unstable, with a half-life of only two minutes, it is difficult to detect it clinically.[2] pro-Gastrin-Releasing Peptide (ProGRP) and GRP are produced by further processing of preproprotein after cleaving of signal peptide. It is known that there are three kinds of molecular structures in proGRP with common C-terminal sequences (31-98).[3] proGRP (31-98) is in stable expression in the blood. A lot of studies show that the level of proGRP (31-98) can represent GRP level and is a tumor-marker in the detection of small cell lung cancer.[4]

proGRP is a molecule related to neuroendocrine-derived tissues and tumor. The serum level of proGRP is correlated with the staging of tumor.[5] proGRP is the most sensitive marker in distinguishing benign lung diseases from SCLC. proGRP has high specificity and sensitivity in the diagnosis of SCLC. Studies show that a combination of proGRP and NSE can further improve the sensitivity in the diagnosis of SCLS and strengthen histological diagnosis and prognosis diagnosis.[6] proGRP is of important value in monitoring the diseases, demonstrating the curative effect and detecting the recurrence of diseases.[7] The proGRP level in blood is not affected by general diseases, but the patients with renal insufficiency will have the false positive elevation of proGRP.[8] Therefore, for clinical use of proGRP for diagnosis guidance, it is necessary to pay attention to whether there is any renal dysfunction in patients. The proGRP level is elevated in the early stage of SCLC, but the incidence of SCLC is low in the general population, therefore, it is not recommended to take proGRP as a screening item in the general population.

**【Principles of the testing method】**

Sandwich principle. Total duration of assay: 9 minutes.

30 μL of sample, biotinylated proGRP monoclonal antibody and the ruthenium (Ru) complex-labeled proGRP monoclonal antibody form an antigen-antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away. Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the concentration of proGRP in the sample is calculated based on the calibration curve.

**【Main Components】**

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional), and different components and reagents of lot numbers should not be used interchangeably.

| Components | Ingredients | Volume (2×50T) | Volume (50T) | Volume (100T) | Volume (2×100T) |
|---|---|---|---|---|---|
| (MB) | Streptavidin coated magnetic particles, 0.45 mg/mL, 0.1M PBS, ProClin300 | 2×1.8mL | 1×1.8mL | 1×3.5mL | 2×3.5mL |
| (RB) | Biotinylated proGRP antibody, 0.8 mg/L, 0.05M PBS, ProClin300 | 2×3.0mL | 1×3.0mL | 1×6.0mL | 2×6.0mL |
| (RA) | Ru complex-labeled proGRP antibody, 1.0 mg/L, 0.05M PBS, ProClin300 | 2×3.0mL | 1×3.0mL | 1×6.0mL | 2×6.0mL |
| Calibrators | proGRP antigen, 0.05M Tris buffer, ProClin300 | High: 1×1.0 mL<br>Low: 1×1.0 mL | | | |
| Control Materials (Optional) | proGRP antigen, 0.05M Tris buffer, ProClin300 | High: 1×1.0 mL<br>Low: 1×1.0 mL | | | |

Traceability:The assignment process of supporting calibrators in this pack can be traced to Roche Elecsys ProGRP.

See the control material value sheet for the target values and ranges of control materials.

**【Instruments and Materials Required but Not Provided】**

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- REF 679009, Concentrated Washing Buffer, 1 L, or REF 679008, Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, o REF 0320510002, Assay Cup/Assay Tip, 1×6×105

**【Applicable Instrument】**

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

**【Storage and Shelf Life】**

Store at 2ºC~8°C.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

| *Stability of the reagents* | |
|---|---|
| Unopened store at 2ºC~8ºC | 18 months |
| Store at 2ºC~8ºC after opening | 8 weeks |
| on the analyzers at 2ºC~15ºC | 8 weeks |

The Calibrators and Control Materials are stable up to the stated expiration date.

| *Stability of the Calibrators and Control Materials* | |
|---|---|
| Unopened store at 2ºC~8ºC | 18 months |
| Store at 2ºC~8ºC after opening | 8 weeks |

**【Specimen collection, preparation and storage】**

It is recommended to use human serum samples or plasma samples collected with EDTA-$K_2$, EDTA-$K_3$, heparin lithium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Serum Samples can be stored for 9 hours at 18~28°C, 12 hours at 2~8°C, and 12 weeks at -15°C or below. Freezing and thawing can be performed twice.

Plasma samples can be stored for 9 hours at room temperature (18~28°C), 72 hours at 2~8°C, and 12 weeks at -15°C or below. Freezing and thawing can be performed twice.

**【Testing Procedure】**

**Testing procedure and precautions**

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. proGRP testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30°C, relative humidity: ≤80%.

**Calibration**

Calibration should be performed using lot-matching reagent and calibrators.

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended as follows :

(1) When using a new lot of reagent;
(2) When the same lot of reagents has been used on the analyzer for more than 28 days;
(3) If the quality control result exceeds the defined limit;
(4) When changing the buffers of different lot numbers.

**Quality Control procedure**

For quality control, use proGRP control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined

limits.
If necessary, repeat the measurement of the samples concerned.
Follow the applicable government regulations and local guidelines for quality control.
Please make sure that the correct values are used.

**Calculation**

The system software can automatically calculate the analyte concentration, with the result in pg/mL.

**Specimen dilution**

proGRP concentration in the sample higher than the upper limit of detection can be diluted manually with sample diluent.
The recommended dilution ratio is 1:10. The results are reported by multiplying the measured values by the dilution ratio.

**【Biological Reference Interval】**

By analyzing the serum samples of 251 healthy test subjects from hospital, it was calculated that the upper limit of the reference interval of 95$^{th}$ percentile is 65.69 pg/mL.
Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

**【Interpretation of results】**

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

**【Limitations】**

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.
The detection range of the kit is 2.0 pg/mL～5000 pg/mL. If the proGRP concentration in the sample is lower than the lower limit of detection, the result is reported as less than this value (for example, <2 pg/mL), if the proGRP concentration in the sample is higher than the upper limit of detection, the result is reported as greater than this value (for example, >5000 pg/mL), or the sample is manually diluted with sample diluent at the dilution ratio of 1:10, and the result is reported with the measured value multiplied by the dilution ratio.
When jaundice (bilirubin) is ≤70 mg/dL, hemolysis (hemoglobin) is ≤250 mg/dL, lipemia (triglyceride) is ≤2500 mg/dL, biotin is ≤20 ng/mL, total protein is ≤12 g/dL, and HAMA is ≤200 ng/mL in the sample, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample is less than or equal to 540 IU/mL, the relative recovery of the test results is between 90% and 110%.
Kinds of drugs are added to the serum samples containing proGRP to make interference samples, and the concentrations of drugs added are as follows: acetaminophen (200 μg/mL), acetylsalicylic acid (1000 μg/mL), ascorbic acid (300 μg/mL), cyclosporin (5 μg/mL), ibuprofen (500 μg/mL), methyldopa (20 μg/mL), metronidazole (200 μg/mL), butazodine (400 μg/mL), theophylline (100 μg/mL), carboplatin (600 μg/mL), cisplatin (180 μg/mL), cyclophosphamide (500 μg/mL), dexamethasone (20 μg/mL), docetaxel (112.5 μg/mL), adriamycin (120 μg/mL), erlotinib (150 μg/mL), etoposide (300 μg/mL), gefitinib (250 μg/mL), gemcitabine (1500 μg/mL), ifosfamide (7200 μg/mL), amethopterin (150 mg/L), metoclopramide (7.5 μg/mL), paclitaxel (330 μg/mL ) and vinorelbine tartrate (53.1 μg/mL), The proGRP contents in the interference sample and the control sample (without drug addition) are measured respectively, and the relative deviation of the measured results is within ±10%.
When the proGRP concentration reaches 100000 pg/mL, there is no high-dose Hook effect.

**【Analytical Performance】**

**Lower limit of measurement**

Limit of Blank = 2 pg/mL.
Limit of Detection = 3 pg/mL.

**Accuracy**

The accuracy control subject to standardized traceability is tested, and the relative deviation of its test results is within ±10%.

**Linearity**

Within the range of 3 pg/mL~5000 pg/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

**Within-run precision (repeatability)**

The coefficient of variation (CV) ≤ 8%.

**Between-lot precision**

The coefficient of variation (CV) ≤10%.

**Measured values of Control Material**

The measured results of supporting assigned Control Material of the kit should be within the Control Material range.

**Homogeneity of calibrator and Control Material**

Within-bottle homogeneity: Coefficient of variation (CV) ≤ 8%,
Between-bottle homogeneity: Coefficient of variation (CV) ≤ 10%.

**Analytical specificity**

When testing specific samples containing 100000 pg/mL gastrin-releasing peptide (GRP), the cross reaction rate was ≤1%.

**Method comparison**

A comparison of the Lifotronic Pro GRP assay (y) with the Roche Elecsys pro GRP method (x) using clinical samples gave the following correlations:
Number of plasma samples measured: 107
Linear regression:
Y=1.0555X+5.3295
The sample concentrations were between 5.15 and 4819 pg/mL.

**【Precaution and Warning】**

The kit is only used for in vitro diagnostics.
Exercise the normal precautions required for handling all laboratory reagents.
All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.
Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/ spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant.

**【References】**

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**【Symbols Explanation】**

| Symbol | Title of Symbol | Symbol | Title of Symbol |
|---|---|---|---|
| | Manufacturer | | Consult instructions for use |
| | Use-by date | IVD | In vitro diagnostic medical device |
| LOT | Batch code | Σ | Sufficient for <n> tests |
| SN | Serial number | | Biological risks |
| | Temperature limit | | This way up |
| EC REP | Authorized representative in the European Community | CE | Indicates this device is in compliance with Europe Directive. |
| REF | Catalogue number | P/N | Material code |
| | Warning | | Date of manufacture |
| UDI | Unique device identifier | | |

**【Manufacturer】**

Shenzhen Lifotronic Technology Co., Ltd.
Lifotronic Tower, No. 8, Qiuzhi East Road, Guancheng Community, Guanhu Street, Longhua, 518110 Shenzhen, PEOPLE'S REPUBLIC OF CHINA
E-mail: inter-service@lifotronic.com

**【European Representative】**

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

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