Instruction for Use

【Product Name】

Pepsinogen I (Electrochemiluminescence Immunoassay)

【Package】

1×100T, 2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of Pepsinogen I in human serum and plasma. This assay is intended for use in assisting diagnosis and monitoring of atrophic gastritis .

Pepsinogen is an inactive precursor of pepsin in gastric juice. It's immunochemically classified into two types, Pepsinogen I (PG I)， and Pepsinogen II (PG II)[1]. PG II is derived from the pyloric glands in the gastric antrum and from the chief and mucus neck cells of the fundic glands in the gastric body[2], while PG I is derived only from the fundic glands[3]. Study have clarified that serum pepsinogen levels reflect the morphological and functional status of the stomach mucosa and the diagnostic value in various gastroduodenal disorders, especially peptic ulcer and atrophic gastritis, has been widely[4-7].

In atrophic gastritis, The gastric chief cells, producting PG I mainly decrease and number of pyloric glands increase resulting in PG I/PG II ratio is lowered.

Therefore, the ratio of pepsinogen I to pepsinogen II (PGR), in combination with pepsinogen I, is predictive of the histologic status of the gastric mucosa. they can be useful for studies of the genetic, immunologic, epidemiologic, and clinical correlates of chronic gastritis[8].

【Test Principle】

Sandwich principle.Total duration of assay: 18 minutes.

- 1st incubation: a sample, biotinylated monoclonal PGI-specific antibody and monoclonal PGI-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin.
- Measurement: The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Buffer. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via calibration and a master curve provided via the reagent barcode.

【Main Component】

The reagent pack consists of MB, RA, RB, calibrators and control materials, different lots cannot be used at the same time or mixed up together.

Component	Ingredients	Volume (1×100T)	Volume (2×100T)
(MB)	Streptavidin-coated microparticles 0.75 mg/mL: 0.1 M PBS; 0.05% ProClin™ 300	1×5.0 mL	2×5.0 mL
(RA)	Anti-PG I-Ab~Ru(bpy)$_3^{2+}$: 0.05 M MES buffer; ProClin™ 300	1×8.0 mL	2×8.0 mL
(RB)	Anti-PG I-Ab~biotin: 0.05 M MES buffer; ProClin™ 300	1×8.0 mL	2×8.0 mL
Calibrator (High)	Tris buffer, BSA,ProClin™ 300	1×1.0 mL	1×1.0 mL
Calibrator (Low)	Tris buffer, BSA,ProClin™ 300	1×1.0 mL	1×1.0 mL
Control Material (High)	Tris buffer, BSA,ProClin™ 300	1×1.0 mL	1×1.0 mL
Control Material (Low)	Tris buffer, BSA,ProClin™ 300	1×1.0 mL	1×1.0 mL

ProClin™ is a trademark of the Dow Chemical Company or an affiliated company of Dow.

The assignment of calibrators value complies strictly with ISO 17511:2003, and this method can be traced back to Abbott ARCHITECT Pepsinogen I.

See the quality control card for the target value and permissible range of quality control.

Materials and instruments required but not provided:

- Auffer
- Buffer
- Concentrated Washing Buffer
- eCL8000 series Automated ECL Immunoassay Analyzer
- Assay Cup

【Storage and Shelf Life】

Unopened reagent rackpack, calibrators and control materials should be placed at 2~8ºC and will be valid until expiration date.

Opened reagents, calibrators and control materials should be used and stored at 2~8ºC in 28 days, otherwise trashed.

The expiration date is labeled on the box, rackpack and bottles.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

Automated ECL Immunoassay Analyzer: eCL8000, eCL8000i, eCL8000p, eCL8000x.

【Specimen collection, handling and storage】

Human serum and plasma added with heparin lithium, heparin sodium and EDTA-K_2, EDTA-K_3, anti-coagulants are recommended. Blood samples should be collected by standard operation of venous puncture; after complete coagulation, the tangible component should be removed by centrifugation. The sample should avoid bubbling during testing. Lipid layer floating on the upper of the sample should be removed. Samples with severe hemolysis are not in suggestion. Samples would better to be tested in 8 hours after collection, otherwise, should be stored 2~8ºC for no more than 7 days or -20ºC, 3 months. Freezing and thawing cycle is permitted only once.

【Assay Procedure】

Testing procedures and precautions

Previous to operation, one should read the operational manual carefully to obtain system operation procedure, sample processing, security precaution, maintenance and other related information.

Set up Pepsinogen I (PG I) test according to the operational manual.

Put the PGI rackpack into the correct analyzer slot to automatically suspend magnetic beads at least 30 min before testing.

The sample volume required for each PG I test is 30 µL.

Calibration

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

Before calibration, the reagent and main curve information should be imported into the analyzer via radio frequency identification (RFID) reagent card (refer to instrument operational manual). The analyzer adjusts the main curve to produce working curve according to the calibrator results, and identifies validity of the working curve automatically.

Recalibration is recommended when:

(1) the reagents of different lot are used;

(2) the same lot of reagents were used on the analyzer beyond 28 days;

(3) quality control misses the target.

Quality control

In order to ensure the reliability of test results, it's recommended to test the control materials every 24 hours. After each calibration, reagent lot change, maintenance or failure repairment, quality control is recommended. The quality control results should fall within the scope of local regulations. If beyond, the analyzer status, reagents, calibration and other factors should be checked.

Calculation

The system software automatically calculates the analyte concentration using particular algorithm, and the result unit is ng/mL .

Specimen dilution

Samples with PG I concentrations above the measuring range can be diluted with PG I negative sample . The recommended dilution ratio is 1:5. After manual dilution, multiply the result by the dilution factor.

【Biological reference interval】

Mike *et al*[9-11] Reporeted, that the combination analysis of PG I level in serum or plasma, and PG I/PG II ratio was useful as an indicator for the degree of atrophy in the fundic gland mucosa. They[12] further reporeted, that less than 70ng/mL for the PGI level and less than 3.0 for the PG I/II ratio as cut-off values gave the hightest detection rate in the diseases with atrophy of fundic gland mucosa. Those cut-off values resulted in 80% specificity with non-atrophy of fundic gland mucosa.

Each laboratory should investigate transferability of the reference range to its local population and if necessary determine its own reference ranges.

【Result Interpretation】

In interpreting the results, the patient's overall clinical situation should be referred to, including symptoms, medical history and other relevant data and information.

【Limitations】

Test results are used only for clinical reference and cannot be used as the basis for diagnosis or rule-out of diseases alone.

The measuring range of the kit is 0.5~250 ng/mL. Values below lower detection limit are reported as <0.5 ng/mL. Values above the measuring range are reported as >250 ng/mL (or up to 250 ng/mL for 5-fold diluted samples).

There is no high-dose hook effect at PG I concentration 1600 ng/mL.

When samples contain the bilirubin concentration of 50 mg/dL or less, lipid concentration of 2000 mg/dL or less, concentration of biotin 25 ng/mL or less, hemoglobin concentration of 200 mg/dL or less, the interference bias

of determination results deviation within ± 10%.

【Analytical Performance】

Lower limit of measurement

The lower detection limit is 0.5 ng/mL.

Accuracy

The relative bias of measuring trueness control materials should fall between 0.90 and 1.10.

Linearity

The correlation coefficient (r) was not less than 0.9900 in the interval of 0.5~ 250 ng/mL.

Within-run precision (repeatability)

The coefficient of variation (CV) is less than 8.0%.

Between-lot precision

The coefficient of variation (CV) is less than 10.0%.

Analytical specificity

Test the following analog-additive blank samples, the results were lower than 0.5 ng/mL.

Analog	Concentration
PG II	500ng/mL

【Method Comparison】

A comparison of Lifotronic PG I assay with Abbott ARCHITECT PG I assay using clinical samples gave the following correlation:

y =1.0072x-1.1681 Pearson's r=0.9856 (concentration range:13.4～242.0 ng/mL)

【Precaution and Warning】

The kit is only used for in vitro diagnostics.

When using the kit, it's necessary to comply with regulations in the laboratory.

All reagents and samples including specimen, calibrators and control materials, should avoid foaming before and during test.

Test results of the kit are for clinical reference only, and clinical evaluation of patients should be combined with their symptoms and signs, medical history, other laboratory examination results and treatment responses.

Due to factors such as methodology or antibody specificity, testing identical samples with reagents from different manufacturers may get different results, and the results from different kits should not compare directly, lest cause the wrong medical explanation; It's suggested that laboratorians should point out the characteristics of used reagent in the test report to the clinician. In serial monitoring, if the reagent type is changed, a continuous parallel test should be performed and comparison of the results to the former to determine new baseline value.

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.

The preservative ProClin™ 300 contains 3% 2-methyl-4-isothiazolin-3-one (MIT) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), will be harmful if inhalation, contact with skin and/or swallow, and may be toxic to aquatic organisms; thus, proper personal protection should be adopt while handling the reagent, and abandoned reagents should be dealed with in compliance with local regulations.

【Symbol】

Symbol	Title of Symbol
	Manufacturer
	Authorized representative in the European Community
	Use by
	Lot number
	Serial number
	Temperature limitation
	Consult instructions for use
	In vitro diagnostic medical device
	Indicates this device is in compliance with Europe Directive.
	Sufficient for <n> tests
	Biological risks
	This way up

【Reference】

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