TiSENC

Pepsinogen I (CLIA)

IVD

[INTENDED USE]

Pepsinogen I (CLIA) is a Chemiluminescence Immunoassay test for the quantitative measurement of circulating pepsinogen I (PG I) in human serum, plasma ,which is used as an aid to evaluate the function of gastric adenocarcinoma cells. The concentration level of PG I and the ratio of PG I/II can also be used to monitor the treatment of gastric cancer.
The test must be performed on the ACCRE series analyzers (including ACCRE 6, ACCRE 8, ACCRE 90, ACCRE 100, ACCRE 120, and all other ACCRE series analyzers).

For *in vitro* diagnostic use only. For professional use only.

[SUMMARY]

PG is an inactive precursor of pepsin. PG can be divided into two subtypes, PG I and PG II, according to biochemical and immunological characteristics. PG I is mainly secreted by the chief cells and cervical mucus cells of the gastric body and fundic glands, while PG II is secreted by the gastric fundic glands, antral pyloric glands and proximal duodenal Brunner glands. PG is a good indicator to reflect the exocrine function of gastric corpus and gastric antrum mucosa. When the gastric mucosa is atrophied, the serum PG I level and/or the PG I/II ratio (PGR) decreases. Therefore, the PG I/II ratio can be used as an indication of gastric fundic mucosa atrophy. Immunological methods are used to detect serum PG I and PG II levels, combined with the PG I/II ratio, can be used to screen for atrophic diseases of the fundic gland mucosa. Among the diseases associated with atrophic diseases of the fundic gland mucosa, particularly atrophic gastritis, is associated with gastric cancer. Therefore, the concentration level of PG I and the ratio of PG I/II can be used for therapeutic monitoring of gastric cancer.

[ASSAY PRINCIPLES]

Pepsinogen I (CLIA) hereinafter also referred to as the "PG I" is a one-step sandwich assay to determine the level of pepsinogen I.
Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent cartridge. All the assay steps are performed automatically by the ACCRE analyzer.
The sample is manually pipetted into the sample well of reagent cartridge, then sample, sample treatment solution, PG I antibody labeled with alkaline phosphatase and PG I antibody coated magnetic particles are mixed thoroughly together, incubating at 37℃. The analyte in the sample, labeled antibody and antibody coated with magnetic particles are bonded together as antibody-antigen sandwich immuno-complex. The immuno-complex is then washed in the magnetic field to eliminate the unbound components in the sample. The substrate is added to trigger the chemiluminescent reaction, the result of chemiluminescent reaction is measured by photomultiplier tube (PMT) as relative light units (RLUs). The concentration of the substance to be detected in the sample is proportional to the luminescence intensity. At the end, the results are automatically calculated by the ACCRE analyzers in relation to the calibration curve stored.

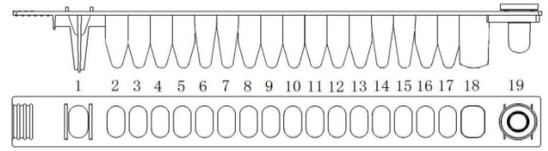
[CONTENT OF THE KIT]

Package: 60 tests/kit with quality controls, 36 tests/kit with quality controls.
Traceability:The calibrators of this kit can be traced to the internal reference substance. For specific calibrator concentration values, see the Calibrator Barcode Sheet for each lot.

Contents	Quantities		Notice
	REF W91A	REF W91C	
PG I reagent cartridge	60 strips	36 strips	Ready to use
PG I Calibrator 1 (CAL 1)	Dissolved to 1.0 mL vial	Dissolved to 1.0 mL vial	Lyophilized powder, PBS buffer; ProClin300; BND, BSA; Pepsinogen I Antigen.
PG I Calibrator 2 (CAL 2)	Dissolved to 1.0 mL vial	Dissolved to 1.0 mL vial	Lyophilized powder, PBS buffer; ProClin300; BND, BSA; Pepsinogen I Antigen.
PG I Quality Control level 1 (QC 1)	Dissolved to 1.0 mL vial	Dissolved to 1.0 mL vial	Lyophilized powder, PBS buffer; ProClin300; BND, BSA; Pepsinogen I Antigen.
PG I Quality Control level 2 (QC 2)	Dissolved to 1.0 mL vial	Dissolved to 1.0 mL vial	Lyophilized powder, PBS buffer; ProClin300; BND, BSA; Pepsinogen I Antigen.
Instruction for Use	1	1	N/A
Reagent Registration Card	1	1	N/A
Calibrator Barcode Sheet	1	1	N/A
Quality Control Sheet	1	1	N/A

Note: Refer to the target value list of the controls from Quality Control Sheet within the kit.

The Reagent Cartridge
The strip consists of 19 wells. The 1st well is the sample well and the last 19th well is the reading well for chemiluminescent signal. From the 2nd to the 18th well are all covered with a labeled, aluminum foil seal. The label comprises a barcode which mainly indicates the reagent assay information, reagent lot number and expiration date. All reagents components needed for the assay are ready-to-use and pre-dispensed in the cartridges. And please do not interchange integral component from different reagents or lots.



Description of the reagent cartridge

Well	Component	Content	Volume
3	Sample Treatment Solution	MES buffer; ProClin300; BND.	100μL
5	Enzyme Label	ALP (alkaline phosphatase) labeled anti-PG Ⅰ antibody, MES Buffer, ProClin300.	50μL
7	Magnetic Particles	Anti- PG Ⅰ antibody coated magnetic particles, MES buffer, ProClin300.	50μL
10, 11, 12, 14	Wash Solutions	Tris buffer, ProClin300, BND.	350μL
16	Substrate Solution	Disodium [(4-chlorophenyl)sulfanyl] (10-methyl-9(10H)-acridinylidene) methyl phosphate.	200μL

Note: Well 1,2,4,6,8,9,13,15,17,18 are empty.

[MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED]

- Sample diluent (manufactured by Tisenc), if needed.
- Pipette with disposable tip to dispense 200 μL.
- Powderless and disposable gloves.
- Disposable tips for ACCRE analyzer
- For other specific materials and disposables, please refer to the Instrument User Manual.
- Instruments of the ACCRE series analyzers.

[WARNINGS AND PRECAUTIONS]

- For *in vitro* diagnostic use only.
- For professional use only by qualified laboratory personnel.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Do not use reagents after the expiration date indicated on the box label.
- Do not use if the packaging of reagent cartridge is damaged or leaked.
- Do not use the reagents if the quantity is obviously insufficient.
- If any component adheres to inside wall of wells or aluminum film of the reagent cartridge, please gently switch the reagent cartridge to let the component back the wells.
- Do not reverse the reagent cartridge. Otherwise, the result will be unreliable.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
- Use powderless gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.

[STORAGE]

- Store the Pepsinogen I (CLIA) upright at 2-8 °C, kit can be stored for 18 months from manufactured date . After the reagent cartridge is taken out from the refrigerator to equilibrate, the testing should be completed within 2 hours.
- Store the calibrators and quality controls at 2-8 °C till expiry date. When the calibrators and/or controls opened and re-dissolved, the testing should be completed within 2 hours, and the calibrators and/or controls will be stable for 30 days at -20℃±5℃, without exceeding 1 freeze/thaw cycle.
- Keep away from sunlight.
- Do not freeze reagents cartridge.

[SAMPLES]

The sample volume for Pepsinogen I (CLIA) assay is 30μL, please note that at least 55μL of sample has to be pipetted manually into the reagent cartridge.

Sample type and collection
Human serum or plasma (Heparin lithium/EDTA-K_2) are recommended.
Types of tubes:
- Standard sampling tube;
- Plastic tube with clot activator,
- Plastic tube with clot activator and separation gel,
- Plastic tube with heparin lithium,
- Plastic tube with EDTA-K_2.

It is recommended to validate collection tubes before using them as some may contain substances which interfere with test results.
Note: Blood sampling tube results may vary from one manufacturer to another depending on the materials and additives used.
It is the responsibility of each laboratory to validate the type of sample tube used and to follow the manufacturer's recommendations for use.

Sample preparation
The current revision of the WHO/DIL/LAB/99.1 document provides recommendations for the preparation of samples.
For use of the sample tubes, follow the tube manufacturer's instructions for use.
The pre-analytical step, including the preparation of blood samples, is an essential part of medical analyses. In accordance with Good Laboratory Practice, this step is performed under the responsibility of the laboratory manager.
Insufficient clot time can result in the formation of fibrin with micro-clots that are invisible to the naked eye. The presence of fibrin, red blood cells, or suspended particles can lead to erroneous results.
Samples containing suspended fibrin particles or erythrocyte stroma should be centrifuged before testing.
If there is a lipid layer over the sample after centrifuging, please transfer the clear sample into a new sample tube without transferring the lipid layer.
Severe hemolysis samples or heat-inactivated samples are not recommended for applying on the reagents.
Preparation of frozen-stored samples: after thawing, these samples must be thoroughly mixed before testing.
Mix using a vortex-type mixer. If necessary, clarify samples by centrifuging before testing.

Sample stability
Samples (serum and plasma) can be stored at room temperature (15-30 ℃) for up to 8 hours or at 2-8 ℃ for up to 12 hours; if longer storage is required, freeze the serum or plasma at -20 °C or below -20 °C for up to 1 month, without exceeding 1 freeze/thaw cycle.

[INSTRUCTIONS FOR USE]

For complete instructions, see the Instrument User Manual.
When using the assay for the first time. Register the assay using the registration code in the reagent kit before any assay is used in the ACCRE analyzer for the first time. Calibration, QC and sample test are only available after assay was registered successfully.
When opening a new lot of reagents. Each new lot of reagent must be registered by using the registration code in Reagent Registration Card within

the reagent kit.

Calibration

Calibration must be performed with the reagents and calibrators from Manufacturer.

Each bottle of lyophilized calibrator should be re-dissolved by distilled water, deionized water, or purified water to 1.0mL before use. Mix it thoroughly and avoid air bubbles. Keep dissolved calibrators upright for about 15 minutes to equilibrate to room temperature (15-30°C) .

Calibration is necessary under the following conditions:

(1). New reagent lot number applied;

(2). Same lot of reagents used more than 4 weeks;

(3). QC out of control;

(4). When necessary, for example: system maintenance or repair that may affect the analytic performance.

Test Procedure

1.Remove the kit from storage at 2-8 °C and take out the required number of reagent cartridges. Allow the reagent cartridges, specimens, calibrators, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

2. Use each assay strips for each sample, calibrator or control respectively.

3. Place the reagent cartridges into the reagent rack.

4. Manually pipette the Sample, Calibrator, or Control into the sample well of each reagent cartridge .

Please note that at least 55μL sample has to be pipetted manually into the reagent cartridge and 30μL sample is needed for each test.

** Before pipetting: Mix the samples using a vortex-type mixer if necessary, and ensure that the samples and sample diluent (if applicable) DO NOT have any bubbles. The same for calibrators and controls.*

5. Put the sample rack into the reagent chamber of ACCRE analyzer.

6. Place the assay tips into the reagent chamber of ACCRE analyzer.

7. Input Sample ID, sample type, dilution etc. into the software as directed by USER MANUAL.

8. Pepsinogen I (CLIA) assay will be performed automatically by the ACCRE analyzer.

9. After assay is completed, remove the reagent rack from the ACCRE analyzer. Dispose the used reagent cartridge into an appropriate recipient.

Dilution

Samples with concentrations above 200ng/mL can be diluted with sample diluent manually. The recommended dilution is 1:10. The concentration of the diluted sample must be >20 ng/mL. After manual dilution, multiply the result by the dilution factor. ACCRE analyzer cannot perform automatic dilution for samples

Quality control

Each bottle of lyophilized Quality Control should be re-dissolved by distilled water, deionized water, or purified water to 1.0mL before use. Mix it thoroughly and avoid air bubbles. Keep dissolved quality controls upright for about 15 minutes to equilibrate to room temperature (15-30°C) .

Quality control should be performed in accordance with local regulations or requirements related to accreditation, as well as requirements defined in the laboratory's quality control procedure.

[RESULTS AND INTERPRETATION]

Calculation of Results

The results are automatically calculated by the ACCRE analyzer using stored calibration curve which generate by 2-point calibration, and calculated according to a predefined mathematical model, the results expressed in ng/mL.

Interpretation of Results

The detection range is from 1.00 ng/mL to 200.00 ng/mL. For samples with Pepsinogen I below the upper limit of detection, quantitative determination can be performed; if the sample is lower than the lower limit of detection, the reported result is <1.00 ng/mL; if the sample is higher than the upper limit of detection, the reported result is >200.00 ng/mL.

Results of Pepsinogen I (CLIA) assays were determined by testing serum specimens from a study in clinical centers with group of totally 306 healthy individuals in China. The 5th percentile value is 70 ng/mL. When PG I<70 ng/mL and PG I/II ratio <3.0, the diagnostic rate of atrophic diseases of the gastric fundus gland mucosa was the highest

** Please note that results may differ between laboratories due to variations in population and test method. If necessary, each laboratory should establish its own reference range.*

In interpreting the results, patient's clinical condition including symptoms, diseases history, and other relevant data and information should be considered.

[LIMITATIONS]

1.For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.

2. Please note that heterophile antibody in human serum can interfere the test result.

3. High concentration of HAMA can also cause a false positive or false negative result.

4. If the test results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

[PERFORMANCE CHARACTERISTICS]

Detection Limit

LoD (Limit of Detection) = 1.00 ng/mL

The Limit of Detection was determined in accordance with the CLSI® EP17-A2 recommendations.

Measurement range

The measurement range is the range of values corresponding to the acceptable performance limits (precision and linearity).

The measurement range of the Pepsinogen I (CLIA) assay is 1.00 ng/mL ~200.00 ng/mL.

Linearity

Linearity was evaluated according to CLSI® EP06-A recommendations. The Pepsinogen I (CLIA) assay is between 1.00 ng/mL ~200.00 ng/mL. The absolute linear correlation coefficient (r) should be bigger than 0.9900.

Precision

The precision study was performed according to CLSI® EP05-A3 recommendations.

The within-lot precision CV% is less than 10%.

Interfering Substances

Potential interference substances were studied according to CLSI ® EP07-A3 recommendations. No significant interference was detected up to the maximum concentrations tested.

Substance	Concentration
Hemoglobin	≤ 200 mg/dL
Bilirubin	≤ 20 mg/dL
Triglyceride	≤ 1000 mg/dL
Rheumatoid factor	≤ 200 IU/mL

Cross-reactivity

When the concentration of Pepsinogen Ⅱ in the sample reached 200ng/mL, the test result was not higher than 1ng/mL.

Hook effect

When the concentration of PG I is ≤ 1500 ng/mL, it has no effect on the detection results.

Accuracy

The relative deviation between the measured value and the calibration concentration is within the range of ±15%.

HAMA

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.

[WASTE DISPOSAL]

Dispose used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.

It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

[INDEX OF SYMBOLS]

Symbol	Meaning	Symbol	Meaning	Symbol	Meaning
	Consult instructions for use		Contains sufficient for <n> tests		Date of manufacture
IVD	*In vitro* diagnostic medical device		Use-by date		Do not re-use
	Temperature limit	LOT	Batch code	REF	Catalogue number
	Keep away from sunlight		Keep dry		Manufacturer
EC REP	Authorized representative in the European Community/ European Union		Do not use if package is damaged and consult instructions for use	BIO	Contains biological material of animal origin
	Biological risks		Caution	CE	CE marking
	Upright		Fragile, handle with care		Do not roll
CONTROL	Control				

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