UMA CO., LTD.

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Matsudo, Chiba, Japan

MEASURE PG I

Reagent for determination of Pepsinogen I

Latex Immunoturbidimetric Method

2 - 8°C	**IVD** *In vitro* Diagnostics	**QUALITY MANAGEMENT SYSTEM (BY TUV)**
DO NOT freeze	18 months/block from light	**ISO 13485:2016**

1. PURPOSE OF USE

Providing a quantitative in vitro assay for the Pepsinogen I (PG I) concentration in serum or plasma.

2. GENERAL INSTRUCTION

a. For in vitro diagnostics use only.

b. Diagnosis should be made in a comprehensive manner, in accordance with other related test results and clinical symptoms by the doctor in attendance.

c. For guaranteed results, usage of this product must comply with the instruction in this manual.

d. If you use automatic analyzers, follow their instructions carefully.

SUMMARY

Serum pepsinogen consists of two biochemically and immunologically distinct types, namely, pepsinogen I (PGI) and pepsinogen II (PGII) (PGI is also called PG"A", and PGII is also called PG"C"). PGI is produced by chief and mucous neck cells in the fundic glands, while PGII is produced by these cells and also by cells in the pyloric glands and Brunner's glands. It is widely accepted that serum pepsinogen levels reflect the functional and morphologic status of the gastric mucosa. As the fundic gland mucosa is reduced, PGI levels gradually decrease, whereas PGII levels remain fairly constant. As a result, a stepwise reduction of the PGI/II ratio is closely correlated with the progression from normal gastric mucosa to extensive atrophic gastritis; this ratio of more than 3 has a sensitivity of 93.3% and specificity of 87.7% for the diagnosis of normal fundic gland mucosa.

3. MATERIALS REQUIRED BUT NOT INCLUDED

- Saline 0.9 % and high grade purified water

- Micropipet and other basic laboratory equipment.

- PGI/PGII Calibrator Set and PGI/PGII Control Set

4. REAGENT COMPOSITION & PREPARATION

- Reagent R-1: Good buffer

  Reagent R-1 ready to use

- Reagent R-2: Anti-human Pepsinogen I antibody-conjugated latex solution

  Reagent R-2 ready to use

- Once open, Reagent stored on board the instrument is stable for 30 days with Hitachi 7180 Analyzers.

- Applicable to various automated analyzers.

- Calibrators PGI/PGII Calibrator Set and Controls PGI/PGII Control Set (separately sold) are ready for use.

5. SAMPLE PREPARATION & STORAGE

- Serum: Wait until the sample is completely coagulated. Take the supernatant to use as specimens.

- Plasma: Treat sample by anticoagulant: Li-Heparin, K2-EDTA plasma; leave sample to stand for 3 hours or centrifuge at 2000 rpm for 2 minutes; take the plasma layer (supernatant) and use as specimen.

- Stability in serum/plasma:

  - 8 hours at 20 - 25°C
  - 3 days at 2 - 8°C
  - 30 days at < -20°C

- See interferences section for details about possible sample interferences.

6. MEASUREMENT PRINCIPLE

When a patient sample is reacted with buffer and latex reagents, specific antigen-antibody reaction is caused by Pepsinogen I in patient samples and anti-human Pepsinogen I mouse monoclonal antibody sensitized latex, then it gives out turbidity.

Degree of turbidity is proportional to concentration of Pepsinogen I in patient samples, so concentration of

Pepsinogen I in patient samples can be obtained by measuring changes of turbidity.

7. ASSAY PROCEDURE

This product is compatible with various types of clinical analyzer. An example of the assay procedure is indicated below.

Sample	+	Reagent 1	$\xrightarrow[280s]{37°C}$	Reagent 2
6.0µL		150µL		50µL
$\xrightarrow[55s]{37°C}$		Meas. Abs I	$\xrightarrow[265s]{37°C}$	Meas. Abs II
		(570nm)		(570nm)

⟶ ΔAbs at 570nm ⟶ Concentration PG I

Perform the assay according to the instructions for operating the automated analyzer Hitachi models. Refer to the **13. INFORMATION FOR AUTOANALYZERS** for the details of the assay method. Contact **HUMA MEDICAL CO., LTD**. for information about the parameters for other automated analyzers.

8. CALCULATION & UNIT CONVERSION

Calculation

- Calculate ΔAbs of specimen & standards vs blank
- Plot a calibration curve PG I = f(ΔAbs)
- Calculate PG I in specimen using the curve

(doing same procedure for Controls)

Unit conversion

N/A

9. PERFORMANCE & CORRELATION TEST

a. Measuring range

- The assay is linear within an PG I concentration range in serum/plasma of 10 - 200 ng/mL.
- If the concentration of sample exceeds assay range, dilute the sample with saline and repeat the measurement.

b. Detection Limit

Limit of Blank (LoB)	=	10 ng/mL
Limit of Detection (LoD)	=	10 ng/mL
Limit of Quantitation (LoQ)	=	10 ng/mL

The LoB, LoD and LoQ were determined in accordance with CLSI EP17-A2 requirements.

The LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

The LoD is determined based on the LoB and standard deviation of low concentration samples. The LoD corresponds to the lowest analyte concentration which can be detected (value above the LoB with a probability of 95%).

The LoQ is the lowest analyte concentration that can be reproducibly measured with a total error of 20%. It has been determined using low concentration samples.

c. Performance

- Sensitivity: Absorbance was less than 0.02 at 570 nm when purified water was used as sample, and change around 0.1 - 0.5 Abs/minutes when standard solution of PG I of 100 ng/mL was used as a sample.
- Accuracy: When measuring a control sample, the result is within ±10% of assigned value.

d. Precision (on Biolis 30i / SK300)

Representative performance data on the analyzers are given below.

Results obtained in individual laboratories may differ.

Precision was determined using controls followed the CLSI Approved Guidline EP5-A2 with repeatability, reproducibility and total precison (1 aliquot per run, 2 run per day, 20 days).The following results were obtained.

Criterion: CV of Repeatability (aka. Within-run precision) is less than 3% and Total Precision is less than 5%.

Repeatability	*Mean ng/mL*	*SD ng/mL*	*CV %*
PG Control Low	49.9	0.60	1.21
PG Control High	149.6	2.37	1.59

Reproducibility	*Mean ng/mL*	*SD ng/mL*	*CV %*
PG Control Low	49.9	0.63	1.26
PG Control High	149.6	2.65	1.77

Total precision	*Mean ng/mL*	*SD ng/mL*	*CV %*
PG Control Low	49.9	0.76	1.52
PG Control High	149.6	3.13	2.09

e. Correlation Test

Company A (same principle)

Regression equation: $y = 1.0043x + 0.9752$ ($n = 55$)

Correlation coefficient: $r = 0.9792$

10. EXPECTED VALUES

PG I ≥ 70 ng/mL and PG I/PG II ≥ 3.0

Reference for Evalution Criterion

Judgment	Measured Value
	Pepsinogen I and PGI/PGII ratio
Strong Positive	below 30.0 and below 2.0
Medium positive	below 50.0 and below 3.0
Positive	below 70.0 and below 3.0

Correct measurement results cannot be obtained in case non-specific reaction materials (heterophile antibodies etc.) exist in the samples.

Reference range should be established at each facility and judgement should be based on measurement results in a comprehensive manner together with clinical symptoms and other measurement results.

11. INTERFERENCES

- Icterus: No significant interference of conjugated/free bilirubin concentration up to 20 mg/dL

- Hemolysis: No significant interference of hemoglobin concentration up to 500 mg/dL

- Lipemia (Intralipid): No significant interference triglycerides concentration up to 3000 FTU

- Ascorbic Acid: No significant interference of ascorbic acid concentration up to 50 mg/dL

- For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. Please use another methods if the result is affected by any factors

12. HANDLING, USAGE & DISPOSAL

Handling

1. Specimen can be potentially positive for infectious agents including hepatitis B virus and HIV. Wear glove and goggle when needed.

2. In case reagents got into skin, eye or mouth by mistake, wash it immediately with plenty of water and consult the doctor if needed.

3. If reagents are spilled, dilute with water and wipe it out. If specimen is spilled, spray 80% of alcohol over the specimen and wipe it out.

Usage

1. Store reagents under specified condition. Do not use after expiration date.

2. Do not use the container and auxiliaries included in this kit for other purposes.

3. Do not mix reagents of different lot for use.

4. Do not add to the reagent being used even if it is the same lot number.

Disposal

1. All specimens, as well as all instruments (e.g. test tubes) that come in contact with the specimens, must be treated by the following methods, or they must be treated according to the manual for infectious medical waste provided in each facility.

・Sterilize with an autoclave, subjecting them to high pressure saturated steam at 121 °C for more than 20 minutes. Do not process waste containing sodium hypochlorite solution with an autoclave.

・Immerse at least one hour in sodium hypochlorite solution (active chloride concentration of over 1000 ppm).

2. This reagent contains sodium azide. Sodium azide can react with lead pipe and/or steel pipe and can generate explosive metal azide. Make sure to use plenty of water at disposal. Concentration of sodium azide in R-2 is 0.05%.

13. INFORMATION FOR AUTOANALYZERS

❖ **For Hitachi Model**

Calculation Method		Two point
Temperature		37°C
Volume (μL)	Specimen	6.0
	R1	150
	R2	50
Wavelength (nm)	Main	570
	Sub	-
Measurement (cycle)	Point 1	10
	Point 2	19
	Point 3	34
Calibration type		Spline
Unit		ng/mL

14. OTHER INSTRUCTIONS AND CAUTION

- Results may differ depending on the sample/reagent ratio. Adjust parameters for different analyzer.

- Prepare the calibration curve on the day of determination.

15. PACKING AND KIT CONFIGURATION

Code	Package	Test/Kit*	Test/Kit**
11P011B	1x30mL; 1x10mL	155	270
11P011B2	2x30mL; 2x10mL	310	540
11P001B	5x30mL; 5x10mL	775	1350

* For middle-scale automatic analyzers such as: SK300; BS series; BA200; BA400. Chemwell Series; Dirui Series; Biolyzer series, HumanStar 300, Erba Series; Bioelab Series, BX 3010; Pictus P500;…

** For large-scale automatic analyzers such as: CA800; CA400; Randox Imola; Randox Modena+; BM 6010; Biolis50i; SK500; AU Series; Pictus P700; C series; Ci series; HumanStar 600; Kenolab series ...

The above-mentioned test's number are calculated base on technical specifications of each analyzer. The real number of test per kit may higher than the calculation's number.

The above-mentioned test's number cover the loss of the dead volume of reagent bottles but not cover the loss of Calibrator and Control.

Please feel free to contact authorized distributor for further confirmation.

16. REFERENCES

1. Y. Xue, ... Navaid Iqbal, in Reference Module in Biomedical Sciences, 2014
2. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A2, 2004
3. CLSI EP17 · Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition, 2017
4. In house data, UMA Diagnostics

17. MANUFACTURER

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