BIOHIT HealthCare

Innovating for Health

PEPSINOGEN I

ELISA kit for the measurement of human pepsinogen I in EDTA plasma and serum

Instructions for use

REF 601 010.01 IVD CE

For *in vitro* diagnostic use

Store at 2-8 °C Upon Receipt

Biohit Oyj Laippatie 1, FI-00880 Helsinki, Finland

Tel. +358 9 773 861, info@biohit.fi, www.biohithealthcare.com

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APPENDIX: QUALITY CONTROL CERTIFICATE

1. INTENDED USE

The pepsinogen I (PGI) kit is a microplate-based quantitative enzyme linked immunosorbent assay (ELISA) for the determination of human pepsinogen I from plasma or serum samples. FOR *IN VITRO* DIAGNOSTIC USE.

2. CLINICAL BACKGROUND

This test is intended to identify patients who have an advanced atrophic gastritis in the gastric corpus and who, correspondingly, are at increased risk for gastric cancer (1, 2). The serum or plasma PGI (P-PGI, S-PGI) assay is a reliable tool for detecting patients with advanced atrophic corpus gastritis (3-6); the sensitivity and specificity of the test are 92% and 90%, respectively.

Pepsinogen I (PGI) is a precursor enzyme of pepsin and is synthesized by the chief cells and neck cells of the gastric corpus (from so-called oxyntic glands of the gastric mucosa). The major part of PGI is secreted into the gastric lumen but a small amount can be found in the blood. The P/S-PGI level reliably correlates with the number of chief cells in the gastric corpus mucosa. Correspondingly, the loss of chief cells results in a linear decrease in P/S-PGI. The loss of chief cells is, on the other hand, a result of atrophic gastritis.

For unknown reasons, atrophic gastritis increases the risk of gastric cancer, the risk being even 5-fold in patients with advanced atrophic gastritis in the corpus and even 90-fold in advanced atrophic pangastritis (both antrum and corpus affected) compared to the cancer risk in persons with normal gastric mucosa (2).

The screening of middle-aged (50-69 years) , smoking men in Finland with the S-PGI test has revealed that a low S-PGI level (<25 µg/l) is detected in 9.8% of men of whom 4.7% revealed either a gastric cancer or precancerous lesion by endoscopy (7). Corresponding results have also been published in earlier studies (8-17).

3. PRINCIPLE OF THE TEST

This PGI ELISA is based on a sandwich enzyme immunoassay technique with a PGI specific capture antibody adsorbed on a microplate and a detection antibody labeled with horseradish peroxidase (HRP).

The assay proceeds according to the following reactions:

1. A monoclonal antibody, specific to human PGI, on the polystyrene surface of the wells binds PGI molecules present in the sample.
2. Wells are washed to remove the residual sample.
3. An HRP-conjugated monoclonal detection antibody is added to the wells and it binds to the PGI molecules.
4. The wells are washed and TMB-substrate is added. The substrate is oxygenized by the enzyme and a blue colored end product is produced.
5. The enzyme reaction is terminated with stop solution. The solution in the microwells should turn yellow. The intensity of the yellowish color developed is directly related to the PGI concentration of the sample.

4. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use

CAUTION: Handle plasma and serum samples as potential biohazardous material.

All samples should be regarded as potentially contaminated and treated as if they were infectious. Please refer to the U. S. department of Health and Human Services (Bethesda, MD., USA) publication Biosafety in Microbiological and Biomedical Laboratories, 1999, 4th ed. (CDC/NIH) and No. (CDC) 88-8395 on reports of laboratory safety procedures on different diseases or any other local or national regulation.

This kit contains reagents manufactured from human blood components. The source materials provided in this kit have been tested for the presence of antibodies to hepatitis B and C as well as antibodies to HIV, and found to be negative. However, as no test method can offer absolute assurance that these pathogens are absent, all recommended precautions for the handling of a blood derivative should be observed.

Always use protective gloves when handling patient samples. Use a safety pipetting device for all pipetting. Never pipette by mouth. Read all instructions prior to performing this assay. All provided reagents of the kit can be disposed by pouring into a sink and flushing with an excess of tap water.

5. SPECIMEN COLLECTION AND HANDLING

Fasting for 10 hours is recommended prior to blood sampling. Blood sample is collected by venipuncture into e.g. a plastic EDTA or serum tube without additives. Plasma blood tubesare mixed immediately by turning them upside down 5-6 times and tubes for serum allowed to clot (for minimum 30 minutes) at room temperature (20...25°C). Serum after clotting and plasma immediately is separated by centrifugation (e.g. plastic tube, acceleration up to 2000 G, 10-15 minutes). Plasma/serum can be stored refrigerated (2...8°C). For longer storage the samples should be stored frozen (preferably at -70°C, alternatively at -20°C). Mix the samples thoroughly after thawing. Avoid repeated freezing and thawing of the samples. Grossly hemolysed, lipemic or turbid specimens should be avoided.

Please refer to Biohit Gastrin-17 Advanced ELISA instructions for use if testing the Biohit GastroPanel ELISA assays (Pepsinogen I, Pepsinogen II, Gastrin-17 Advanced, *Helicobacter pylori* IgG antibodies) from the same sample.

6. KIT CONTENTS, REAGENT PREPARATION AND STABILITY FOR MATERIALS PROVIDED

The reagents are sufficient for 96 wells and three separate runs. Reagents of different kit lots should not be mixed.

6.1. Microplate

Contents: 12 x 8 strips in frame coated with high-affinity, monoclonal anti-human -PGI IgG_1.
Preparation: Ready for use.
Stability: Stable until expiry date. Discard the strips after use.

6.2. Washing Buffer Concentrate (10 x)

Contents: 120 ml of 10 x phosphate buffer saline (PBS) concentrate containing Tween 20 and 0.1 % ProClin 300 as preservative.
Preparation: Dilute 1 to 10 (e.g. 100 ml+ 900 ml) with distilled water and mix well.
Stability: The diluted solution is stable for two weeks refrigerated (2…8 °C).

6.3. Diluent Buffer

Contents: 100 ml of phosphate buffer containing bovine serum albumin, Tween 20, 0.1% ProClin 300 as preservative and red dye extract.

Preparation: Ready for use.
Stability: Stable until expiry date.

6.4. Blank Solution
Contents: One vial containing 1.5 ml of human serum-based phosphate buffer with 0.1% ProClin 300 as preservative.
Preparation: Ready for use.
Stability: Stable until expiry date.

6.5. Calibrators
Contents: Three vials each containing 1.5 ml of human serum-based calibrators with 0.1% ProClin 300 as preservative. The calibrators have lot-specific PGI values of approximately 25, 100 and 200 µg/l. The exact PGI concentration of the calibrators is labelled on the vials.
Preparation: Ready for use.
Stability: Stable until expiry date.

6.6. Control
Contents: One vial containing 1.5 ml of human serum-based PGI control with 0.1% ProClin 300 as preservative. The expected PGI level of the control is indicated on the label.
Preparation: Ready for use.
Stability: Stable until expiry date.

6.7. Conjugate Solution
Contents: 15 ml of HRP-conjugated monoclonal anti-human-PGI in stabilizing buffer with 0.02% methylisothiazolone, 0.02% bromonitrodioxne and 0.002% other active isothiazolones as preservatives.
Preparation: Ready for use.
Stability: Stable until expiry date.

6.8. Substrate Solution
Contents: 15 ml of tetramethylbenzidine (TMB) in aqueous solution.
Preparation: Ready for use.
Stability: Stable until expiry date. Avoid exposure to direct light.

6.9. Stop Solution
Contents: 15 ml of 0.1 mol/l sulphuric acid.
Preparation: Ready for use.
Stability: Stable until expiry date.

6.10. Incubation Covers
Three plastic sheets to cover the microplate during incubation.

6.11. Instructions for Use

7. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water
- Micropipettes and disposable tips, to accurately deliver 20 - 1000 µl
- Pipettes to accurately deliver 1 - 10 ml
- 8-channel pipette delivering 100 µl
- Graduated cylinder, 1000 ml
- Vortex mixer for sample dilutions
- Test tubes for specimen dilutions
- Microplate washer
- Paper towels or absorbent paper
- Timer
- Incubator, 37°C
- Microplate reader, 450 nm
- e.g. plastic blood collection tube for plasma or serum
- Container for ice

8. STORAGE AND STABILITY

Store the Pepsinogen I kit refrigerated (2…8°C). When stored at these temperatures the kit is stable until the expiration date printed on the box label and the label of each individual kit component. Do not freeze or expose the kit to high temperatures or store at above 8°C when not in use. The substrate solution is sensitive to light. The microplate or individual strips should not be removed from the foil pouch until equilibrated to room temperature (20…25°C). Unused strips must be returned to the foil pouch, sealed and stored at 2…8°C.

Do not use reagents after the expiration date printed on the label. Do not use reagents from kits with different lot numbers or substitute reagents from kits of other manufacturers. Use only distilled or deionized water. The components of the kit are provided at precise concentrations. Further dilution or other alterations of the reagents may cause incorrect results.

Indication of Kit Deterioration
Liquid components should not be visibly cloudy or contain precipitated material. At 2…8°C, the washing buffer concentrate may, however, partially crystallize, but the crystals will dissolve by mixing at room temperature (20…25°C). The substrate solution should be colourless or pale blue. Any other color indicates deterioration of the substrate solution.

9. TEST PROCEDURE

PRELIMINARY PREPARATIONS
Allow all reagents and the microplate to reach room temperature (20…25°C). Warm the incubator to 37°C. Dilute the washing buffer concentrate 1 to 10 (e.g. 100 ml + 900 ml) with distilled or deionized water. **Read the complete assay procedure before starting. It is recommended that all calibrators and samples are applied on the plate as duplicates. It is necessary to use calibrators and the control in each test run**.

Mix all the reagents and samples well before use.

SPECIMEN DILUTION
Dilute serum or plasma samples 1 to 10 (50 µl + 450 µl) with the diluent buffer, **mix well.**

STEP I
Mix and pipette 100 µl of the blank solution (BS), the calibrators (CAL1-CAL3), the control and diluted samples (S1, S2 etc.) into the wells as duplicates (see Figure 1). Cover the plate with the incubation cover. Incubate for 60 minutes at 37°C.

Figure 1. Pipetting Order.

	1	2	3	4	5
A	BS	BS			
B	CAL1	CAL1			
C	CAL2	CAL2			
D	CAL3	CAL3			
E	Control	Control			
F	S1	S1			
G	S2	S2			
H	etc.	etc.			

WASHING
Wash the wells three times with 350 µl of the diluted (1 to 10) washing buffer and gently tap the inverted plate a few times on a clean paper towel.

STEP II
Pipette 100 µl of the mixed conjugate solution into the wells, preferably with an 8-channel pipette. Cover the plate with the incubation cover. Incubate for 30 minutes at 37°C.

WASHING
Wash the wells three times with 350 µl of the diluted (1 to 10) washing buffer and gently tap the inverted plate a few times on a clean paper towel.

STEP III
Pipette 100 µl of the mixed substrate solution into wells with an 8-channel pipette. Start the incubation time after pipetting the substrate solution into the first strip and continue the incubation for 30 minutes at room temperature (20…25°C). Avoid direct exposure to light during incubation.

STEP IV
Pipette 100 µl of the mixed stop solution with an 8-channel pipette into the wells.

MEASURING
Measure the absorbance at 450 nm within 30 minutes.

10. RESULTS

10.1. Quality Control Values

Good Laboratory Practice requires the use of appropriate controls to establish that all the reagents and protocols are performing as designated. The Pepsinogen I ELISA is provided with the control serum. Quality control charts should be maintained to follow the performance of the control or appropriate statistical methods should be used for analyzing control values, which should fall within the appropriate confidence intervals employed in each laboratory.

10.2. Calculation of the Results

Assay results can be analyzed by using a) manual method or b) automated methods, where the absorbance readings are converted to pepsinogen I concentrations. Since the calibrators are ready to use, the results of the patient samples are not multiplied by the dilution factor.

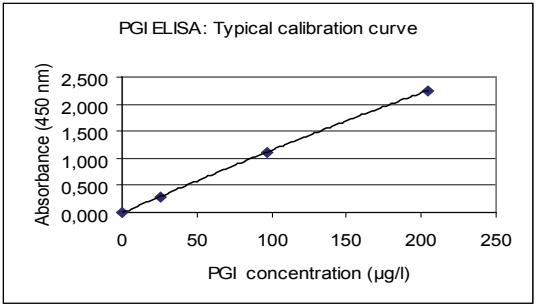
a) Manual Method

Calculate the mean absorbance of the duplicate determinations of the blank solution, the calibrators, the control and samples. Subtract the mean of the blank solution from itself (consider this as the first point of the calibration curve), the calibrators, the control and samples. Graph the calibrator curve by plotting the mean absorbance for the first point and each calibrator (y-axis) against the PGI concentrations given for the calibrators (x-axis). Draw a best fit curve to construct a calibration curve. Use the mean absorbance value for each sample and the control to interpolate the PGI value from the calibration curve.

b) Automated Methods

There are several computer programs available for interpolating the unknown concentrations, automatically. A simple 2nd order polynomial fit is adequate for interpolating unknown concentrations within the calibrator range. However, if sample absorbance value exceeds the absorbance value of the highest calibrator, a more complex extrapolating algorithm may be more appropriate. A typical calibration curve is shown in Figure 2.

Figure 2. Example of a Typical Calibration Curve.



10.3. Prevalence

In an elderly population (age above 50 years), 10% shows advanced atrophic corpus gastritis and abnormal S-PGI levels (S-PGI<25 µg/l). Approximately 5% of these patients show gastric cancer or precancerous lesions in endoscopy (7).

10.4. Interpretation of the Results

- A low P/S-PGI result (P/S-PGI<30 µg/l) indicates advanced (moderate and severe) atrophic gastritis of the corpus mucosa. This cut-off level has been determined using the Biohit Pepsinogen I ELISA kit based on large clinical material. A low P/S-PGI is an indicator for upper gastrointestinal endoscopy (gastroscopy) because of the increased risk of these patients of developing cancer prelesions and gastric cancer.
- It is recommended that the given limits are considered as guidelines. Also the PGI results determined for given specimen with assays from different manufacturers can vary due to differences in standardization, assay methods and reagent specificity. Results obtained from other manufacturers' assay method should not be used interchangeably.
- This Pepsinogen I ELISA assay enables wide range measurements of both low and high concentrations of P/S-PGI.

11. LIMITATIONS OF THE PROCEDURE

As with any diagnostic procedure the Biohit Pepsinogen I ELISA test results must be interpreted together with the patient's clinical presentation and any other information available to the physician.

Samples suspected of having PGI concentrations greater than the highest calibrator should be further diluted (final dilution 1 to 20) before assay.

12. PERFORMANCE CHARACTERISTICS

Within-Assay Imprecision:
The within-assay imprecision was determined with four serum samples. These samples were run as 17 replicates in one run.

Sample	Mean PGI (µg/l)	CV%
1	9.3	5.9
2	31.3	3.0
3	78.1	2.4
4	137.2	2.4

Between-Assay Imprecision:
The between-assay imprecision was evaluated in six assays using four serum samples. The PGI concentration of these samples was measured as duplicates.

Sample	Mean PGI (µg/l)	CV%
1	10.3	4.9
2	47.4	3.0
3	80.4	3.4
4	133.9	2.9

Specificity/Cross-Reactivity:
The cross-reaction and interference by PGII was tested by spiking five serum samples with PGII (Company Z) at the concentrations of up to 200 µg/l. The test showed no significant increase or reduction in the signal of the samples with a PGII concentration of 200 µg/l.

Sample	PGII added (µg/l in sample)	PGI observed (µg/l)	Added/ 0-sample (%)
1	- 10 50 200	4.2 4.2 4.4 4.4	- 100 105 105
2	- 10 50 200	26.3 24.6 27.3 24.5	- 93.5 104.0 93.2
3	- 10 50 200	56.2 59.6 60.9 53.3	- 106.0 108.0 94.8
4	- 10 50 200	72.7 70.8 72.5 71.9	- 97.4 99.7 98.9
5	- 10 50 200	100.1 98.3 99.9 98.7	- 98.2 99.8 98.6

Sensitivity:
The sensitivity of the test was determined in two different ways:

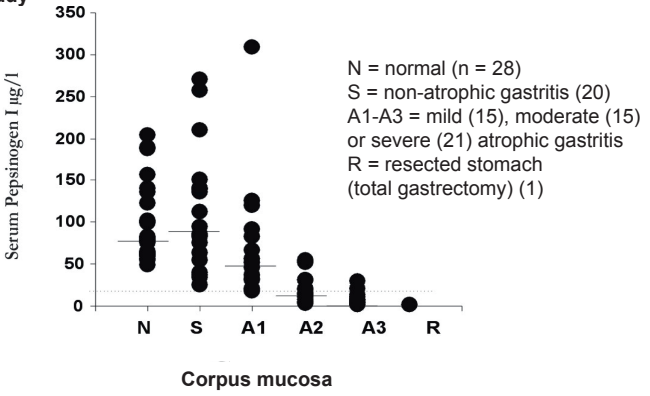
1) Dilution of a kit calibrator with PGI concentration 10.0 µg/l with diluent buffer gives a 10% CV limit at a concentration of 0.6 µg/l.
2) Mean of 25 zero replicates + 2 standard deviations corresponds to 1.9 µg/l PGI.

Recovery:
Four serum samples were spiked with 6.2, 31.0 and 60.8 µg/l human pepsinogen I (purified human PGI, Biohit Diagnostics).

The average recovery was:
6.2 µg/l 97.5%
31.0 µg/l 89.1%
60.8 µg/l 72.6%

Correlation: Correlation was shown with the relationship between the serum levels of pepsinogen I and histological status of the corpus mucosa (18).

Serum Pepsinogen I and Corpus Mucosa - Finnish Case-Control study



Linearity:
Three serum samples were assayed in serial dilutions with the diluent buffer to determine the linearity of Biohit Pepsinogen I ELISA. Results are listed in the following table.

Sample	Dilution factor	Observed (µg/l)	Expected (µg/l)	Recovery (%)
1	1 2 4	212.4 105.4 52.8	- 106.2 53.1	- 99 99
2	1 2 4	146.4 72.8 39.3	- 73.2 36.6	- 99 107
3	1 2 4	59.0 29.7 14.8	- 29.5 14.7	- 101 101

13. REFERENCES

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14. DATE OF ISSUE

Pepsinogen I kit insert.
Version 06, 28.11.2012.

15. WARRANTY

The Manufacturer shall remedy all defects discovered in any Product (the "Defective Product") that result from unsuitable materials or negligent workmanship and which prevent the mechanical functioning or intended use of the Products including, but not limited to, the functions specified in the Manufacturer's specifications for the Products. ANY WARRANTY WILL, HOWEVER, BE DEEMED AS VOID IF FAULT IS FOUND TO HAVE BEEN CAUSED BY MALTREATMENT, MISUSE, ACCIDENTIAL DAMAGE, INCORRECT STORAGE OR USE OF THE PRODUCTS FOR OPERATIONS OUTSIDE THEIR SPECIFIED LIMITATIONS OR OUTSIDE THEIR SPECIFICATIONS, CONTRARY TO THE INSTRUCTIONS GIVEN IN THE INSTRUCTION MANUAL.
The period of this warranty for the Distributor is defined in the instruction manual of the Products and will commence from the date the relevant Product is shipped by the Manufacturer. In case of interpretation disputes the English text applies.

This Biohit diagnostic kit has been manufactured according to our ISO 9001 / ISO 13485 quality management protocols and has passed all relevant Quality Assurance procedures related to this product.

16. ORDERING INFORMATION

Pepsinogen I ELISA test kit.
Cat. No. 601 010.01.

Headquarters
BIOHIT OYJ
Laippatie 1
00880 Helsinki, Finland
Tel: +358-9-773 861
Fax: +358-9-773 86200
E-mail: info@biohit.fi
www.biohithealthcare.com

17. EXPLANATION OF THE SYMBOLS USED IN LABELS

IVD	For *in vitro* diagnostic use
REF	Catalogue number
LOT	Batch code
	Use by
	Consult instructions for use
 +2...+8°C	Temperature limitation. Store at +2...8°C
	96 determinations
	Do not re-use

18. SHORT OUTLINE OF THE PROCEDURE

Allow all the reagents to reach room temperature (20…25°C)
Remember to mix all the reagents and samples well just before pipetting
*

After mixing, pipette 100 µl of the blank solution, the calibrators, the control and diluted (1 to 10) patient samples into the wells
*

Incubate for **60 min at 37°C**
*

Wash the wells 3 times with 350 µl of the diluted washing buffer
*

Pipette 100 µl of the mixed conjugate solution into the wells
*

Incubate for **30 min at 37°C**
*

Wash the wells 3 times with 350 µl of the diluted washing buffer
*

Pipette 100 µl of the mixed substrate solution into the wells
*

Incubate for **30 min** at room temperature **(20…25°C)**
*

Pipette 100 µl of the mixed stop solution into the wells
*

Read at **450 nm** within 30 minutes