BIOHIT HealthCare

Innovating for Health

PEPSINOGEN II

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

Instructions for use

REF 601 020.02 IVD CE

Pepsinogen II ELISA   Cat. No. 601 020.02

CONTENTS

APPENDIX: QUALITY CONTROL CERTIFICATE

1. INTENDED USE

The pepsinogen II (PGII) kit is a microplate-based quantitative enzyme immunoassay for the determination of human pepsinogen II from plasma or serum samples. FOR *IN VITRO* DIAGNOSTIC USE.

2. CLINICAL BACKGROUND

Pepsinogen II is produced by chief cells and mucous neck cells of the gastric mucosa, in pyloric glands in the gastric antrum, and Brunner's glands in the proximal duodenum. The ratio of concentration of Pepsinogen I (PGI) to PGII in plasma or serum of normal subjects is about 4:1 (1).

The PGI/PGII ratio decreases linearly with increasing grade of atrophic gastritis in the corpus (2, 3). The ratio is < 3.0 when atrophic gastritis is advanced (moderate or severe) in the gastric corpus (3). It has been shown that the risk of gastric cancer is increased (5-fold) when the PGI/PGII ratio is low (5-14). This test is intended as an additional tool in the diagnosis of atrophic corpus gastritis, which is also a risk state for gastric cancer (2, 4). The Pepsinogen II assay is used together with the Pepsinogen I assay by which the PGI/PGII ratio is determined. As will as with Gastrin-17 confirms atrophic corpus gastritis.

3. PRINCIPLE OF THE TEST

This PGII ELISA is based on a sandwich enzyme immunoassay technique with a PGII specific capture antibody adsorbed on a microwell plate and detection antibody labeled with horseradish peroxidase (HRP). The assay proceeds according to the following reactions:

1. Human PGII specific monoclonal antibodies attached to polystyrene surface bind PGII molecules present in the sample.
2. Wells are washed to remove residual sample.
3. HRP-conjugated detection antibodies are added into the wells and they bind to PGII molecules.
4. The wells are washed and TMB-substrate is added. The substrate is oxidized 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 PGII concentration of the sample.

4. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use

CAUTION: Handle plasma and serum samples as potentially 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 for 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 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 of by pouring them 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. The blood sample is collected by venipuncture into e.g. a plastic EDTA or serum tube without additives. Plasma blood tubes are mixed immediately by turning them upside down 5-6 times, and tubes for serum are 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, relative centrifugal force up to 2000 G, 10-15 minutes). Plasma/serum can be stored refrigerated (2-8°C) for three days. 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 specimen should be avoided.

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-PGII 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 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 casein, Tween 20, 0.1% Pro-Clin 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 PGII values of approximately 6.3, 12.5 and 50 µg/l. The exact PGII concentration of the calibrators is labeled 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 PGII control with 0.1% ProClin 300 as preservative. The expected PGII level of the control serum is indicated on the label of the vial.

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

6.7. Conjugate Solution
Contents: 15 ml of HRP-conjugated anti-human-PGII in stabilizing buffer with 0.1% ProClin 300 as preservative.
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 direct exposure to light.

6.9. Stop Solution
Contents: 15 ml of 0.1mol/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
- Microplate reader, 450 nm
- e.g. plastic blood collection tube for plasma or serum
- Container for ice

8. STORAGE AND STABILITY

Store the Pepsinogen II 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 any of the 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 colorless 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 (e.g. 20-25°C). Dilute the washing buffer concentrate 1 to 10 (e.g. 100 ml + 900 ml) with distilled or deionized water. Frozen samples should be thawn fast in a room temperature water bath with occational mixing. Once they are almost thawed, place them in a crushed ice bath. **Read the complete assay procedure before starting. It is recommended that the 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.

STEP 1: SPECIMEN DILUTION
Dilute the mixed plasma or serum samples 1 to 5 (e.g. 100 µl + 400 µl) with the diluent buffer, **mix well**. Return the rest of the sample back to the freezer.

STEP 2: SAMPLE
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 60 minutes at room temperature (20-25°C).

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

Figure 1. Pipetting Order.

STEP 3: 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 4: CONJUGATE
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 60 minutes at room temperature (20-25°C).

STEP 5: 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 6: SUBSTRATE
Pipette 100 µl of the mixed substrate solution into the 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 7: REACTION STOP
Pipette 100 µl of the mixed stop solution with an 8-channel pipette into the wells.

STEP 8: MEASURING OF RESULTS
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 II ELISA is provided with control serum. Quality control charts should be maintained to follow the performance of the control, or appropriate statistical methods should be used to analyze 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 II 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 the samples. Graph the calibration curve by plotting the mean absorbance for the first point and each calibrator (y-axis) against the PGII 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 PGII 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 values 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.
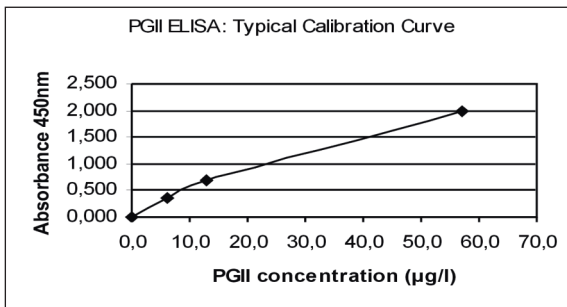
Figure 2. Example of a Typical Calibration Curve.

10.3. Prevalence

A PGI/PGII ratio lower than 3.0 indicates that the patient has advanced corpus atrophy and an increased risk of gastric cancer. It is recommended that the given limits are considered as guidelines. Also the PGII results determined for a given specimen with assays from different manufacturers can vary due to differences in standardization, assay methods and reagent specificity. Results obtained by different manufacturers' assay methods should not be used interchangeably.

11. LIMITATIONS OF THE PROCEDURE

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

12. PERFORMANCE CHARACTERISTICS

Within-Assay Imprecision:
The within-assay imprecision was determined with four serum samples. These samples were run as 18 replicates in one run.

Sample	Mean PGII (µg/l)	CV%
1	4.6	3.2
2	8.8	5.6
3	13.4	5.1
4	21.7	4.0

Between-Assay Imprecision:

The between-assay imprecision was evaluated in six assays using four serum samples. The PGII concentration of these samples was measured as duplicates.

Sample	Mean PGII (μg/l)	CV%
1	3.3	2.8
2	6.7	3.4
3	16.3	2.3
4	16.7	3.4

Specificity/Cross-Reactivity:

The cross-reaction and interference by PGI was tested by spiking three serum samples with PGI (rPGI, company X) at concentrations up to 500 μg/l. The test showed no significant increase or reduction in the signal of samples with a PGI concentration of up to 500 μg/l.

Sample	PGI added (μg/l)	Observed (μg/l)	Added/ 0-Sample (%)
1	- 50 100 500	30.5 30.2 31.5 30.2	- 99 103 99
2	- 50 100 500	12.3 12.8 12.5 12.8	- 104 102 104
3	- 50 100 500	5.7 5.9 5.8 6.0	- 104 102 105

Analytical Sensitivity:

The sensitivity of the PGII ELISA was determined according to the NCCLS (CLIA) guideline Consensus Standards for Medical Testing (NCCLS EP17-A).

The limit of blank (LoB) was determined as the 95th percentile under which the measurement results of 60 blank samples (blank solution) fall. The LoB was found to be 0.6 μg/l.

The limit of detection (LoD), which is the lowest actual amount of analyte that may be detected with 95% confidence, was assessed using four EDTA plasma and serum samples that contain PGII levels close to the observed LoB value.

The LoD was found to be 0.7 µg/l for EDTA plasma and 0.8 µg/l for serum samples.

The limit of quantitation (LoQ), i.e. the lowest amount of analyte in a sample that can be reliably detected, was calculated assuming a coefficient of variation (CV%) of 20% at the level of LoD. Accordingly, the LoQ wascalculated to be 1.2 x LoD = 0.8 µg/l for EDTA plasma and 1.0 µg/l for serum samples.

Recovery:
Five serum samples were spiked with 10, 20 and 40 µg/l of human pepsinogen II (purified human PGII, Biohit Diagnostics).

The average recovery was:
10 µg/l   95.1%
20 µg/l   96.3%
40 µg/l   98.9%

Correlation:
Correlation was shown with the relationship between serum levels of pepsinogen II and histological status.

Ratio of Pepsinogen I to pepsinogen II – atrophic corpus gastritis – a population sample (Borch et al, University Hospital of Linköping, Sweden).
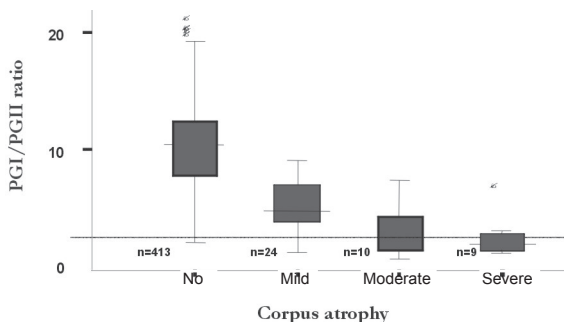
Linearity:

Four serum samples were assayed in serial dilutions with the diluent buffer to determine the linearity of Biohit Pepsinogen II ELISA. Results are listed in the table below.

Sample	Dilution Factor	Observed (µg/l)	Expected (µg/l)	Recovery (%)
1	1 2 4	23.8 12.8 6.4	- 11.9 6.0	- 108 107
2	1 2 4	9.4 5.1 2.6	- 4.7 2.3	- 109 113
3	1 2 4	11.0 5.5 3.0	- 5.5 2.8	- 100 107
4	1 2 4	28.6 15.7 7.9	- 14.3 7.1	- 110 111

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14. DATE OF ISSUE

Pepsinogen II 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, ACCIDENTAL 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.

All Biohit diagnostic kits have been manufactured according to our ISO 9001 / ISO 13485 quality management protocols and have passed all relevant Quality Assurance procedures related to these products.

16. ORDERING INFORMATION

Pepsinogen II ELISA test kit.
Cat. No. 601 020.02.

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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	96 determinations
②	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 5) patient samples into the wells

*

Incubate for 60 min at room temperature (20-25°C)

*

Wash 3 times with 350 µl of the diluted washing buffer

*

Pipette 100 µl of the mixed conjugate solution into the wells

*

Incubate for 60 min at room temperature (20-25°C)

*

Wash 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