

BIOHIT HealthCare

Innovating for Health

GASTRIN-17

ELISA kit for the measurement of human
Gastrin-17 in EDTA plasma and serum

Instructions for use

REF 601 035

IVD



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 Gastrin-17 Advanced ELISA test is an enzyme-linked immunosorbent assay (ELISA) for the quantitative measurement of gastrin-17 (G-17) in human EDTA plasma (P-G-17) or serum (S-G-17) samples. FOR *IN VITRO* DIAGNOSTIC USE.

2. CLINICAL BACKGROUND

The antral hormone gastrin (gastrin-17) regulates gastric acid secretion and growth of the gastric mucosa (1). As a result of the cellular post-translational maturation process of progastrin, the G cells in the antrum release a mixture of different acid stimulatory gastrins and other precursor fragments into the circulation (2). This mixture comprises gastrin-71, -52, -34, -17, -14, and -6, all of which are carboxyamidated and circulate in an O-sulfated and nonsulfated form. The dominant gastrin forms in plasma/serum healthy humans are amidated gastrin-34 and -17 [for a review, see Ref. (3)], of which the latter, G-17 is the predominant and potent tissue form in healthy antral mucosa and almost exclusively produced by the antrum G-cells.

This test is intended to identify *Helicobacter pylori* infected patients who have an advanced atrophic gastritis in the gastric antrum – these patients have an abnormally low P/S-G-17 - and who, correspondingly, are at an increased risk of gastric cancer and of peptic ulcer disease [Ref. (4-8), for reviews see Ref. (9,10)]. For unknown reasons, atrophic antrum gastritis increases the risk of gastric cancer, the risk being even 18-fold in patients with advanced atrophic antrum gastritis compared to the cancer risk in persons with normal antrum mucosa. The risk of peptic ulcer is approximately 25-fold in patients with antrum-limited moderate or severe atrophic gastritis compared to normal population (5). On the other hand, abnormally high P/S-G-17 concentrations can be used as a biomarker of hypo- or achlorhydria and can be seen as a sign of atrophic gastritis that is limited to the gastric corpus. Moreover, P/S-G-17 levels can be used in differentiation of hypergastrinemias of neoplastic origin from those of non-neoplastic origin - G-17 does not rise, in contrast to high molecular mass forms of gastrins, in patients with gastrinoma tumors. The measurement of plasma/serum G-17 may also be used to monitor patients who have undergone successful gastric surgery - secretion of G-17 into circulation is practically zero after successful antrectomy.

In patients without *H. pylori* infection, a low fasting level of gastrin-17 may be an indicator of the high acid output, as well as of the risk of gastroesophageal reflux disease and Barrett's esophagus. A low fasting G-17 (<1 pmol/l) raises the pre-test odds of Barrett's esophagus 3 to 4 -fold, and a high fasting G-17 (>5 pmol/l) strongly opposes or excludes the presence of Barrett's esophagus (11).

The secretion of G-17 from the G-cells in the antrum mucosa is a result of a stimulation by various factors e.g. of dietary protein stimulus. High acidity in the stomach inhibits the secretion of G-17 (12). In a normal stomach, protein stimulation or the lack of acid will result in an increase in the P/S-G-17 level. In advanced or severe atrophic gastritis of the antrum, the fasting (basal) level of G-17 in plasma/serum is low and no increase will occur following protein stimulation. The magnitude of the decline of the G-17 concentration and its response to stimulus depends on the degree of atrophy: the more severe the atrophy, the lower the concentration of G-17, and the weaker the G-17 response to stimulus.

The G-17 ELISA method is specific for amidated gastrin-17 in plasma and serum (13).

3. PRINCIPLE OF THE TEST

This G-17 Advanced ELISA is based on a sandwich enzyme immunoassay technique with a G-17 specific capture antibody adsorbed to a microwell plate and a detection antibody labeled with horseradish peroxidase (HRP). The assay proceeds according to the following reactions:

1. A monoclonal antibody, specific to human G-17, on the polystyrene surface of the wells binds to G-17 molecules present in the sample.
2. Wells are washed after incubation to remove residual sample.
3. HRP-conjugated monoclonal antibody binds to the G-17 molecules bound to the surface of the wells.
4. The wells are washed after incubation and TMB substrate is added. The substrate is oxidized by the enzyme (HRP), resulting in the formation of blue end product.
5. The enzyme reaction is terminated with the stop solution. The optical density of the developed yellow color is directly related to the G-17 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.

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 reagents provided in the kit can be disposed by pouring into a sink and flushing with an excess of tap water.

5. SPECIMEN COLLECTION AND GASTRIN STIMULATION

The blood sample is drawn after overnight fasting (approx. 10 hours) into an EDTA or serum tube without additives. Blood tubes for plasma are mixed immediately by turning them upside down 5-6 times. Coagulation of tubes for serum must not exceed 30 minutes at room temperature (20 – 25 °C). The coagulation phase on ice should not exceed 60 minutes. Serum after clotting and plasma within 30 minutes is separated by centrifugation (e.g. plastic tube, relative centrifugal force up to 2000 G, 10-15 minutes.) Plasma/serum is divided into a separate plastic tube. Samples are frozen immediately. For temporary storage, the plasma samples can be stored frozen at –20°C, but long-term storage of over two weeks, the storage should be at –70°C. The samples should be mixed thoroughly after quick thawing. Multiple freeze and thaw cycles of samples should be avoided. Grossly lipemic or cloudy specimens must not be used.

Alternatively, the Gastrin-17 stabilizer (Gastrin-17 Stabilizer, Cat. No. 601 050) can be used. The addition of the stabilizer into the plasma or serum sample immediately after separation (100µl / 2 ml plasma or serum) enables the storage of the sample for 3 days in the refrigerator at 2-8°C.

When a postprandial, protein stimulated blood sample is needed, a drink made from Biohit protein powder (Biohit Oyj., Cat. No. 610099.01) should be taken after fasting for a minimum of 10 hours. 20 minutes after the protein drink, blood is drawn into an EDTA or serum tube without additives.

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

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

6.1. Microplate

Contents: 12 x 8 strips in frame coated with high-affinity, monoclonal anti-human G-17 peptide antibody.

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 buffered 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: Stable until expiry date.

6.3. Sample Diluent Buffer

Contents: 100 ml of phosphate buffer containing blocking protein and 0.1% ProClin 300 as preservative. **Note:** The buffer is slightly opaque.

Preparation: Ready for use.

Stability: Stable until expiry date.

6.4. Blank Solution

Contents: One vial containing 1.5 ml of 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 gastrin-17 calibrators in phosphate buffer with 0.1% ProClin 300 as preservative. The calibrators have lot specific G-17 values of approximately 5, 10 and 40 pmol/l. The exact G-17 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 control in phosphate buffer with 0.1% ProClin 300 as preservative. The expected G-17 concentration of the control is labeled on the vial.

Preparation: Ready for use.

Stability: Stable until expiry date.

6.7. Conjugate

Contents: 0.2 ml of HRP-conjugated anti-human gastrin-17 in stabilizing buffer with 0.02% methylisothiazolone and 0.02% bromonitrodioxane, and 0.002% other active isothiazolones as preservatives.

Preparation: Dilute 1 to 100 (e. g. for 4 strips 40 µl + 3960 µl) with the **conjugate diluent buffer**.

Stability: Stable until expiry date.

6.8. Conjugate Diluent Buffer

Contents: 15 ml of phosphate buffer solution containing 0.1% ProClin 300 as preservative.

Preparation: Ready for use.

Stability: Stable until expiry date.

6.9. 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.

6.10. Stop Solution

Contents: 15 ml of 0.1 mol/l sulphuric acid.

Preparation: Ready for use.

Stability: Stable until expiry date.

6.11. Incubation Covers

Three plastic sheets to cover the microplate during incubation.

6.12. Instructions for Use

7. MATERIALS REQUIRED BUT NOT PROVIDED

- ▶ Distilled or deionized water
- ▶ Micropipettes and disposable tips, to accurately deliver 20 - 1000 μ l
- ▶ Pipettes to accurately deliver 1-10 ml
- ▶ 8-channel pipette delivering 100 μ l
- ▶ 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-water bath

8. STORAGE AND STABILITY

Store the Gastrin-17 Advanced ELISA 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 diluent buffer is slightly opaque. The calibrators and control may also seem slightly opaque. 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 (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 thawed fast in a room temperature water bath with occasional mixing. Once they are almost thawed, place them in a crushed ice bath. **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 reagents well before use.

STEP 1: SPECIMEN DILUTION

Dilute the mixed plasma or serum samples 1 to 2 (e. g. 150 µl + 150 µl) with the sample diluent buffer, mix well. Samples with G-17 levels greater than the highest calibrator can be further diluted with sample diluent buffer (up to 1 to 8 dilution). Return the rest of the sample back to the freezer.

STEP 2: SAMPLE

Mix and pipette 100 µl of the blank solution (BS), 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. Note: It is recommended that the samples are dispensed into the wells within 10 minutes to avoid assay drift within the plate.

	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.			

Figure 1. Pipetting Order.

STEP 3: WASHING

Wash the strips with 3 x 350 µl of the diluted washing buffer.

STEP 4: CONJUGATE

Pipette 100 µl of the mixed dilution (1 to 100 diluted) of conjugate solution into the emptied wells with an 8-channel pipette. Cover the plate with the incubation cover. Incubate for 60 minutes at 37°C.

STEP 5: WASHING

Wash the strips with 3 x 350 µl of the diluted washing buffer.

STEP 6: SUBSTRATE

Pipette 100 µl of the 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 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 Gastrin-17 Advanced ELISA kit is provided with the control. 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. Expected control result must be obtained in order that the results can be accepted.

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 G-17 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, 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 G-17 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 G-17 value from the calibration curve. A typical calibration curve is shown in Figure 2.

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 the 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.

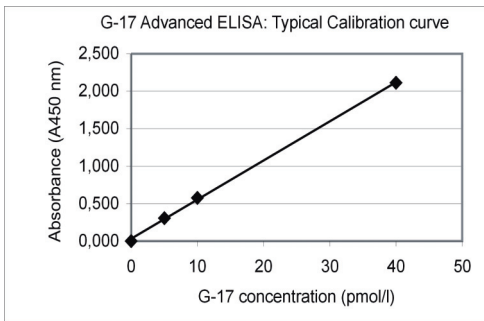


Figure 2. Example of a Typical Calibration Curve.

10.3. Interpretation of the Results

In patients with *H. pylori* infection, a low fasting level of plasma/serum gastrin-17 (<1.0 pmol/l) suggests two possibilities: 1) the antral mucosa is atrophic (moderate or severe atrophic antral gastritis) and/or 2) the stomach is strongly acidic - the acid secretion is so high that it inhibits the release of gastrin-17 into the circulation from antral G-cells.

In patients without *H. pylori* infection, a low fasting level of gastrin-17 (<1.0 pmol/l) is an indicator only of the high acid output and of the risk of gastroesophageal reflux disease and Barrett's esophagus. Consequently, an additional postprandial gastrin-17 test is not needed.

A low fasting level of gastrin-17 in both *H. pylori*-negative and positive patients is an indication for gastroscopy and examination of biopsy specimens. In the former cases, due to the high acid output, there is an increased risk of gastroesophageal reflux (GERD)-related diseases. In the latter cases, due to the atrophic gastritis in antrum, there is an increased risk of neoplastic lesions in the stomach, or an increased risk of peptic ulcer diseases.

If in patients with *H. pylori* -infection the postprandial gastrin-17 level is low, the antral mucosa is atrophic (moderate or severe atrophic antral gastritis). In spite of this, these patients can also be hyperacidic, providing that the plasma/serum pepsinogen I is at normal level or elevated (50

µg/l or more).

Gastrin-17 Advanced ELISA method is specific for amidated G-17 measurements in plasma or serum. The G-17 results cannot be compared with the results of total gastrin assays or used interchangeably. This Biohit Gastrin-17 Advanced ELISA assay should not be used for diagnosis of gastrinoma, since the circulating gastrins e.g. in gastrinoma patients vary extensively, the molecular heterogeneity is accordingly unpredictable, and tends to shift toward larger, less processed gastrin forms (13).

11. LIMITATIONS OF THE PROCEDURE

As with any diagnostic procedure, the Biohit Gastrin-17 Advanced ELISA test results must be interpreted together with the patient's clinical presentation and any other information available to the physician.

12. PERFORMANCE CHARACTERISTICS

Imprecision

For determination of inter-assay variation, a panel of ten matching serum and EDTA plasma samples was assayed 40 times (assayed in duplicate) over 20 days for each level of the test material. In the inter-assay variation for serum samples, the range for the means was from 1.2 pmol/L to 35.2 pmol/L, the standard deviation from 0.2 pmol/L to 1.6 pmol/L, and the %CV from 4.6% to 14.6%. For EDTA-plasma samples, the means, standard deviations and ranges were from 1.0 pmol/L to 32.6 pmol/L, from 0.2 pmol/L to 1.5 pmol/L, and from 4.4% to 17.6%, respectively.

A panel consisting of four matching serum and EDTA plasma samples were run in twenty one replicates in three separate runs. Results were used for the determination of intra-assay variation. In the intra-assay variation for serum samples, the range for means was from 2.1 pmol/L to 39.0 pmol/L, the standard deviations from 0.2 pmol/L to 1.7 pmol/L, and the %CV from 4.2% to 9.5%. For EDTA plasma samples, the means ranged from 2.3 pmol/L to 39.8 pmol/L, standard deviations from 0.2 pmol/L to 1.2 pmol/L, and %CV from 3.1% to 10.1%.

Analytical sensitivity

The sensitivity of the Gastrin-17 Advanced 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. LoB was found to be 0.5 pmol/l.

The limit of detection (LoD) that 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 G-17 levels close to the observed LoB value. The LoD was calculated according to the formula that is presented in NCCLS EP17-A, and was found to be 0.7 pmol/l for EDTA plasma and 0.8 pmol/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 was calculated to be $1.2 \times \text{LoD} = 0.8 \text{ pmol/l}$ for EDTA plasma and 1.0 pmol/l for serum samples.

Linearity

The EDTA plasma samples were tested in serial dilutions with the sample diluent buffer to determine the linearity of Biohit Gastrin-17 Advanced ELISA. Results are listed in a table below.

Sample	Dilution factor	Observed pmol/l	Expected pmol/l	Recovery (%)
1	1	52.3	-	-
	2	29.7	26.2	113.6
	4	14.7	13.1	112.4
2	1	38.2	-	-
	2	20.6	19.1	107.9
	4	11.2	9.6	117.3

Recovery

Four EDTA plasma samples were spiked with 2.3, 9.7 and 30.0 pmol/l of human Gastrin-17.

The average recovery was:

2.3 pmol/l	97.8%
9.7 pmol/l	93.3%
30.0 pmol/l	98.9%

Correlation with other Methods

Gastrin-17 Advanced ELISA test was compared with an in-house total carboxyamidated gastrin radioimmunoassay giving the following linear regression formula:

$$y=1.451x-6.761; r=0.951$$

13. REFERENCES

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14. DATE OF ISSUE

Gastrin-17 Advanced ELISA kit insert.

Version 04, 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

Gastrin-17 Advanced ELISA test kit

Cat. No. 601 035

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

	For <i>in vitro</i> diagnostic use
	Catalogue number
	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 2 dilution) patient samples into the wells

*

Incubate for **60 min at 37°C**

*

Wash 3 times with 350 µl of the diluted washing buffer

*

Pipette 100 µl of the diluted (1 to 100 dilution) and mixed conjugate solution into the wells

*

Incubate for **60 min at 37°C**

*

Wash 3 times with 350 µl of the diluted washing buffer

*

Pipette 100 µl of the mixed substrate solution into the wells

*

Incubate **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