

# **ab133033 – Human Gastrin I ELISA Kit (GAST)**

## Instructions for Use

For quantitative detection of Gastrin I (GAST) in tissue culture media, Human serum and Plasma (citrate).

This product is for research use only and is not intended for diagnostic use.

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## 1. BACKGROUND

Abcam's Gastrin 1 (GAST) *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Gastrin 1 (GAST) in tissue culture media, Human serum and Plasma (citrate).

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated Gastrin 1 antigen and a polyclonal rabbit antibody specific to Gastrin 1. After incubation the excess reagents are washed away. pNpp substrate is added and catalyzed by AP to produce a yellow color. The optical density of the yellow coloration at 405 nm is inversely proportional to the amount of Gastrin 1 captured in the plate.

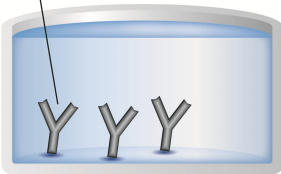
Gastrins are a family of sequence-related carboxyamided peptides produced by endocrine G Cells of the antrum mucosa in response to a number of stimuli associated with digestion. Antral distension, partially digested proteins, amino acids, and vagal stimulation resulting from smelling, tasting, chewing or swallowing food all contribute to gastrin release from G Cell storage. In addition, caffeine, alcohol, hypoglycemia, antacids and elevated calcium levels will also stimulate gastrin release. Increased serum gastrin levels are associated with duodenal ulcers, *Helicobacter pylori* infections, colorectal carcinomas, and other tumors and cancerous lesions. Gastrin 1 is the most potent stimulator of gastric acid secretion.

Gastrin 1 is synthesized as a 101 residue pre-pro-peptide on the rough endoplasmic reticulum, then post-translationally modified by cleavage and alpha-amidation to result in the active forms G34, G17 and G13/14; Big, Little and Mini-Gastrins respectively. Other forms also exist but are not considered biologically significant. There are two types of G17 and G34, type II is sulfated at the tyrosine residue, while

type I is not. Both G34 and G17 circulate and contribute to the stimulation of gastric acid secretion but have different clearance rates. In man, G17 has a circulating half-life of about 9 minutes while G34 has a half-life of about 35 minutes. G34 is the major circulating Gastrin 1 in fasting serum, but with G17, increases two to three-fold after feeding until both are present in approximately equal amounts.

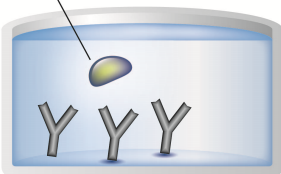
## 2. ASSAY SUMMARY

Capture Antibody



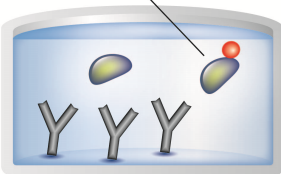
Prepare all reagents and samples as instructed.

Sample



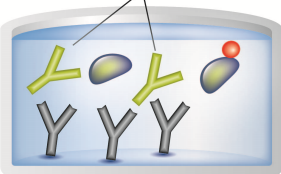
Add standards and samples to appropriate wells.

Labeled AP-Conjugate



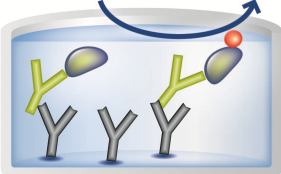
Add prepared labeled AP-conjugate to appropriate wells.

Target Specific Antibody



Add Gastrin 1 antibody to appropriate wells. Incubate at room temperature.

Substrate      Colored Product



Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

### 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- The activity of the alkaline phosphatase conjugate is dependent on the presence of  $Mg^{2+}$  and  $Zn^{2+}$  ions. The activity of the conjugate is affected by concentrations of chelators ( $>10$  mM) such as EDTA and EGTA
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results
- The Gastrin 1 Standard provided, is supplied in ethanolic buffer at a pH optimized to maintain Gastrin 1 integrity. Care should be taken handling this material because of the known and unknown effects of Gastrin 1.

#### 4. STORAGE AND STABILITY

**Store kit at +4°C immediately upon receipt. Avoid multiple freeze-thaw cycles.**

Refer to list of materials supplied for storage conditions of individual components.

#### 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 Wells	+4°C
Gastrin 1 Alkaline Phosphatase Conjugate	5 mL	+4°C
Gastrin 1 Antibody	5 mL	+4°C
Human Gastrin 1 Standard	500 µL	+4°C
Assay Buffer	27 mL	+4°C
20X Wash Buffer Concentrate	27 mL	+4°C
pNpp Substrate	20 mL	+4°C
Stop Solution (Trisodium phosphate)	5 mL	+4°C

### **6. MATERIALS REQUIRED, NOT SUPPLIED**

These materials are not included in the kit, but will be required:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Deionized water
- 37 °C Incubator

### **7. LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted



### 8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

## 9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

### 9.1 **Gastrin 1 Conjugate 1:10 Dilution for Total Activity Measurement**

Prepare the Conjugate 1:10 Dilution by diluting 50 µL of the supplied conjugate with 450 µL of Assay Buffer. The dilution should be used within 3 hours of preparation. This is intended for use in the Total Activity wells only.

### 9.2 **Gastrin 1 Alkaline Phosphatase Conjugate**

Allow the Gastrin 1 Alkaline Phosphatase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20 °C.

### 9.3 **1X Wash Buffer**

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

## 10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation.

10.1 For:

10.1.1 **Serum/Plasma** samples dilute the Gastrin 1 standard with Assay Buffer.

10.1.2 **Tissue culture media** samples dilute the Gastrin 1 standard with tissue culture media.

10.2 Allow the reconstituted 100,000 pg/mL Gastrin 1 Stock Standard solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.3 Label five tubes with numbers 1 – 5.

10.4 Add 900 µL of appropriate diluent (Assay Buffer or Tissue Culture Media) to tube #1

10.5 Add 750 µL of appropriate diluent to tubes #2-#5

10.6 Prepare a 10,000 pg/mL **Standard 1** by adding 100 µL of the 10,000 pg/mL Stock Standard to tube 1. Vortex thoroughly.

10.7 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Vortex thoroughly.

10.8 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Vortex thoroughly.

10.9 Using the table below as a guide, repeat for tubes 4 and 5.

## ASSAY PREPARATION

Standard	Sample to Dilute	Volume to Dilute (μL)	Volume of Diluent (μL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Standard	100	900	100,000	10,000
2	Standard 1	250	750	10,000	2,500
3	Standard 2	250	750	2,500	625
4	Standard 3	250	750	625	156.25
5	Standard 4	250	750	156.25	39.1



## 11. SAMPLE COLLECTION AND STORAGE

- The Gastrin 1 (human), EIA is compatible with human Gastrin samples in a number of matrices after dilution in Assay Buffer. Sufficient dilution of samples in this Assay Buffer may allow them to be read directly without extraction.
- Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer. There may be a small change in binding associated with running the standards and samples in media.
- Users should only use standard curves generated in media or buffer to calculate concentrations of Gastrin 1 in the appropriate matrix. The end user must verify that the recommended dilutions are appropriate for their samples.
- Samples containing rabbit IgG may interfere with the assay.
- We recommend extraction of samples for accurate determinations of human Gastrin 1 if the sample cannot be sufficiently diluted without being too dilute to measure. An extraction protocol is outlined below. Because of the labile nature of Gastrin, we recommend several precautions in collecting and analyzing samples.
- Blood samples should be drawn into chilled EDTA (1 mg/mL blood) or serum tubes containing Aprotinin (500 KIU/mL or 10.6 TIU/mL of blood). Centrifuge the samples at 1,600 x g for 15 minutes at 0 °C. Transfer the plasma or serum to a plastic tube and store at -70 °C or lower for long term storage. Avoid repeated freeze/thaw cycles. The stability of some peptides is improved by the addition of a protease inhibitor cocktail to the sample before freezing.
- Extraction of the sample, if necessary, should be carried out using a similar protocol to the one described below:

- 11 Add an equal volume of 1% trifluoroacetic acid (TFA) in water to the sample. Centrifuge at 17,000 x g for 15 minutes at 4°C to clarify and save the supernatant.
- 11.2 Equilibrate a 200 mg C18 Sep-Pak column with 1 mL of acetonitrile, followed by 10-25 mL of 1% TFA in water
- 11.3 Apply the supernatant to the Sep-Pak column and wash with 10-20 mL of 1% TFA in water. Discard wash
- 11.4 Elute the sample slowly by applying 3 mL of acetonitrile: 1% TFA in water 60:40. Collect the eluant in a plastic tube.
- 11.5 Evaporate to dryness using a centrifugal concentrator under vacuum. Store at -20°C.
- 11.6 Reconstitute with Assay Buffer and measure immediately.

## 12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

	1	2	3	4
A	B <sub>s</sub>	Std 1	Std 5	Sample 4
B	B <sub>s</sub>	Std 1	Std 5	Sample 4
C	TA	Std 2	Sample 1	Sample 5
D	TA	Std 2	Sample 1	Sample 5
E	NSB	Std 3	Sample 2	etc
F	NSB	Std 3	Sample 2	etc
G	B <sub>0</sub>	Std 4	Sample 3	
H	B <sub>0</sub>	Std 4	Sample 3	

Plate layout shows controls, blanks and standards required for each assay. Use additional strips of wells to assay all your samples.

### Key:

**B<sub>s</sub>** = Blank; contains substrate only.

**TA** = Total Activity; contains conjugate (5 µL) and substrate.

**NSB** = Non-specific binding; contains standard diluent, assay buffer, conjugate and substrate.

**B<sub>0</sub>** = 0 pg/mL standard; contains standard diluent, conjugate, antibody and substrate

## 13. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use
- It is recommended to assay all standards, controls and samples in duplicate
- Refer to the recommended plate layout in Section 12 before proceeding with the assay

13.1 Add 150  $\mu$ L appropriate diluent\* into the NSB (non-specific binding) wells. (\*Use the same diluent used to prepare standards in section 10, either Assay Buffer or Tissue Culture Media).

13.2 Add 100  $\mu$ L appropriate diluent (Assay Buffer or tissue culture media) into the B<sub>0</sub> (0 pg/mL standard) wells.

13.3 Add 100  $\mu$ L of standards and samples into the appropriate wells.

13.4 Add 50  $\mu$ L of Gastrin 1 Alkaline Phosphatase Conjugate (blue) into NSB, B<sub>0</sub>, standard and sample wells, i.e. not the Total Activity (TA) and B<sub>s</sub> wells.

13.5 Add 50  $\mu$ L of Gastrin 1 antibody (yellow) into B<sub>0</sub>, standard and sample wells, i.e. not B<sub>s</sub>, TA and NSB wells.

*Note:* Every well used should be green in color except the NSB wells which should be blue. The B<sub>0</sub> and TA wells are empty at this point and have no color.

13.6 Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided.

13.7 Empty the contents of the wells and wash by adding 200  $\mu$ L of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.8 Add 5  $\mu$ L of the Gastrin 1 Alkaline Phosphatase Conjugate to the TA wells only.



- 13.9 Add 200  $\mu$ L of the pNpp Substrate solution to every well. Incubate at 37°C for 3 hours.
- 13.10 Add 50  $\mu$ L Stop Solution into each well. The plate should be read immediately.
- 13.11 Blank the plate reader against the blank wells, read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the blank wells, manually subtract the mean optical density of the blank wells from all readings.

## 14. CALCULATIONS

15. Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

$$\text{Average Net OD} = \text{Average Bound OD} - \text{Average NSB OD}$$

- 14.2 Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells ( $B_0$ ), using the following formula

$$\text{Percent Bound} = \frac{\text{Average Net OD}}{\text{Average Net } B_0 \text{ OD}} \times 100$$

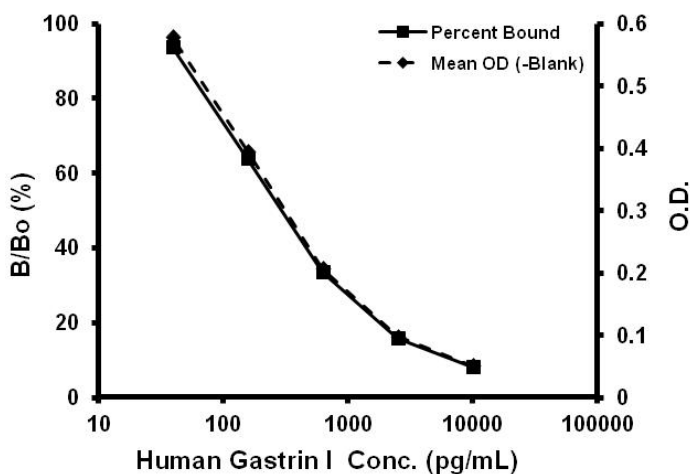
- 14.3 Plot the Percent Bound ( $B/B_0$ ) and the net OD versus concentration of Gastrin I for the standards. The concentration of Gastrin I in the unknowns can be determined by interpolation of net OD values.

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor

## 16. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Mean OD (-B <sub>s</sub> )	Percent Bound	Human Gastrin 1 (pg/mL)
B <sub>s</sub>	(0.093)		
TA	0.274		
NSB	0	0	
B <sub>0</sub>	0.616	100	0
Standard 1	0.051	8.20	10,000
Standard 2	0.098	15.9	2,500
Standard 3	0.207	33.5	625
Standard 4	0.395	64.0	156.25
Standard 5	0.578	93.8	39.1
Unknown 1	0.250	40.6	439
Unknown 2	0.153	24.8	1,068

## TYPICAL QUALITY CONTROL PARAMETERS –

Total Activity Added	= $0.274 \times 10 \times 10 = 27.4$
%B <sub>0</sub> /TA	= 2.2%
Quality of Fit	= 1.0000 (Calculated from 4 parameter logistic curve fit)
20% Intercept	= 1,605 pg/mL
50% Intercept	= 282 pg/mL
80% Intercept	= 78 pg/mL

## 17. TYPICAL SAMPLE VALUES

### SENSITIVITY –

The sensitivity, minimum detectable dose of Gastrin 1 using this Abcam ELISA kit was found to be 7.27 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 5. The detection limit was determined as the concentration of Gastrin 1 measured at two standard deviations from the zero along the standard curve.

### SAMPLE RECOVERY –

Recovery was determined by Gastrin 1 into tissue culture media. Mean recoveries are as follows:

Sample Type	Average Recovery (%)	Recommended Dilution
Tissue Culture Media	100.6	None
Human Serum	101.9	≥ 1:8
Human Plasma (Citrate)	107.9	≥ 1:8

## LINEARITY OF DILUTION –

A sample containing 4,576 pg/mL Gastrin 1 was diluted 6 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual Gastrin 1 concentration versus measured Gastrin 1 concentration.

The line obtained had a slope of 0.9734 and a correlation coefficient of 0.999.

## PRECISION –

	Gastrin 1 (pg/mL)	Intra-Assay %CV
Low	278	8.8
Medium	471	3.7
High	920	5.7

	Gastrin 1 (pg/mL)	Inter-Assay %CV
Low	278	4.4
Medium	328	6.5
High	1,065	3.4

## 18. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant Gastrin 1.

### CROSS REACTIVITY –

The cross reaction of the antibody calculated at 50% is:

Gastrin 1 (G17-1)	100 %
Minigastrin (G13-I)	74.6 %
Rat Gastrin 1	70.7 %
Gastrin 1I (G17-II, sulfated)	9.3%
Cholesystokinin 26-33 (CCK-8)	2.67 %
Gastrin Tetrapeptide (CCK-4)	1.6 %
Big Gastrin (G34-I)	0.8 %
Gastrin Releasing Peptide (GRP)	<0.001 %
Gastrin 1inhibitory Polypeptide (GIP)	<0.001 %
Glucagon	<0.001 %
Bombesin	<0.001 %
Pancreatic Polypeptide	<0.001 %
Vasoactive Intestinal Peptide (VIP)	<0.001 %
Somatostatin-14	<0.001 %

## 19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

### 20. NOTES









**For all technical and commercial enquires please go to:**

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)