ab133049 – Gastrin 1 Rat ELISA Kit

Instructions for Use

For quantitative detection of Gastrin 1 in Tissue Culture Media, Rat Serum and Plasma (EDTA).

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

Version 3 Last Updated 6 February 2019

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1. BACKGROUND

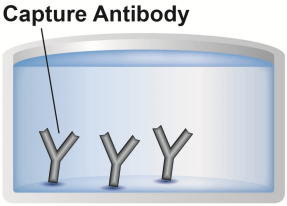
Abcam's Gastrin 1 Rat *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Gastrin 1 in tissue.

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 I antigen and a polyclonal rabbit antibody specific to Gastrin I. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the coloration is inversely proportional to the amount of Gastrin I captured in the plate.

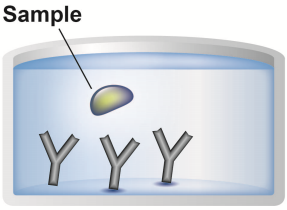
Gastrins are a family of sequence-related carboxyamidated peptides produced by endocrine G Cells of the antrum mucosa and duodenum in response to a number of stimuli associated with digestion. Antral distension, partially digested proteins, amino acids, and vagal stimulation resulting from smelling, 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 is the most potent stimulator of gastric acid secretion. Gastrin is synthesized as a 104 residue pre-pro-peptide on the rough endoplasmic reticulum, and then post-translationally modified by cleavage and alpha-amidation to result in the active forms G34 and G17. 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 12 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. G34 is the major circulating Gastrin in fasting

serum, but with G17, increases two to three-fold after feeding until both are present in approximately equal amounts.

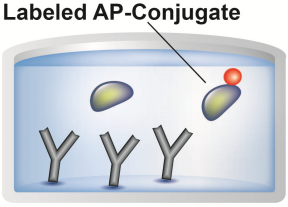
2. ASSAY SUMMARY



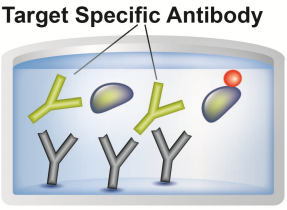
Prepare all reagents and samples as instructed.



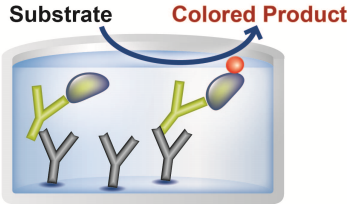
Add standards and samples to appropriate wells.



Add prepared labeled AP-conjugate to appropriate wells.



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



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 (Before Preparation)
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 Wells	+4°C
Gastrin 1 Alkaline Phosphatase Conjugate	6 mL	+4°C
Gastrin 1 Antibody	6 mL	+4°C
Rat Gastrin 1 Standard	250 µL	+4°C
Assay Buffer	30 mL	+4°C
20X Wash Buffer Concentrate	30 mL	+4°C
pNpp Substrate	23 mL	+4°C
Stop Solution	6 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
- 1% trifluroacetic acid (TFA)
- 200 mg C18 Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of Gastrin 1)
- Deionized water

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.

9.2 **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 plasma/serum** samples: dilute Gastrin 1 standards with Assay Buffer.

10.2 **For cell culture supernatant** samples: dilute Gastrin 1 standards with tissue culture media.

10.3 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.

10.4 Label seven tubes with numbers #1 – #7

10.5 Add 475 µL of appropriate diluent (Assay Buffer or Tissue Culture Media) to tube #1.

10.6 Add 250 µL appropriate diluent to tubes #2 through #7

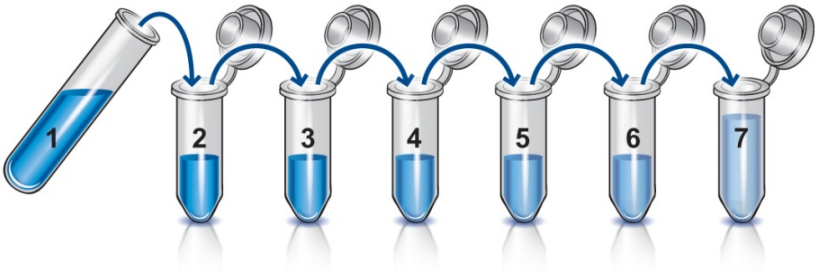
10.7 Prepare a 5,000 pg/mL **Standard 1** by adding 25 µL of the 100,000 pg/mL Stock Standard tube 1. Vortex thoroughly.

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

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

10.10 Using the table below as a guide, repeat for tubes #4 through to #7.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock	25	475	100,000	5,000
2	Standard 1	250	250	5,000	2,500
3	Standard 2	250	250	2,500	1,250
4	Standard 3	250	250	1,250	625
5	Standard 4	250	250	625	313
6	Standard 5	250	250	313	156
7	Standard 6	250	250	156	78.1



11. SAMPLE COLLECTION AND STORAGE

- The Gastrin 1 kit is compatible with Gastrin 1 samples in a wide range of matrices after dilution in Assay Buffer. However, the end user must verify that the recommended dilutions are appropriate for their samples. Samples containing mouse IgG may interfere with the assay
- 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 will be a small change in binding associated with running the standards and samples in media
- Blood samples should be drawn into chilled EDTA (1mg/mL blood) or serum tubes containing Aprotonin (500 KIU/mL). 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

11.1. Extraction of the sample should be carried out using a similar protocol below:

11.1.1. 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.

11.1.2. Equilibrate a 200 mg C 18 Sep-Pak column with 1 mL of acetonitrile, followed by 10-25 mL of 1% TFA in water.

11.1.3. Apply the supernatant to the Sep-Pak column and wash with 10-20 mL of 1% TFA in water. Discard wash.

11.1.4. Elute the sample slowly by applying 3 mL of acetonitrile: 1% TFA in water 60:40. Collect eluant in a plastic tube.

11.1.5. Evaporate to dryness using a centrifugal concentrator under vacuum. Store at -20 °C

11.1.6. 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 each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- 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_s	Std 1	Std 5	Sample 1
B	B_s	Std 1	Std 5	Sample 1
C	TA	Std 2	Std 6	Sample 2
D	TA	Std 2	Std 6	Sample 2
E	NSB	Std 3	Std 7	etc
F	NSB	Std 3	Std 7	etc
G	B_0	Std 4	Std 8	
H	B_0	Std 4	Std 8	

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

Key:

B_s = 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_0 = 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 50 µL of standard diluent (Assay Buffer or tissue culture media) into the NSB and B_O wells.

13.2 Add 50 µL of prepared standards and diluted samples to appropriate wells.

13.3 Add 25 µL of Assay Buffer into the NSB wells.

13.4 Add 25 µL of Gastrin 1 Alkaline Phosphatase Conjugate (blue) into NSB, B0, standard and sample wells, i.e. not the Total Activity (TA) and Bs wells.

13.5 Add 25 µL of Gastrin 1 Antibody (yellow) into B_0, standard and sample wells, i.e. not B_s, TA and NSB wells.
Note: Every well used should be green in color except the NSB wells which should be blue. The B_s 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 400 µL 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 µL of the light blue conjugate 1:10 dilution (see step 9.1 Reagent Preparation section) to the TA wells only.

13.9 Add 200 µL of the pNpp Substrate solution to every well. Incubate at 37°C for 3 hours without shaking.

13.10 Add 50 µL Stop Solution into each well.

13.11 Read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm.

14. CALCULATIONS

14.1 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.

Average Net OD = Average Bound OD - Average NSB OD

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

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

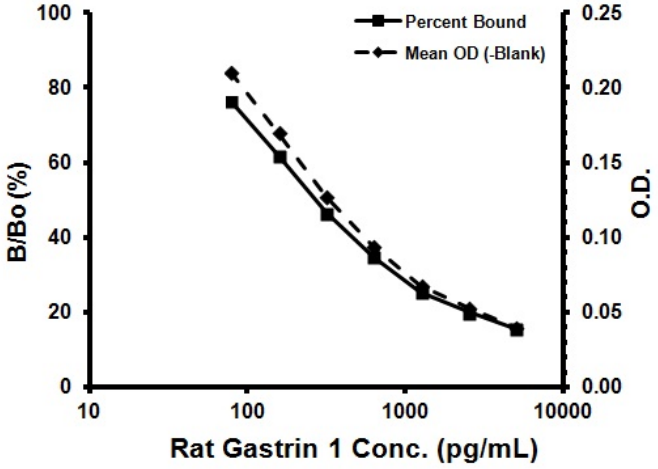
14.3 Plot the Percent Bound (B/B_0) and the net OD versus concentration of Gastrin 1 for the standards. The concentration of Gastrin 1 in the unknowns can be determined by interpolation of net OD values.

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.

15. 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_s)	Bound (%)	Gastrin 1 (pg/mL)
B_s	(0.104)	-	-
TA	0.352	-	-
NSB	0.000	0	-
Standard 1	0.040	15.6	5,000
Standard 2	0.053	20	2,500
Standard 3	0.068	25.4	1,250
Standard 4	0.094	34.8	625
Standard 5	0.127	46.6	313
Standard 6	0.170	62	156
Standard 7	0.210	76.34	78.1
B_0	0.276	100	0
Unknown1	0.205	74.8	84.8
Unknown 2	0.082	30.3	842

Typical Quality Control Parameters

Total Activity Added	= 0.352 x 10 x 10 = 35.2
NSB	= 0.0%
$\%B_0/TA$	= 7.9%
Quality of Fit	= 1.0000 (Calculated from 4 parameter logistic curve fit)
20% Intercept	= 2,346 pg/mL
50% Intercept	= 269 pg/mL
80% Intercept	= 65 pg/mL

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

Sensitivity was calculated by determining the average optical density bound for 16 wells run as B_0 (Standard 8), and comparing to the average optical density for 16 wells run with Standard 7. The detection limit was determined as the concentration of cGMP measured at 2 standard deviations from the zero along the standard curve was determined to be 78.10 pg/mL.

SAMPLE RECOVERY –

Recovery was determined by Gastrin 1 into Tissue Culture Media, rat serum and plasma (EDTA). Mean recoveries are as follows:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	92	None
Rat serum	93	≥1:16
Rat Plasma (EDTA)	95	≥1:16

LINEARITY OF DILUTION –

A sample containing 3,241 pg/mL Gastrin 1 was diluted 5 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 0.905 with a correlation coefficient of 0.999.

PRECISION –

Intra-Assay

	Gastrin 1 (pg/mL)	%CV
Low	79.9	8.3
Medium	260	8.8
High	937	8.5

Inter-Assay

	Gastrin 1 (pg/mL)	%CV
Low	86.5	14.7
Medium	233	12.6
High	904	7.0

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The cross reactivities for a number of related compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 5,000,000 to 0.5 pg/mL. These samples were then measured in the rat Gastrin I assay, and the measured Gastrin I concentration at 50% B/B_0 calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage:

Compounds	Cross Reactivity (%)
Rat Gastrin 1	100
Cholesystokinin 26-33 (CCK-8)	100
Human Gastrin 1	30
mouse Gastrin 1	20.5
Minigastrin (G13-I)	11
Gastrin Tetrapeptide (CCK-4)	0.75
Glucagon	0.5
Gastrin Inhibitory Polypeptide (GIP)	<0.2
Bombesin	<0.2
Somatostatin-14	<0.2
Gastrin Releasing Peptide (GRP)	<0.2

18. 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

19. NOTES

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