# Gast (Rat) ELISA Kit

Catalog Number KA0319

96 assays

Version: 02

Intended for research use only

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## Introduction

### Background

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[3]. 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[3-6]. In addition, caffeine, alcohol, hypoglycemia, antacids and elevated calcium levels will also stimulate gastrin release[4,7]. Increased serum gastrin levels are associated with duodenal ulcers[6], Helicobacter pylori infections[9], colorectal carcinomas[10-12], and other tumors and cancerous lesions[13]. Gastrin is the most potent stimulator of gastric acid secretion.

rat Gastrin I (G17) Pyr-Arg-Pro-Pro-Met-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH2

Gastrin is synthesized as a 104 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 and G17[3,6.] 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[13]. 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. Normal serum levels in rats have been reported over a range of 40-169 pg/mL with possible variations between animal sources[3].

### Principle of the Assay

The Gast (Rat) ELISA Kit is a competitive immunoassay for the quantitative determination of Gastrin in samples. The kit uses a polyclonal antibody to Gastrin I to bind, in a competitive manner, the Gastrin I in the sample or an alkaline phosphatase molecule which has Gastrin I covalently attached to it. After a simultaneous incubation at room temperature the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of Gastrin I in either standards or samples. The measured optical density is used to calculate the concentration of Gastrin I. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

## General Information

### Materials Supplied

List of component

| Component | Description | Amount |
|---|---|---|
| Goat anti-Rabbit IgG Microtiter Plate | A plate using break-apart strips coated with goat antibody specific to rabbit IgG. | One Plate of 96 wells |
| Gastrin I Conjugate | A blue solution of alkaline phosphatase conjugated with Gastrin I. | 6 mL |
| Gastrin I Antibody | A yellow solution of a rabbit polyclonal antibody to Gastrin I. | 6 mL |
| Assay Buffer | Tris buffered saline containing proteins and sodium azide as preservative. | 30 mL |
| Wash Buffer Concentrate | Tris buffered saline containing detergents. | 30 mL |
| rat Gastrin I Standard | A solution of 100,000 pg/mL rat Gastrin I. | 0.25 mL |
| pNpp Substrate | A solution of p-nitrophenyl phosphate. | 23 mL |
| Stop Solution | A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic. | 6 mL |
| Plate Sealer | - | 1 each |

### Storage Instruction

All components of this kit are stable at 4℃ until the kit's expiration date.

### Materials Required but Not Supplied

- ✓ Deionized or distilled water.
- ✓ Precision pipets for volumes between 5 µL and 1,000 µL.
- ✓ Repeater pipets for dispensing 50 µL and 200 µL.
- ✓ Disposable beakers for diluting buffer concentrates.
- ✓ Graduated cylinders.
- ✓ A microplate shaker.
- ✓ A 37℃ Incubator.
- ✓ Adsorbent paper for blotting.
- ✓ Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

## Precautions for Use

- Precautions

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- ✓ 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 rat Gastrin I Standard provided is supplied in ethanolic buffer at a pH optimized to maintain Gastrin integrity. Care should be taken in handling this material because of the known and unknown effects of Gastrin I.

- Procedural Notes:

- ✓ Do not mix components from different kit lots or use reagents beyond the kit expiration date.
- ✓ Allow all reagents to warm to room temperature for at least 30 minutes before opening.
- ✓ Standards can be made up in either glass or plastic tubes.
- ✓ Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
- ✓ Pipet 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 ℃ 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.

## Assay Protocol

### Reagent Preparation

- ✓ rat Gastrin I Standard

  Allow the 100,000 pg/mL rat Gastrin I standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 500 µL of standard diluent (Assay Buffer or Tissue Culture Medium) into tube #1. Pipet 250 µL of standard diluent into tubes #2 through #7. Remove 25 µL of diluent from tube #1. Add 25 µL of the 100,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 and #7.

  The concentration of rat Gastrin I in tubes #1 through #7 will be 5,000, 2,500, 1,250, 625, 313, 156 and 78.1 pg/mL, respectively. See rat Gastrin I Assay Layout Sheet for dilution details.

  Diluted standards should be used within 60 minutes of preparation.

- ✓ Gastrin I 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.

- ✓ Wash Buffer

  Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration date, or for 3 months, whichever is earlier.

### Sample Preparation

The rat Gastrin I enzyme immunoassay is compatible with rat Gastrin I 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 I in the appropriate matrix. Please refer to the Sample Recovery recommendations for details of suggested dilutions. However, 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 Gastrin I if the sample cannot be sufficently diluted without being too dilute to measure in the assay range. Normal rat sera and plasma require extraction. An extraction protocol is outlined below. Because of the labile nature of Gastrin I, we recommend

several precautions in collecting and analyzing samples.

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℃. Transfer the plasma or serum to a plastic tube and store at -70℃ 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 should be carried out using a similar protocol to the one described below.

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℃ to clarify and save the supernatant.
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.
3. Apply the supernatant to the Sep-Pak column and wash with 10-20 mL of 1% TFA in water. Discard wash.
4. Elute the sample slowly by applying 3 mL of acetonitrile: 1% TFA in water 60:40. Collect the eluant in a plastic tube.
5. Evaporate to dryness using a centrifugal concentrator under vacuum. Store at -20℃.
6. Reconstitute with Assay Buffer and measure immediately.

Please note that recovery of peptides from extraction processes can be variable. It is important to optimize any process to obtain optimum recoveries. Extraction efficiencies can be determined by a number of methods, including the use of radioactive peptide, or by spiking into paired samples and determining the recovery of this known amount of added Gastrin I.

**Assay Procedure**

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4℃.
2. Pipet 50 µL of standard diluent (Assay Buffer or Tissue Culture Medium) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 50 µL of Standards #1 through #7 into the appropriate wells.
4. Pipet 50 µL of the Samples into the appropriate wells
5. Pipet 25 µL of Assay Buffer into the NSB wells.
6. Pipet 25 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 25 µL of yellow Antibody into each well, except the Blank, TA and NSB wells.

*Note: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.*

8. 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, if so desired.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every well. Repeat the wash 2 more times for a total of 3 Washes.
10. 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.
11. Add 5 µL of the light blue Conjugate 1:10 dilution (see step 3, Reagent Preparation) to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Seal plate and incubate at 37℃ for 3 hours.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density 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.

## Data Analysis

### Calculation of Results

Several options are available for the calculation of the concentration of Gastrin I in the samples. We recommend that the data be handled by an immunoassay software package utilizing a weighted 4 parameter logistic curve fitting program. If this type of data reduction software is not readily available, the concentration of Gastrin I can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   Average Net OD = Average Bound OD - Average NSB OD

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

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

3. Using Logit-Log paper plot Percent Bound (B/Bo) versus Concentration of Gastrin I for the standards. Approximate a straight line through the points. The concentration of Gastrin I in the unknowns can be determined by interpolation.

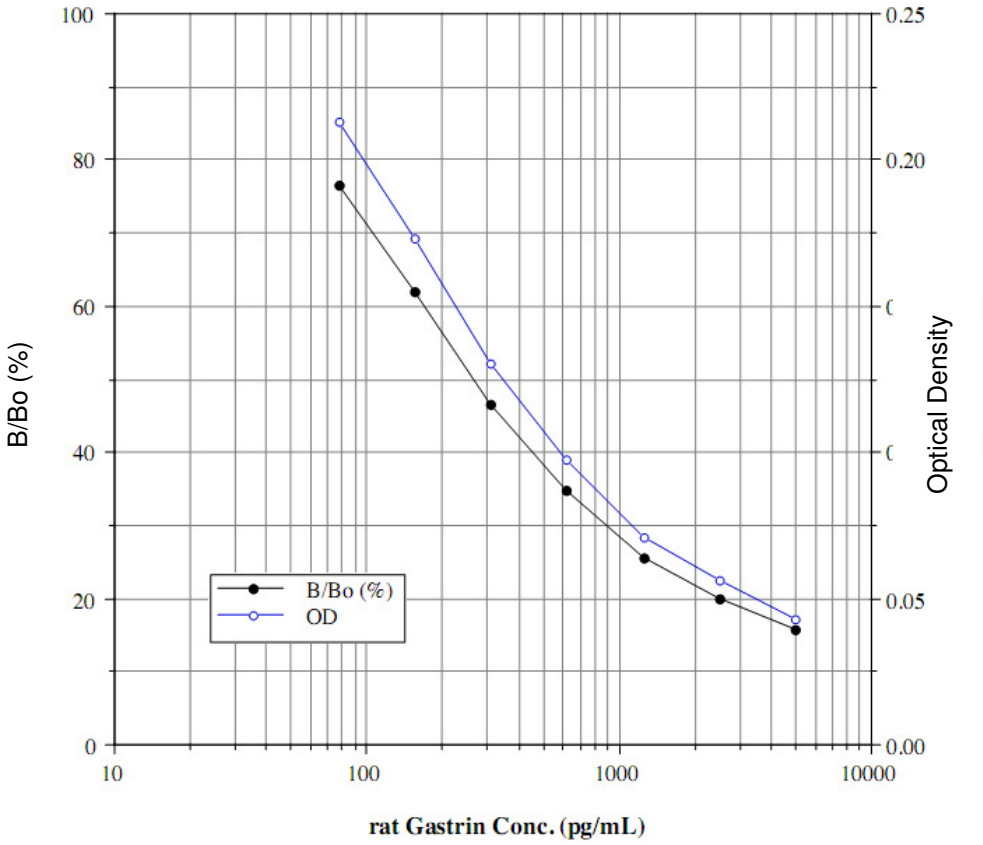
- Typical Results

  The results shown below are for illustration only and should not be used to calculate results from another assay.

| Sample | Mean OD (-Blank) | Average Net OD | Percent Bound | Gastrin I (pg/mL) |
|---|---|---|---|---|
| Blank OD | (0.104) | | | |
| TA | 0.352 | 0.355 | | |
| NSB | 0.000 | -0.003 | | |
| Bo | 0.276 | 0.279 | 100% | 0 |
| S1 | 0.040 | 0.043 | 15.6% | 5,000 |
| S2 | 0.053 | 0.056 | 20.0% | 2,500 |
| S3 | 0.068 | 0.071 | 25.4% | 1,250 |
| S4 | 0.094 | 0.097 | 34.8% | 625 |
| S5 | 0.127 | 0.130 | 46.6% | 313 |
| S6 | 0.170 | 0.173 | 62.0% | 156 |
| S7 | 0.210 | 0.213 | 76.4% | 78.1 |
| Unknown 1 | 0.205 | 0.208 | 74.8% | 84.8 |
| Unknown 2 | 0.082 | 0.085 | 30.3% | 842 |

- Typical Standard Curve

  Typical standard curves are shown below. These curves must not be used to calculate Gastrin I concentrations; each user must run a standard curve for each assay.




- Typical Quality Control Parameters

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

**Performance Characteristics**

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols[15].

✓ Sensitivity

Sensitivity was calculated by determining the average optical density bound for twenty (20) wells run as Bo, and comparing to the average optical density for twenty (20) wells run with Standard #7. The detection limit was determined as the concentration of Gastrin I measured at two (2) standard deviations from the zero along the standard curve.

| | | | | |
|---|---|---|---|---|
| Average Optical Density for the Bo | = | 0.241 ± 0.024 (9.96%) | | |
| Average Optical Density for Standard #7 | = | 0.193 ± 0.012 (6.22%) | | |
| Delta Optical Density (0-78.1 pg/mL) | = | 0.048 | | |
| 2 SD's of the Zero Standard | = | 2 x 0.024 | = | 0.048 |
| Sensitivity | = | $\frac{0.048}{0.048}$ x 78.10 pg/mL | = | 78.10 pg/mL |

✓ Linearity

A sample containing 3,241 pg/mL Gastrin I was serially diluted 5 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual Gastrin I concentration versus measured Gastrin I concentration.

The line obtained had a slope of 0.905 with a correlation coefficient of 0.999.

✓ Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of Gastrin I and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of Gastrin I in multiple assays (n=12).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of Gastrin I determined in these assays as calculated by a 4 parameter logistic curve fitting program.

| | Gastrin I (pg/mL) | Intra-assay %CV | Inter-assay %CV |
|---|---|---|---|
| Low | 79.9 | 8.3 | |
| Medium | 260 | 8.8 | |
| High | 937 | 8.5 | |
| Low | 86.5 | | 14.7 |
| Medium | 233 | | 12.6 |
| High | 904 | | 7.0 |

✓ Cross Reactivities

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/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

| Compound | Cross Reactivity |
|---|---|
| rat Gastrin I | 100% |
| Cholesystokinin 26-33 (CCK-8) | 100% |
| human Gastrin I | 30% |
| mouse Gastrin I | 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% |

✓ Sample Recoveries

Please refer to Sample Handling recommendations and Standard preparation.

Gastrin I concentrations were measured in a variety of different samples including tissue culture media, and rat serum and plasma. Gastrin I was spiked into the undiluted samples which were diluted with the appropriate diluent and then assayed in the kit. The following results were obtained:

| Sample | % Recovery* | Recommended Dilution* |
|---|---|---|
| Tissue Culture Media | 92% | None |
| rat Serum | 93% | ≥1:16 |
| rat EDTA Plasma | 95% | ≥1:16 |

* See Sample Handling instructions.

## Resources

### References


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**Plate Layout**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Blank | Std1 | Std5 | | | | | | | | | |
| B | Blank | Std1 | Std5 | | | | | | | | | |
| C | TA | Std2 | Std6 | | | | | | | | | |
| D | TA | Std2 | Std6 | | | | | | | | | |
| E | NSB | Std3 | Std7 | | | | | | | | | |
| F | NSB | Std3 | Std7 | | | | | | | | | |
| G | Bo | Std4 | | | | | | | | | | |
| H | Bo | Std4 | | | | | | | | | | |