

RayBio[®] Mouse/Rat Gastrin-releasing peptide Enzyme Immunoassay Kit

Catalog #: EIAM-GRP, EIAR-GRP

User Manual
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Caution:
Extraordinarily useful information enclosed



ISO 13485 Certified

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Please read the entire manual carefully before starting your experiment

I. Introduction

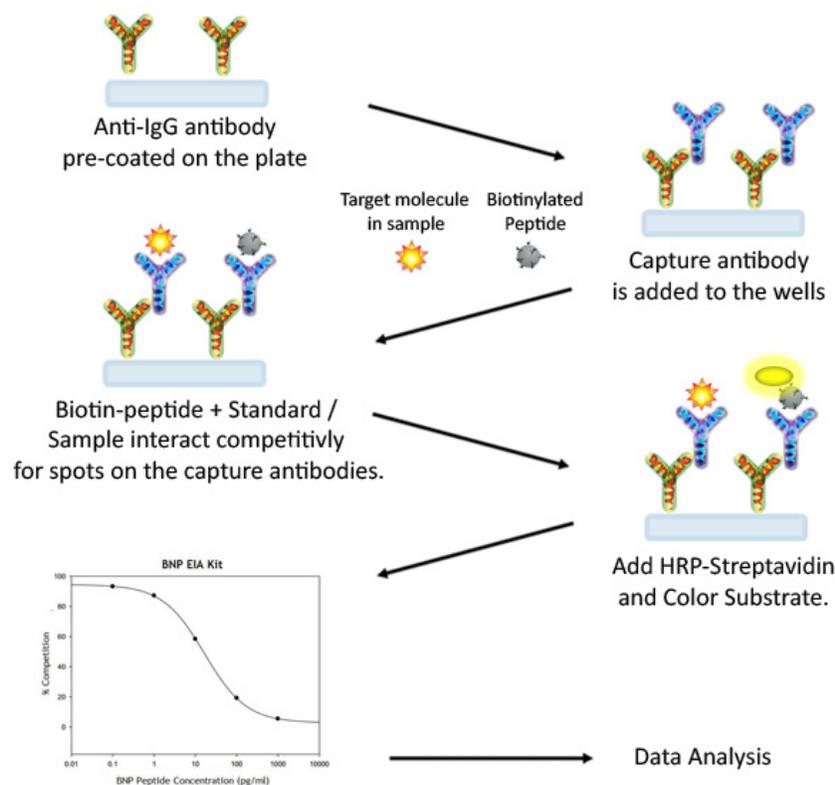
Gastrin-releasing peptide (GRP), is a 27-amino acid neuropeptide. Gastrin-releasing peptide stimulates the release of Gastrin from the G cells of the stomach, and regulates gastric acid secretion and enteric motor function. Gastrin-releasing peptide is also an important growth-modulating factor in developing lung epithelium. It has been used as a tumor marker in the diagnosis of small-cell lung carcinoma, since it is known to be produced by these cancer cells. GRP is also involved in the biology of the circadian system, playing a role in the signaling of light to the master circadian oscillator in the suprachiasmatic nuclei of the hypothalamus. Furthermore, GRP seems to mediate certain aspects of stress.

II. General Description

The RayBio® GRP Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting GRP peptide based on the competitive enzyme immunoassay principle.

In this assay, a biotinylated GRP peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated GRP peptide competes with endogenous (unlabeled) GRP for binding to the anti-GRP antibody. After a wash step, any bound biotinylated GRP then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated GRP peptide and inversely proportional to the amount of endogenous GRP in the standard or samples. A standard curve of known concentration of GRP peptide can be established and the concentration of GRP peptide in the samples can be calculated accordingly.

III. How It Works



IV. Storage

The entire kit may be stored at -20°C to -80°C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at -80°C. **Avoid repeated freeze-thaw cycles.** For prepared reagent storage, see table below.

V. Reagents

Component	Size / Description	Storage / Stability After Preparation
EIA Microplate (Item A)	96 wells (12 strips x 8 wells) coated with secondary antibody.	1 month at 4°C*
Wash Buffer Concentrate (20X) (Item B)	25 ml of 20X concentrated solution.	1 month at 4°C
Standard GRP Peptide (Item C)	2 vials of Lyophilized GRP Peptide. 1 vial is enough to run each standard in duplicate.	Do not store and reuse
Anti-GRP Polyclonal Antibody (Item N)	2 vials of Lyophilized anti-GRP.	Do not store and reuse
Assay Diluent C (Item L)	30 ml diluent for standards and serum or plasma.	N/A
Assay Diluent B (Item E)	15 ml of 5X concentrated buffer. Diluent for standards, cell culture media or other sample types, and HRP-Streptavidin.	1 month at 4°C
Biotinylated GRP Peptide (Item F)	2 vials of Lyophilized Biotinylated GRP Peptide, 1 vial is enough to assay the whole plate.	Do not store and reuse
HRP-Streptavidin Concentrate (Item G)	600 µl 100x concentrated HRP-conjugated streptavidin.	Do not store and reuse
Positive Control (Item M)	1 vial of Lyophilized Positive Control.	Do not store and reuse
TMB One-Step Substrate Reagent (Item H)	12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.	N/A
Stop Solution (Item I)	8 ml of 0.2 M sulfuric acid.	N/A

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

VI. Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes

3. Adjustable 1-25 ml pipettes for reagent preparation
4. 100 ml and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions
9. Orbital shaker
10. Aluminum foil
11. Plastic wrap

VII. Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

Note: **Assay Diluent C** should be used for dilution of samples, Item F and Item C when testing **plasma or serum samples**. **1X Assay Diluent B** should be used for dilution of samples, Item F and Item C when testing **cell culture media or other sample types**.

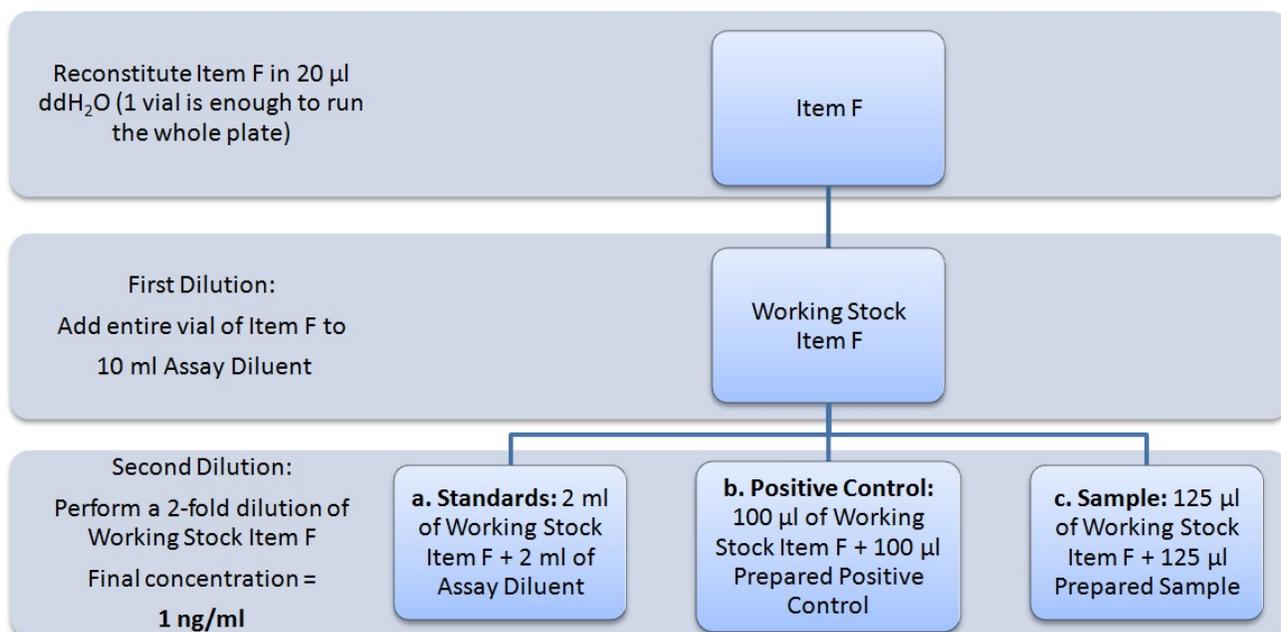
A. Preparation of Plate and Anti-GRP Antibody

1. Equilibrate plate to room temperature before opening the sealed pouch.
2. Label removable 8-well strips as appropriate for your experiment.
3. 5X Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.
4. Briefly centrifuge the anti-GRP antibody vial (Item N) and reconstitute with 55 μ l of 1X Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.
5. The antibody concentrate should then be diluted 100-fold with 1X Assay Diluent B. This is your anti-GRP antibody working solution, which will be used in step 2 of Assay Procedure (Section VIII).

Note: The following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure)

B. Preparation of Biotinylated GRP (Item F)

6. Briefly centrifuge the vial of Biotinylated GRP (Item F) and reconstitute with 20 μ l of ddH₂O before use.
7. See the image below for proper preparation of Item F. Transfer the entire contents of the Item F vial into a tube containing 10 ml of the appropriate Assay Diluent. This is your Working Stock of Item F. Pipette up and down to mix gently. *The final concentration of biotinylated GRP will be 2 ng/ml.*
 - a. Second Dilution of Item F for Standards: Add 2 ml of Working Stock Item F to 2 ml of the appropriate Assay Diluent. The final concentration of biotinylated GRP will be **1 ng/ml**.
 - b. Second Dilution of Item F for Positive Control: Add 100 μ l of Working Stock Item F to 100 μ l of the prepared Positive Control (Item M). (See section D for Positive Control preparation) The final concentration of biotinylated GRP will be **1 ng/ml**.
 - c. Second Dilution of Item F for samples: Add 125 μ l of Working Stock Item F to 125 μ l of prepared sample (see section E for sample preparation). This is a 2-fold dilution of your sample. The final concentration of biotinylated GRP will be **1 ng/ml**.

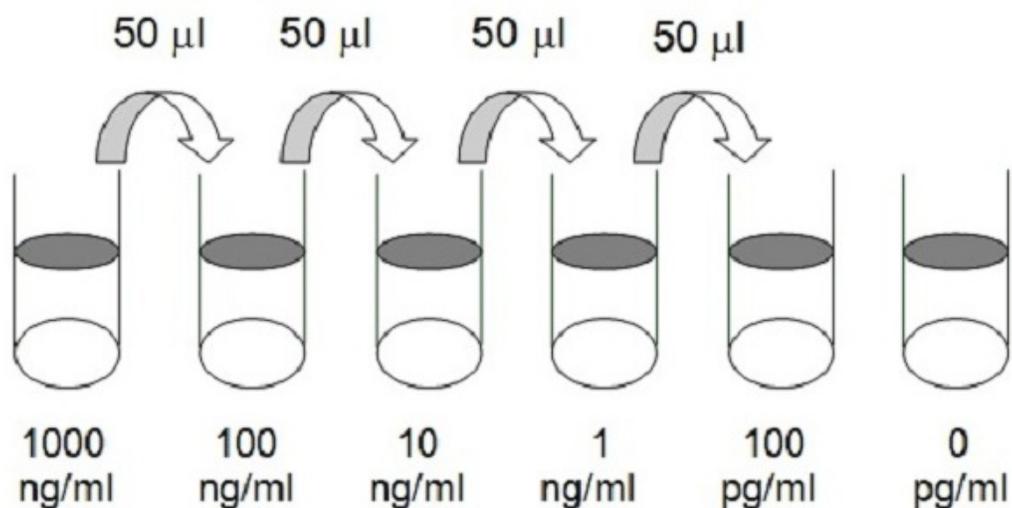


C. Preparation of Standards

- Label 6 microtubes with the following concentrations: 1,000 ng/ml, 100 ng/ml, 10ng/ml, 1 ng/ml, 100 pg/ml and 0 pg/ml. Pipette 450 μ l of biotinylated GRP Item F working solution (prepared in step 7a) into each tube, except the 1,000 ng/ml (leave this one empty).

It is very important to make sure the concentration of biotinylated GRP is 1 ng/ml in all standards.

- Briefly centrifuge the vial of GRP Standard (Item C). Reconstitute with 10 μ l of ddH₂O and briefly vortex if desired. Pipette 8 μ l of Item C and 792 μ l of 1 ng/ml biotinylated GRP working solution (prepared in step 7a) into the tube labeled 1000 ng/ml. Mix thoroughly. This solution serves as the first standard (1,000 ng/ml GRP standard, 1 ng/ml biotinylated GRP).
- To make the 100 ng/ml standard, pipette 50 μ l of the 1000 ng/ml GRP standard into the tube labeled 100 ng/ml. Mix thoroughly.
- Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 μ l of biotinylated GRP and 50 μ l of the prior concentration until the 100 pg/ml is reached. Mix each tube thoroughly before the next transfer.



D. Positive Control Preparation

12. Briefly centrifuge the Positive Control vial (Item M) and reconstitute with 100 μ l of ddH₂O.
13. Refer to step 7b. This is a 2-fold dilution of the Positive Control. The final concentration of biotinylated GRP should still be 1 ng/ml.

The Positive Control is a mouse serum sample sample that serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations; if no positive competition is observed please contact RayBiotech Technical Support. The Positive Control may be diluted further if desired, but be sure the final concentration of biotinylated GRP is 1 ng/ml.

E. Sample Preparation

14. If you wish to perform a 2-fold dilution of your sample, proceed to step 7c. If you wish to perform a higher dilution of your sample, dilute your sample with the appropriate Assay Diluent before performing step 7c.

EXAMPLE (to make a 4-fold dilution of sample):

- a. Dilute sample 2-fold (62.5 μ l of sample + 62.5 μ l of the appropriate Assay Diluent.).
- b. Perform step 7c (125 μ l of working solution Item F + 125 μ l of sample prepared above).

The total volume is 250 μ l, enough for duplicate wells on the microplate. It is very important to make sure the final concentration of the biotinylated GRP is **1 ng/ml**.

Note: Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for serum: Mouse=4x
Rat=2x.

You may also contact technical support (888-494-8555; techsupport@raybiotech.com) to obtain additional recommended dilution factors for serum.

F. Preparation of Wash Buffer and HRP

15. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved.
16. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
17. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use.
18. Dilute the HRP-Streptavidin concentrate 100-fold with 1X Assay Diluent B.
*Note: do **not** use Assay Diluent C for HRP-Streptavidin preparation in step 18*

VIII. Assay Procedure

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μ l of Anti-GRP Antibody (Item N) (See Reagent Preparation step 5) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycle/sec). You may also incubate overnight at 4°C.
3. Discard the solution and wash wells 4 times with 1X Wash Solution Buffer (200-300 μ l each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μ l of each standard (see Reagent Preparation Section C), Positive Control (see Reagent Preparation Section D) and sample (see Reagent Preparation Section E) to appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) overnight or at 4°C.
5. Discard the solution and wash 4 times as directed in Step 3.

6. Add 100 μ l of prepared HRP-Streptavidin solution (see Reagent Preparation step 18) to each well. Incubate for 45 minutes at room temperature with gentle shaking. It is recommended that incubation time should not be shorter or longer than 45 minutes.
7. Discard the solution and wash 4 times as directed in Step 3.
8. Add 100 μ l of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
9. Add 50 μ l of Stop Solution (Item I) to each well. Read at 450 nm immediately.

IX. Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 μ l anti-GRP to each well. Incubate 1.5 hours at room temperature or overnight at 4°C.
3. Add 100 μ l standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.
4. Add 100 μ l prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 μ l TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 μ l Stop Solution to each well. Read at 450 nm immediately.

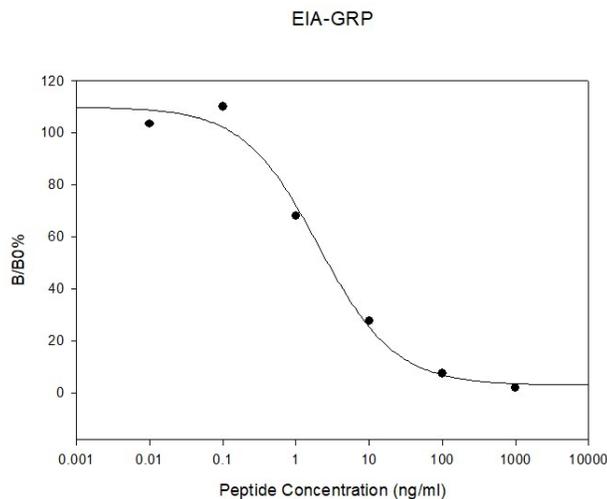
X. Calculation of Results

Calculate the mean absorbance for each set of duplicate stands, controls, and samples and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = $(B - \text{blank OD}) / (B_0 - \text{blank OD})$ where
B = OD of sample or standard and
B₀ = OD of zero standard (total binding)

A. Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. Sensitivity

The minimum detectable concentrations of GRP is 0.7 ng/ml.

C. Standard Curve Range

0.1-1,000 ng/ml

D. Reproducibility

Intra-Assay: CV<10%

Inter-Assay: CV<15%

E. Assay Diagram

Recommended Plate Layout:

Blank	Blank	SA1	SA1	SA9	SA9	SA17	SA17	SA25	SA25	SA33	SA33
Total Binding	Total Binding	SA2	SA2	SA10	SA10	SA18	SA18	SA26	SA26	SA34	SA34
Standard1	Standard1	SA3	SA3	SA11	SA11	SA19	SA19	SA27	SA27	SA35	SA35
Standard2	Standard2	SA4	SA4	SA12	SA12	SA20	SA20	SA28	SA28	SA36	SA36
Standard3	Standard3	SA5	SA5	SA13	SA13	SA21	SA21	SA29	SA29	SA37	SA37
Standard4	Standard4	SA6	SA6	SA14	SA14	SA22	SA22	SA30	SA30	SA38	SA38
Standard5	Standard5	SA7	SA7	SA15	SA15	SA23	SA23	SA31	SA31	SA39	SA39
Pos Control	Pos Control	SA8	SA8	SA16	SA16	SA24	SA24	SA32	SA32	SA40	SA40

Key:

Blank = Buffer Only

Total Binding = Biotin- GRP only

Standard 1 = 1000 ng/ml

Standard 2 = 100 ng/ml

Standard 3 = 10 ng/ml

Standard 4 = 1 ng/ml

Standard 5 = 100 pg/ml

Pos Control = Biotin with Item M

XI. Specificity

This EIA kit is designed to detect mouse, and rat Gastrin-releasing peptide.

XIV. Publications Citing This Product

1. Indumathi S., et al. Nonobese diabetic mice, hypoglycaemia and liver necrosis: a case report. Comparative Clinical Pathology August 2014, released 07 Aug 2014. DOI 10.1007/s00580-014-1969-8
Species: Mouse
Sample Type: Plasma
2. Pujol-Autonell I, Ampudia RM, Monge P, et al. Immunotherapy with Tolerogenic Dendritic Cells Alone or in Combination with Rapamycin Does Not Reverse Diabetes in NOD Mice. ISRN Endocrinol. 2013;2013:346987.
<http://dx.doi.org/10.1155/2013/346987>
Species: Mouse
Sample Type: Serum
3. Bhatt MP, Lim Y-C, Hwang J, et al. C-peptide prevents hyperglycemia-induced endothelial apoptosis through inhibition of reactive oxygen species-mediated transglutaminase 2 activation. Diabetes. 2013;62(1):243–253.
Species: Mouse
Sample Type: Serum

XIII. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	<ul style="list-style-type: none"> ○ Inaccurate pipetting ○ Improper standard dilution 	<ul style="list-style-type: none"> ○ Check pipettes ○ Briefly centrifuge Item C and dissolve the powder thoroughly by gently mixing
Low signal	<ul style="list-style-type: none"> ○ Improper preparation of standard and/or biotinylated antibody ○ Too brief incubation times ○ Inadequate reagent volumes or improper dilution 	<ul style="list-style-type: none"> ○ Briefly spin down vials before opening. Dissolve the powder thoroughly. ○ Ensure sufficient incubation time; assay procedure step 2 may be done overnight ○ Check pipettes and ensure correct preparation
Large CV	<ul style="list-style-type: none"> ○ Inaccurate pipetting ○ Air bubbles in wells 	<ul style="list-style-type: none"> ○ Check pipettes ○ Remove bubbles in wells
High background	<ul style="list-style-type: none"> ○ Plate is insufficiently washed ○ Contaminated wash buffer 	<ul style="list-style-type: none"> ○ Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. ○ Make fresh wash buffer
Low sensitivity	<ul style="list-style-type: none"> ○ Improper storage of the ELISA kit ○ Stop solution 	<ul style="list-style-type: none"> ○ Follow storage recommendations in sections IV and V. Keep substrate solution protected from light. ○ Add stop solution to each well before reading plate

RayBio[®] ELISA Kits

Over 3,000 ELISA kits available, visit www.RayBiotech.com/ELISA-Kits.html for details.

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