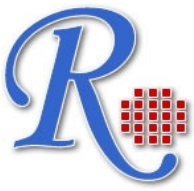
RayBio®
Human/Mouse/Rat GIP Enzyme Immunoassay Kit

Please Read the Manual Carefully Before Starting your Experiment

User Manual 2.2
(Revised April 18, 2013)

RayBio® GIP Enzyme Immunoassay Kit Protocol

(Cat#: EIA-GIP-1)



RayBiotech, Inc.

We Provide You With Excellent Protein Array System and Service

Tel: (Toll Free) 1-888-494-8555 or 770-729-2992; Fax: 770-206-2393;
Web: www.raybiotech.com Email: info@raybiotech.com

RayBiotech, Inc.

RayBio® Human/Mouse/Rat GIP Enzyme Immunoassay Kit Protocol

TABLE OF CONTENTS

I. INTRODUCTION

Gastric inhibitory polypeptide (GIP), also known as the glucose-dependent insulinotropic peptide belongs to the secretin family. GIP is derived from a 153-amino acid proprotein and circulates as a biologically active 42-amino acid peptide. GIP is synthesized by K cells, which are found in the mucosa of the duodenum and the jejunum of the gastrointestinal tract as well as in beta-cells in the pancreas.

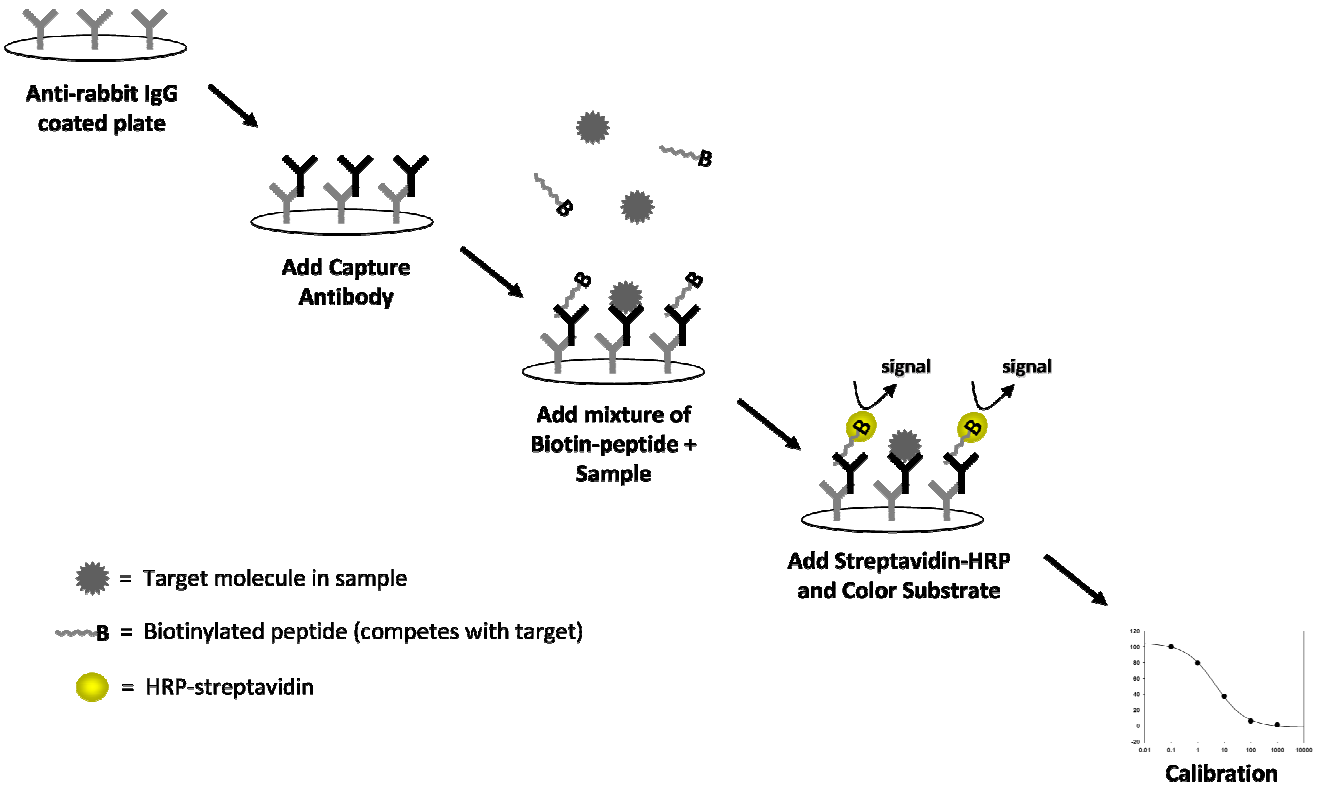
GIP has been reported to play a role in neutralizing stomach acid to protect the small intestine from acid damage, reducing the rate at which food is transferred through the stomach, and inhibiting the GI motility and secretion of acid. Recently it has been believed that the function of GIP is to induce insulin secretion, which is primarily stimulated by hyperosmolarity of glucose in the duodenum. In addition, GIP has been reported to have significant effects on fatty acid metabolism through stimulation of lipoprotein lipase activity in adipocytes. GIP release has been demonstrated in the ruminant animal and may play a role in nutrient partitioning in milk production (lipid metabolism).

GIP also has clinical applications. It has been reported that Type 2 diabetics are not responsive to GIP. Additionally, studies with knockout mice suggested that absence of GIP receptors correlates with resistance to obesity.

II. GENERAL DESCRIPTION

The RayBio® GIP Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting GIP peptide based on the principle of Competitive Enzyme Immunoassay.
The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-GIP antibody, both biotinylated GIP peptide and peptide standard or targeted peptide in samples interacts competitively with the GIP antibody. Uncompeted (bound) biotinylated GIP peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP) which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of GIP peptide in the standard or samples. This is due to the competitive binding to GIP antibody between biotinylated GIP peptide and peptides in standard or samples. A standard curve of known concentration of GIP peptide can be established and the concentration of GIP peptide in the samples can be calculated accordingly.

Principle of Competitive EIA



III. REAGENTS

1. GIP Microplate (Item A): 96 wells (12 strips x 8 wells) coated with secondary antibody.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml
3. Standard GIP Peptide (Item C): 2 vials, 10 µl/vial
4. Anti-GIP polyclonal antibody (Item N): 2 vials, 5 µl/vial
5. Assay Diluent A (Item D): 30 ml, contains 0.09% sodium azide as preservative. Diluent for standards and serum or plasma samples.
6. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. Diluent for standards and cell culture media or other sample types.
7. Biotinylated GIP peptide, (Item F): 2 vials, 20 µl/vial
8. HRP-Streptavidin concentrate (Item G): 600 µl 400x concentrated HRP-conjugated Streptavidin.
9. Positive control (Item M): 1 vial, 100 µl
10. TMB One-Step Substrate Reagent (Item H): 12 ml of 3, 3', 5, 5'- tetramethylbenzidine (TMB) in buffered solution.
11. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
12. Assay Diagram (Item J)
13. User Manual (Item K)

IV. STORAGE

- Standard, Biotinylated GIP peptide, and Positive Control should be stored at -20°C or -80°C (recommended at -80°C) after arrival. **Avoid multiple freeze-thaws.**
- The remaining kit components may be stored at -20°C.
- Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
- If stored in this manner, RayBiotech warranties this kit for 6 months from the date of shipment.

V. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450nm.
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. Saran Wrap

VI. REAGENT PREPARATION

If testing plasma or serum samples, use Assay Diluent A to dilute Item F and Item C. If testing cell culture media or other sample types, use Assay Diluent B to dilute Item F and Item C. For sample and positive control dilutions, refer to steps 6, 7, 8 and 10 of Reagent Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

3. Briefly centrifuge the Anti-GIP Antibody vial (Item N) before use. Add 50 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.

4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is your anti-GIP antibody working solution, which will be used in step 2 of the Assay Procedure.

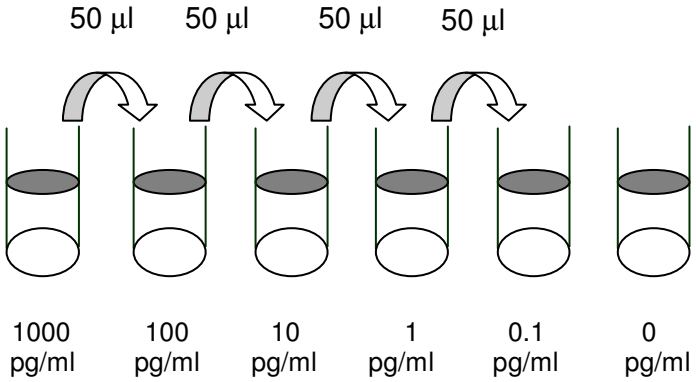
NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure).

5. Briefly centrifuge the vial of Biotinylated GIP (Item F) before use. Add 5 µl of Item F to 5 ml of the appropriate Assay Diluent. Pipette up and down to mix gently. *The final concentration of biotinylated GIP will be 10 pg/ml.* This solution will only be used as the diluent in step 6 of Reagent Preparation.

6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 pg/ml, 100 pg/ml, 10 pg/ml, 1 pg/ml, 0.1 pg/ml and 0 pg/ml. Pipette 450 µl of biotinylated GIP solution into each tube, except for the 1000 pg/ml (leave this one empty). *It is very important to make sure the concentration of biotinylated GIP is 10 pg/ml in all standards.*

a. Briefly centrifuge the vial of GIP (Item C). In the tube labeled 1000 pg/ml, pipette 8 µl of Item C and 792 µl of 10 pg/ml biotinylated GIP solution (prepared in step 5 above). This is your GIP stock solution (1000 pg/ml GIP, 10 pg/ml biotinylated GIP). Mix thoroughly. This solution serves as the first standard.
b. To make the 100 pg/ml standard, pipette 50 µl of GIP stock solution into the tube labeled 100 pg/ml. Mix thoroughly.
c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 µl of biotinylated GIP and 50 µl of the prior concentration until 0.1 pg/ml is reached. Mix each tube thoroughly before the next transfer.

d. The final tube (0 pg/ml GIP, 10 pg/ml biotinylated GIP) serves as the zero standard (or total binding).



7. Prepare a 10-fold dilution of Item F. To do this, add 2 µl of Item F to 18 µl of the appropriate Assay Diluent. This solution will be used in steps 8 and 10.

8. Positive Control Preparation: briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 µl 1x Assay Diluent B. Also add 2 µl of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10% and 30% of total binding (70-90% of competition) if diluted as described above. It may be diluted further if desired, but be sure the final concentration of biotinylated GIP is 10 pg/ml.

9. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

10. Sample Preparation: Use Assay Diluent A + biotinylated GIP to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated GIP as the diluent. *It is very important to make sure the final concentration of the biotinylated GIP is 10 pg/ml in every sample.* EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 µl of 10-fold diluted Item F (prepared in step 7), 185 µl of appropriate Assay Diluent, and 62.5 µl of your sample; mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate.
    Do not use Item F diluent from Step 5 for sample preparation.
    If you plan to use undiluted samples, you must still add biotinylated GIP to a final concentration of 10 pg/ml. EXAMPLE: Add 2.5 µl of 10-fold diluted Item F to 247.5 µl of sample. NOTE: Optimal sample dilution factors should be determined empirically, however you may contact technical support (888-494-8555; techsupport@raybiotech.com) to obtain recommended dilution ranges for serum or plasma.

11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 400-fold with 1X Assay Diluent B.

    Note: Do not use Assay Diluent A for HRP-Streptavidin preparation in Step 11.

VII. 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 µl anti-GIP antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room temperature with

gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4 degrees C.

3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 µl 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 µl of each standard (see Reagent Preparation step 6), positive control (see Reagent Preparation step 8) and sample (see Reagent Preparation step 10) into 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) or overnight at 4℃.

5. Discard the solution and wash 4 times as directed in Step 3.

6. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 11) to each well. Incubate for 45 minutes with gentle shaking at room temperature. 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 µl 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 µl of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

VIII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

⇩

2. Add 100 µl anti- GIP antibody to each well. Incubate 1.5 hours at room temperature.

⇩

3. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.

⇩

4. Add 100 µl prepared streptavidin solution. Incubate 45 minutes at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

⇩

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately

IX. CALCULATION OF RESULTS

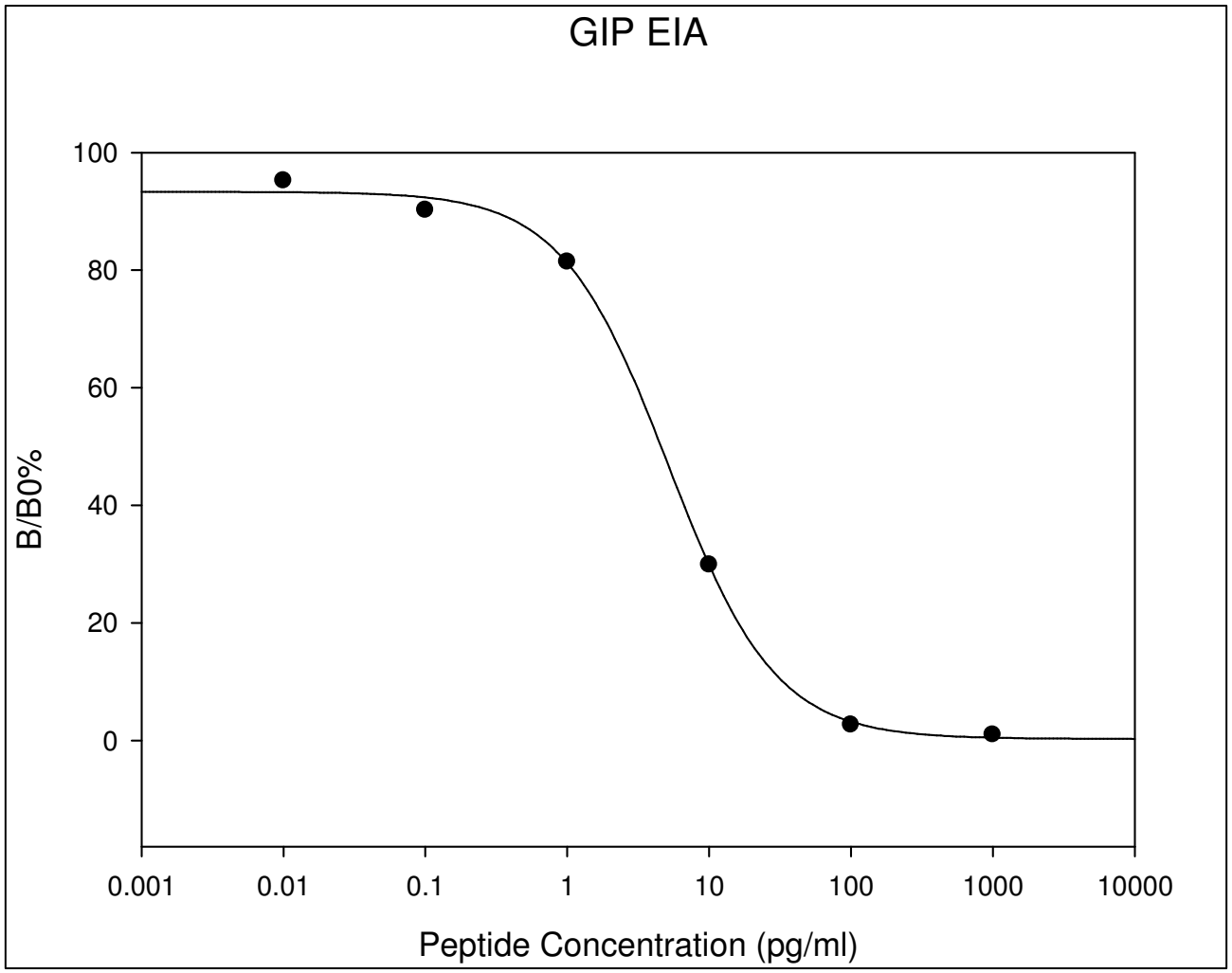
Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis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

calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = (B – blank OD)/(B_o – blank OD) where
B = OD of sample or standard and
B_o = 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 concentration of GIP is 1.25 pg/ml.

C. DETECTION RANGE

0.1-1,000 pg/ml

D. REPRODUCIBILITY

Intra-Assay: CV<10%
Inter-Assay: CV<15%

X. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

XI. REFERENCES

1. Meier JJ, Nauck MA (2005). "Glucagon-like peptide 1(GLP-1) in biology and pathology". *Diabetes Metab. Res. Rev.* **21** (2): 91–117.
2. Yamada Y, Seino Y (2004). "Physiology of GIP--a lesson from GIP receptor knockout mice". *Horm. Metab. Res.* **36** (11–12): 771–4.

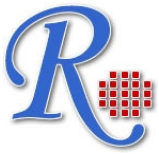
XII. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 change to over night
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the EIA kit	1. Store your standard at ≤ -20°C after receipt of the kit.
	2. Stop solution	2. Stop solution should be added to each well before measure

RayBio® EIA kits:
If you are interested in other EIA kits, please visit www.raybiotech.com for details.

Notes:

This product is for research use only.



3607 Parkway Lane, Suite 200
Norcross, GA 30092
Tel: 770-729-2992, 1-888-494-8555
Fax: 770-206-2393
Web: www.raybiotech.com