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Product Information

Gastric Inhibitory Peptide (GIP) EIA Kit
for serum, plasma, culture supernatant, and cell lysates

Catalog Number **RAB0209**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Gastric Inhibitory Peptide (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.

Components

1. 96 well plate coated with secondary antibody (Item A) - RAB0209A: 96 wells (12 strips × 8 wells) coated with secondary antibody.
2. 20x Wash Buffer (Item B) - RABWASH3: 25 mL
3. EIA Gastric Inhibitory Peptide standard (Item C) - RAB0209C: 2 vials, 10 µL/vial.
4. Anti-Gastric Inhibitory Peptide Detection Antibody (Item N) - RAB0209F: 2 vials, 5 µL/vial.
5. EIA Assay Diluent A (Item D) - RABDIL9: 30 mL, contains 0.09% sodium azide as preservative. Diluent for standards, and serum or plasma samples.
6. EIA 5x Assay Diluent B (Item E) - RABDIL10: 15 mL of 5x concentrated buffer. Diluent for standards and cell culture media or other sample types.
7. Biotinylated Gastric Inhibitory Peptide (Item F) - RAB0209G: 2 vials, 20 µL/vial.
8. HRP-streptavidin (Item G) - RABHRP3: 600 µL of 400x concentrated HRP-conjugated Streptavidin.
9. Gastric Inhibitory Peptide Positive Control Sample, Lyophilized (Item M) - RAB0209K: 1 vial, 100 µL.
10. TMB Substrate solution (Item H) - RABTMB2: 12 mL of 3,3′,5,5′- tetramethylbenzidine (TMB) in buffered solution.
11. Stop Solution (Item I) – RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

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.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices

Preparation Instructions

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 Preparation steps 6, 7, 8, and 10.

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 the anti-GIP antibody working solution, which will be used in Procedure, step 2.

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

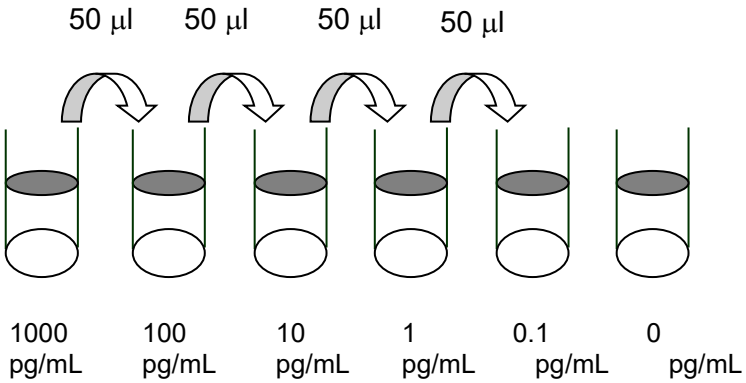
5. Briefly centrifuge the vial of Biotinylated GIP (Item F) before use. Add 5 µL of Item F to 5 mL of the appropriate Assay Diluent. Pipette up and down to mix gently. The final concentration of biotinylated GIP will be 10 pg/mL. This solution will only be used as the diluent in Preparation, step 6.

6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1,000 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 1,000 pg/mL (leave this one empty).

   Note: 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 1,000 pg/mL, pipette 8 µL of Item C and 792 µL of 10 pg/mL biotinylated GIP solution (Preparation, step 5). This is the GIP stock solution (1,000 pg/mL GIP and 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 the tube labeled 100 pg/mL. Mix thoroughly.
   c. Repeat this step with each successive concentration, preparing a dilution series as shown in Figure 1. 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 and 10 pg/mL biotinylated GIP) serves as the zero standard (or total binding).

Figure 1.
Dilution Series for Standards



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 of 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 10–30% 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 plus biotinylated GIP to dilute serum/plasma samples. For cell culture medium and other sample types, use 1x Assay Diluent B plus biotinylated GIP as the diluent.

    Note: It is very important to make sure the final concentration of the biotinylated GIP is 10 pg/mL in every sample.

    For 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 the 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 undiluted samples are used, biotinylated GIP must be added to a final concentration of 10 pg/mL. For example, Add 2.5 µL of 10-fold diluted Item F to 247.5 µL of sample.

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.

Storage/Stability

Standard, Biotinylated GIP peptide, and Positive Control should be stored at –20 °C or –70 °C (recommended at –70 °C) after arrival. **Avoid multiple freeze-thaws.**

The remaining kit components may be stored at –20 °C.

Opened microplate strips and antibody (Item N) may be stored for up to 1 month at 2–8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

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 Preparation, step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or overnight at 4 °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 Preparation, step 6), positive control (see Preparation, step 8), and sample (see 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 °C.

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

6. Add 100 µL of prepared HRP-Streptavidin solution (see 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 absorbance at 450 nm immediately.

Results

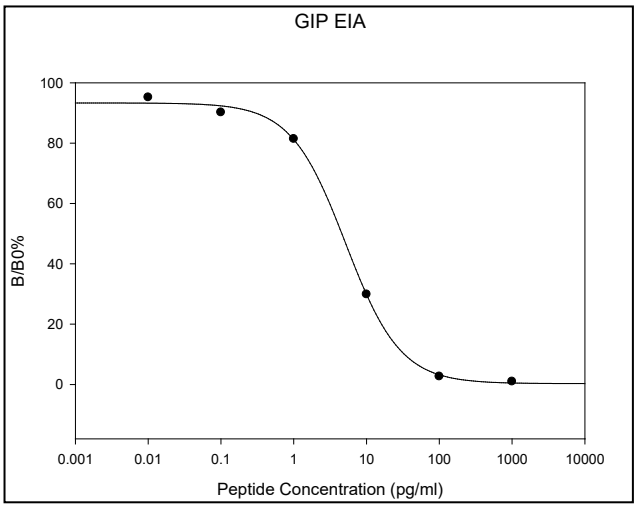
Calculations

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 straight line 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)

Typical Data

Standard curve(s) is for demonstration only. Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable concentration of GIP is 1.25 pg/mL.

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

Specificity

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

References

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

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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