DRG® GIP (Rat/Mouse) ELISA (EIA-4528)

Revised 24 Apr. 2011 rm (Vers. 2.1) **Research Use Only**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

This kit is not intended for diagnostic purposes.

1 INTENDED USE

This Rat/Mouse GIP (Total) ELISA kit is used for the non-radioactive quantification of Rat/Mouse GIP in rat or mouse serum, plasma, tissue extract and cell culture samples.

This kit has 100% cross reactivity to rat GIP and mouse GIP and measures both GIP(1-42) and GIP(3-42).

One kit is sufficient to measure 39 unknown samples in duplicate.

This kit is for research purposes only.

2 PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of rat or mouse GIP molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-GIP monoclonal antibodies, 2) wash away of unbound materials from samples, 3) binding of a second biotinylated anti-GIP polyclonal antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) incubation of streptavidin-Horseradish peroxidase conjugate to bind to the immobilized biotinylated antibodies, 6) wash away of free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured rat or mouse GIP in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of Rat GIP.

3 REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

A. **GIP ELISA Plate**
Coated with monoclonal anti-GIP Antibodies
Quantity: 1 plate
Preparation: Ready to Use
Note: Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C.

B. **Adhesive Plate Sealer**
Quantity: 2 sheets
Preparation: Ready to Use

C. **10X HRP Wash Buffer Concentrate**
10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.
Quantity: 2 bottles containing 50 mL each
Preparation: Dilute 1:10 with distilled or deionized water.

D. **Rat/Mouse GIP Standard**
Rat GIP(1-42), 2000 pg/mL, 0.5 mL/vial, lyophilized.
Quantity: 0.5 mL/vial upon hydration
Preparation: Contents lyophilized. Reconstitute with 0.5 mL distilled or deionized water.

E. **Rat/Mouse GIP Quality Controls 1 and 2**
Rat GIP(1-42), 0.5 mL/vial, lyophilized.
Quantity: 0.5 mL/vial upon hydration
Preparation: Contents lyophilized. Reconstitute with 0.5 mL distilled or deionized water.

F. **Assay Buffer**
Buffer containing BSA and 0.08% Sodium Azide
Quantity: 12 mL
Preparation: Ready to Use

G. GIP **Detection Antibody**
Pre-titered Biotinylated Rabbit anti-GIP Polyclonal Antibody
Quantity: 11 mL
Preparation: Ready to Use

H. **Enzyme Solution** Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer
Quantity: 12 mL
Preparation: Ready to Use

I. **Substrate** (*Light sensitive, avoid unnecessary exposure to light*)
3, 3', 5, 5'-tetramethylbenzidine in buffer
Quantity: 12 mL
Preparation: Ready to Use.

J. **Stop Solution** (*Caution: Corrosive Solution*)
0.3 M HCl
Quantity: 12 mL
Preparation: Ready to Use

K. **Matrix Solution**
Quantity: 1 mL/vial
Preparation: Ready to Use

4 STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C.
For longer storage (> 2 weeks), freeze Wash Buffer, Assay Buffer, Matrix Solution, and reconstituted Standards and Controls at ≤ –20°C.

Minimize repeated freeze and thaw of the GIP Standards and Quality Controls.

Unused microtiter strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C.

Refer to expiration dates on all reagents prior to use.

Do not mix reagents from different kits unless they have the same lot numbers.

5 REAGENT PRECAUTIONS

A. Sodium Azide

Sodium azide has been added to certain reagents as a preservative. Although the concentrations are low, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

B. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

6 MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10 µL - 20 µL or 20 µL - 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 5 ~ 50 µL and 50 ~ 300 µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

7 SAMPLE COLLECTION AND STORAGE

NOTE: Although DPPIV inhibitor is not required to be added to serum/plasma samples for measurement of total GIP, we recommend that DPPIV inhibitor be added to the serum/plasma samples during the sample collection so that the same samples could be used in the future for the measurement of intact (1-42) GIP with an assay that is capable of selectively measuring only the intact GIP.

1. To prepare **serum samples**, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.

   Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2oC.

   Transfer and store serum samples in separate tubes. Date and identify each sample.

   Use freshly prepared serum or aliquot and store samples at £ –20oC for later use. For long-term storage, keep at -70°C. Avoid freeze/thaw cycles.

2. To prepare **plasma samples**, whole blood should be collected into centrifuge tubes containing enough K3EDTA to achieve a final concentration of 1.735 mg/ml and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.

3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

4. Avoid using samples with gross hemolysis or lipemia.

8 SAMPLE PREPARATION

1. No dilution or preparation is needed for normal serum or plasma samples. In the event that any sample is above 2000 pg/mL range, dilutions should be performed using the Matrix Solution provided.
2. Tissue extracts or cell culture samples may require dilution. Dilutions should be performed using the assay buffer provided.

9 STANDARD AND QUALITY CONTROLS PREPARATION

9.1 Rat/Mouse GIP Standard Preparation

1. Use care in opening the lyophilized Standard vial.
   Using a pipette, reconstitute the Rat/Mouse GIP Standard with 0.5 mL distilled or deionized water into the glass vial to give a 2000 pg/mL concentration of Standard.
   Invert and mix gently, and let sit for 5 minutes then mix well.
2. Label five tubes 666.7, 222.2, 74.1, 24.7, and 8.2 pg/mL.
   Add 100 µL Assay Buffer to each of the five tubes.
   Prepare 3 times serial dilutions by adding 50 µL of the 2000 pg/mL reconstituted standard to the 666.7 pg/mL tube, mix well and transfer 50 µL of the 666.7 pg/mL standard to the 222.2 pg/mL tube,
   mix well and transfer 50 µL of the 222.2 pg/mL standard to the 74.1 pg/mL tube,
   mix well and transfer 50 µL of the 74.1 pg/mL standard to the 24.7 pg/mL tube,
   mix well and transfer 50 µL of the 24.7 pg/mL standard to the 8.2 pg/mL tube and mix well.

Note:
Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Standard Concentration pg/mL	Volume of Deionized Water to Add	Volume of Standard to Add
2000	0.5 mL	0
Standard Concentration pg/mL	**Volume of Assay Buffer to Add**	**Volume of Standard to Add**
666.7	100 µL	50 µL of 2000 pg/mL
222.2	100 µL	50 µL of 666.7 pg/mL
74.1	100 µL	50 µL of 222.2 pg/mL
24.7	100 µL	50 µL of 74.1 pg/mL
8.2	100 µL	50 µL of 24.7 pg/mL

9.2 Rat/Mouse GIP Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials.

Using a pipette, reconstitute each of the Rat/Mouse GIP Quality Control 1 and Quality Control 2 with 0.5 mL distilled or deionized water into the glass vials.

Invert and mix gently, let sit for 5 minutes then mix well.

10 ASSAY PROCEDURE

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire contents of both buffer bottles with 900 mL deionized or distilled water.
2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble strips in an empty plate holder and fill each well with **300 µL of diluted Wash Buffer**. **Incubate at room temperature for 5 minutes**.
   Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol.**
3. Add in duplicate, **90 µL Assay Buffer** to the **blank** wells and **sample** wells.
   (See plate well map for suggested well orientation).
4. Add in duplicate, **80 µL Assay Buffer** to **Standard** wells, **QC1 and QC2** wells.
5. Add **10 µL Matrix Solution** to the **Blank** wells, **Standard** wells, and **QC1 and QC2** wells.

6. Add in duplicate, **10 µL** Rat/Mouse GIP **Standards** in the order of ascending concentration to the appropriate wells.
   Add in duplicate, **10 µL QC1 and 10 µL QC2** to the appropriate wells.
   Add sequentially, **10 µL of the unknown samples** in duplicate to the remaining wells.
   For best result all additions should be completed within 30 minutes.
7. Cover the plate with plate sealer and incubate at **room temperature for 1.5 hours** on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.
8. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
9. **Wash** wells **3 times** with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
10. Add **100 µL Detection Antibody to all** wells. Cover the plate with plate sealer and incubate at **room temperature for 1 hour** on an orbital microtiter **plate shaker** set to rotate at moderate speed, approximately 400 to 500 rpm.
11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.
12. **Wash wells 3 times** with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
13. Add **100 µL Enzyme Solution** to each well. Cover plate with sealer and incubate with moderate shaking at **room temperature for 30 minutes** on the microtiter **plate shaker**.
14. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.
15. **Wash wells 3 times** with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.
16. Add **100 µL of Substrate Solution** to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 20 minutes (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of GIP standards with intensity proportional to increasing concentrations of GIP.
    NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.
    One can monitor color development using 370 nm filter, if available on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.
17. Remove sealer and add **100 µL Stop Solution** [CAUTION: CORROSIVE SOLUTION] and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification.
    Read absorbance at 450 nm and 590nm in a plate reader **within 5 minutes** and ensure that there are no air bubbles in any well.
    Record the difference of absorbance units.
    The absorbance of the highest GIP standard should be approximately 2.0-3.2, or not to exceed the capability of the plate reader used.

11 MICROTITER PLATE ARRANGEMENT

Rat/Mouse GIP (Total) ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	222.2 pg/mL	QC 2									
B	Blank	222.2 pg/mL	QC 2									
C	8.2 pg/mL	666.7 pg/mL	Sample									
D	8.2 pg/mL	666.7 pg/mL	Sample									
E	24.7 pg/mL	2000 pg/mL	Sample									
F	24.7 pg/mL	2000 pg/mL	Sample									
G	74.1 pg/mL	QC 1	Sample									
H	74.1 pg/mL	QC 1	Etc.									

12 CALCULATIONS

The dose-response curve of this assay fits best to a 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function.

Note:

When sample volumes assayed differ from 10 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 5 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 µL, compensate the volume deficit with matrix solution.

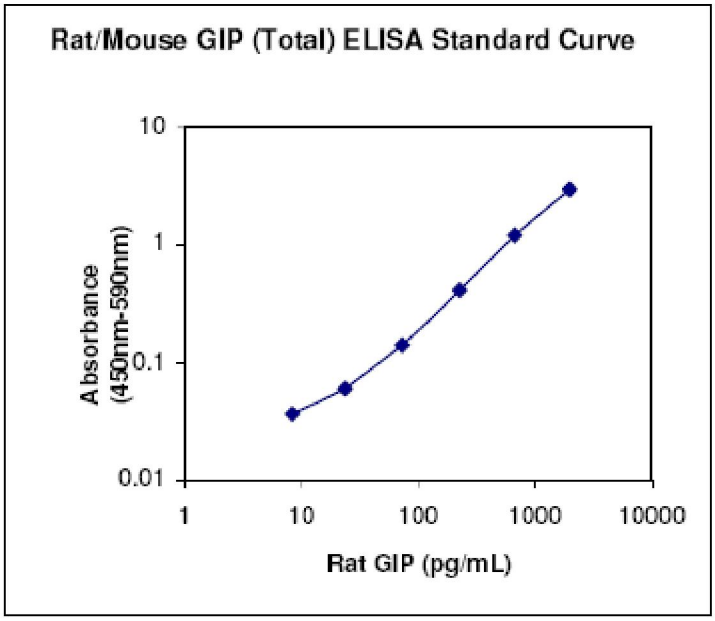
13 INTERPRETATION

Acceptance Criteria

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.
3. The limit of sensitivity of this assay is 8.2 pg/mL Rat/Mouse GIP (10 µL sample size).

4. The appropriate range of this assay is 8.2 pg/mL to 2000 pg/mL Rat/Mouse GIP (10 µL sample size).
   Any result greater than 2000 pg/mL in a 10 µL sample should be diluted using matrix solution, and the assay repeated until the results fall within range.
   Tissue extracts or cell culture media samples greater than 2000 pg/mL in a 10 µL sample should be diluted in Assay Buffer.

14 STANDARD CURVE



Typical Standard Curve, not to be used to calculate results.

15 QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert.

16 TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to
   1) cross well contamination by standard solution or sample or
   2) inadequate washing of wells with Wash Buffer or
   3) overexposure to light after substrate has been added.

17 ORDERING INFORMATION

Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to human or animals.

Material Safety Data Sheets (MSDS)

Material safety data sheets for DRG products may be ordered by fax or phone.