



**RAT/MOUSE GIP (TOTAL) ELISA KIT
96-Well Plate (Cat. # EZRMGIP-55K)**

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RAT/MOUSE GIP (TOTAL) ELISA KIT 96-Well Plate (Cat. #EZRMGIP-55K)

I. INTENDED USE

This Rat/Mouse GIP (Total) ELISA kit is used for the non-radioactive quantification of Rat/Mouse GIP (Total) in rat or mouse serum, plasma, tissue extracts or cell culture media samples. This kit has 100% crossreactivity 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 purpose only.***

II. 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.

III. 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

III. REAGENTS SUPPLIED (continued)

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 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

IV. 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 $\leq -20^{\circ}\text{C}$. 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.

V. 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.

VI. 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

VII. 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 $4 \pm 2^{\circ}\text{C}$.

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

Use freshly prepared serum or aliquot and store samples at $\leq -20^{\circ}\text{C}$ 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 K_3EDTA 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.

VIII. 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.

IX. STANDARD AND QUALITY CONTROLS PREPARATION

A. 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, 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 μ L Assay Buffer to each of the five tubes. Prepare 3 times serial dilutions by adding 50 μ L of the 2000 pg/mL reconstituted standard to the 666.7 pg/mL tube, mix well and transfer 50 μ L of the 666.7 pg/mL standard to the 222.2 pg/mL tube, mix well and transfer 50 μ L of the 222.2 pg/mL standard to the 74.1 pg/mL tube, mix well and transfer 50 μ L of the 74.1 pg/mL standard to the 24.7 pg/mL tube, mix well and transfer 50 μ L 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 $\leq -20^{\circ}\text{C}$. 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 μ L	50 μ L of 2000 pg/mL
222.2	100 μ L	50 μ L of 666.7 pg/mL
74.1	100 μ L	50 μ L of 222.2 pg/mL
24.7	100 μ L	50 μ L of 74.1 pg/mL
8.2	100 μ L	50 μ L of 24.7 pg/mL

B. Rat/Mouse GIP Quality Control 1 and 2 Preparation

1. 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.

X. 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 μ L 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 μ L Assay Buffer to the blank wells and sample wells. (See plate well map for suggested well orientation).
4. Add in duplicate, 80 μ L Assay Buffer to Standard wells, QC1 and QC2 wells.
5. Add 10 μ L Matrix Solution to Blank wells, Standard wells, and QC1 and QC2 wells.
6. Add in duplicate, 10 μ L Rat/Mouse GIP Standards in the order of ascending concentration to the appropriate wells. Add in duplicate, 10 μ L QC1 and 10 μ L QC2 to the appropriate wells. Add sequentially, 10 μ L 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 μ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
10. Add 100 μ L 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.

X. ASSAY PROCEDURE (continued)

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 μ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
13. Add 100 μ L 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 μ L per well per wash. Decant and tap firmly after each wash to remove residual buffer.
16. Add 100 μ L of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 5 to 18 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 μ L 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.

Assay Procedure for Rat/Mouse GIP (Total) ELISA kit (Cat. #EZRMGIP-55K)

	Step 1	Step 2	Step 3-4	Step 5	Step 6	Step 7-9	Step 10	Step 11-12	Step 13	Step 14-15	Step 16	Step 16	Step 17	Step 17
Well #	Dilute both bottles of 10X Wash Buffer with 900 mL Deionized Water.	Wash plate with 300 μL Wash Buffer and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels	Assay Buffer	Matrix	Standards/ Controls/ Samples	Seal, Agitate, Incubate 1.5 hour at Room Temperature. Wash 3X with 300 μL Wash Buffer	Detection Ab	Seal, Agitate, Incubate 1 hour at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels. Wash 3X with 300 μL Wash Buffer	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature . Wash 3X with 300 μL Wash Buffer	Substrate	Seal, Agitate, Incubate 5-18 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, B1			90 μ L	10 μ L	-----		100 μ L		100 μ L		100 μ L			
C1, D1			80 μ L	10 μ L	10 μ L of 8.2 pg/mL Standard		↓		↓		↓			
E1, F1			80 μ L	10 μ L	10 μ L of 24.7 pg/mL Standard									
G1, H1			80 μ L	10 μ L	10 μ L of 74.1 pg/mL Standard									
A2, B2			80 μ L	10 μ L	10 μ L of 222.2 pg/mL Standard									
C2, D2			80 μ L	10 μ L	10 μ L of 666.6 pg/mL Standard									
E2, F2			80 μ L	10 μ L	10 μ L of 2000 pg/mL Standard									
G2, H2			80 μ L	10 μ L	10 μ L of QC 1									
A3, B3			80 μ L	10 μ L	10 μ L of QC 2									
C3, D3			90 μ L	-----	10 μ L of Sample									
E3, F3			90 μ L	-----	10 μ L of Sample									
G3, H3 ↓			90 μ L	-----	10 μ L of Sample									

XI. 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.									

XII. 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.

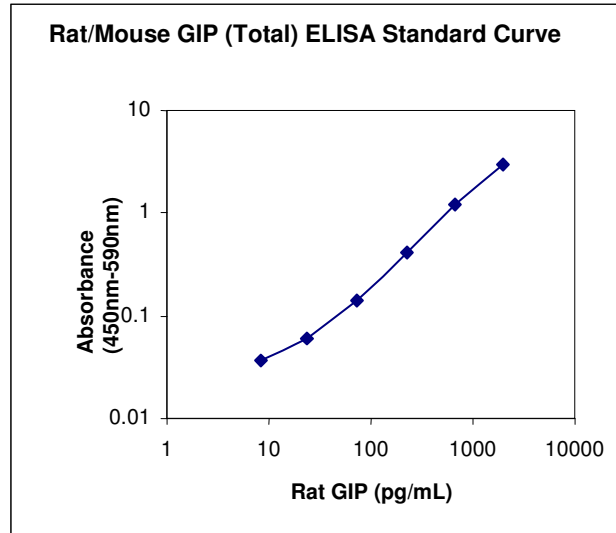
Note: When sample volumes assayed differ from 10 μL , an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 5 μL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 10 μL , compensate the volume deficit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

XIII. INTERPRETATION

A. 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.

XIV. STANDARD CURVE



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

XV. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Rat/Mouse GIP that can be detected by this assay is 8.2 pg/mL when using a 10 μ L sample size.

B. Specificity

The antibody pair used in this assay is specific to rat and mouse GIP and does not significantly cross-react with Glucagon, Oxyntomodulin, GLP-1, and GLP-2.

XV. ASSAY CHARACTERISTICS (continued)

C. Precision

Intra-Assay Variation

Sample No.	Mean GIP Levels (pg/mL)	Intra-Assay % CV
1	92	1.0
2	98	2.4
3	173	5.9
4	150	1.1

Inter-Assay Variation

Sample No.	Mean GIP Levels (pg/mL)	Inter-Assay % CV
1	80	5.9
2	113	1.1
3	178	4.1

The assay variations of Millipore Rat/Mouse GIP ELISA kits were studied on four rat serum samples with varying concentrations of endogenous GIP. The mean intra-assay variation was calculated from results of six duplicate determinations in each assay of the indicated samples. The mean inter-assay variations of each sample was calculated from results of four separate assays with duplicate samples in each assay.

XV. ASSAY CHARACTERISTICS (continued)

D. Recovery

Spike & Recovery of Rat GIP in Rat Serum Samples

Sample No.	GIP Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	% of Recovery
1	0	85	85	100
	49.4	134.4	136	101
	148.9	233.9	238	102
	444.4	529.4	567	107
2	0	93	93	100
	49.4	142.4	146	103
	148.9	241.9	249	103
	444.4	537.4	597	111
3	0	14	14	100
	49.4	63.4	62	98
	148.9	162.9	165	101
	444.4	458.4	512	112

Varying amounts of rat GIP were added to three rat serum samples and the GIP content was determined. The % of recovery = observed GIP concentrations/expected GIP concentrations x 100%.

XV. ASSAY CHARACTERISTICS (continued)

E. Linearity

Effect of Serum Dilution

Sample No.	Volume Sampled (µL)	Expected (ng/mL)	Observed (ng/mL)	% Of Expected
1	10	43	43	100
	5	22	21	98
	2.5	10.8	11	102
	1.25	5.4	--	--
2	10	62	62	100
	5	31	29	94
	2.5	15.5	14	90
	1.25	7.8	9	116
3	10	21	21	100
	5	10.5	11	105
	2.5	5.25	--	--
	1.25	2.6	--	--

Three rat serum samples with the indicated sample volumes were assayed. Required amounts of matrix were added to compensate for lost volumes below 10 µL. The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 representing 10 µL, 5 µL, 2.5 µL, and 1.25 µL sample volumes assayed, respectively, were applied in the calculation of observed GIP concentrations. % expected = observed/expected x 100%.

XVI. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com.

XVII. 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.

XVIII. REPLACEMENT REAGENTS

Reagents	Cat. #
GIP ELISA Plate	EP54
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
Rat/Mouse GIP Standards	E8055-K
Rat/Mouse GIP Quality Controls 1 and 2	E6055-K
Assay Buffer	EABGLP
Matrix Solution	EMTX
GIP Detection Antibody	E1054
Enzyme Solution	EHRP
Substrate	ESS-TMB2
Stop Solution	ET-TMB

XIX. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

TELEPHONE ORDERS:

Toll Free US (866) 441-8400

(636) 441-8400

FAX ORDERS: (636) 441-8050

MAIL ORDERS: Millipore Corporation
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

B. 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. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.