Human GIP (Total)

96-Well Plate Assay

Cat. # EZHGIP-54K

HUMAN GIP (TOTAL) ELISA KIT
96-Well Plate (Cat. # EZHGIP-54K)

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I. INTENDED USE

This Human GIP (Total) ELISA kit is used for the non-radioactive quantification of Human GIP in human serum, plasma, tissue extract and cell culture samples. This kit has 100% cross reactivity to human GIP (1-42) and GIP (3-42). One kit is sufficient to measure 39 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of human 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 human 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 human 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 anti-GIP Monoclonal 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.

III. REAGENTS SUPPLIED (continued)

D. Human GIP Standard
Human GIP (1-42), 0.5 mL/vial, lyophilized.
Quantity: 0.5 mL/vial upon hydration.
Preparation: Reconstitute with 0.5 mL distilled or deionized water. The actual concentration of Human GIP present in the vial will be lot dependent. Please refer to the analysis sheet for exact Human GIP concentration present in a specific lot.

E. Human GIP Quality Controls 1 and 2
Human 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. Human GIP Detection Antibody
Pre-titered Biotinylated Rabbit anti-Human 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

Recommended storage for kit components is 2 - 8°C.

All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use but repeated freeze thaws should be avoided. 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 or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

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.

Full labels of hazardous components in this kit:

Ingredient, Cat #		**Full Label**	
Enzyme Solution	ET-TMB		**Danger.** May be corrosive to metals.
Human GIP Standard	E8054-K		**Warning.** Harmful if swallowed. Toxic to aquatic life with long lasting effects. Avoid release to the environment.
Quality Controls 1 & 2	E6054-K		**Warning.** Harmful if swallowed. Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Wash Buffer	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

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 ± 2°C.

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

   Use freshly prepared serum or aliquot and store samples at ≤ –20°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 media samples may require dilution. Dilutions should be performed using the assay buffer provided.

IX. STANDARD AND QUALITY CONTROLS PREPARATION

A. Human GIP Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human GIP Standard with 0.5 mL distilled or deionized water into the vial to give a concentration prescribed in the analysis sheet. Invert and mix gently, and let sit for 5 minutes then mix well.

2. Label five tubes 1, 2, 3, 4 and 5. Add 100 µL Assay Buffer to each of the five tubes. Perform 3 times serial dilutions by adding 50 µL of the reconstituted standard to Tube 1, mix well and transfer 50 µL of Tube 1 to Tube 2, mix well and transfer 50 µL of Tube 2 to Tube 3, mix well and transfer 50 µl of Tube 3 to Tube 4, mix well and transfer 50 µL of Tube 4 to Tube 5 and mix well.

Note: Do not use a repeater pipette. Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted 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
X (refer to the analysis sheet for exact concentration)	0.5 mL	0

Tube #	Standard Concentration pg/mL	Volume of Assay Buffer to Add	Volume of Standard to Add
1	X/3	100 µL	50 µL of Reconstituted Standard
2	X/9	100 µL	50 µL of Tube 1
3	X/27	100 µL	50 µL of Tube 2
4	X/81	100 µL	50 µL of Tube 3
5	X/243	100 µL	50 µL of Tube 4

B. Human GIP Quality Control 1 and 2 Preparation

1. Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Human GIP Quality Control 1 and Quality Control 2 with 0.5 mL distilled or deionized water into the 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 with the desiccant provided 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, 80 µL Assay Buffer to the blank wells and sample wells. (See plate well map for suggested well orientation).

4. Add in duplicate, 60 µL Assay Buffer to Standard wells, QC1 and QC2 wells.

5. Add 20 µL Matrix Solution to the Blank wells, Standard wells, and QC1 and QC2 wells.

6. Add in duplicate, 20 µL Human GIP Standards in the order of ascending concentration to the appropriate wells. Add in duplicate, 20 µL QC1 and 20 µL QC2 to the appropriate wells. Add sequentially, 20 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.

X. ASSAY PROCEDURE (continued)

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. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. 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 Human GIP (Total) ELISA Kit (Cat. # EZHGIP-54K)

	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 5-20 minutes at Room Temperature.**	Stop Solution	**Read Absorbance at 450 nm and 590 nm.**
A1, B1			80 µL	20 µL	---------		100 µL		100 µL		100 µL		100 µL	
C1, D1			60 µL	20 µL	20 µL of Tube 5									
E1, F1			60 µL	20 µL	20 µL of Tube 4									
G1, H1			60 µL	20 µL	20 µL of Tube 3									
A2, B2			60 µL	20 µL	20 µL of Tube 2									
C2, D2			60 µL	20 µL	20 µL of Tube 1									
E2, F2			60 µL	20 µL	20 µL of Reconstituted Standard									
G2, H2			60 µL	20 µL	20 µL of QC 1									
A3, B3			60 µL	20 µL	20 µL of QC 2									
C3,D3			80 µL	---------	20 µL of Sample									
E3, F3			80 µL	---------	20 µL of Sample									
G3, H3 ↓			80 µL	---------	20 µL of Sample									

XI. MICROTITER PLATE ARRANGEMENT

Human GIP (Total) ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 2	QC 2									
B	Blank	Tube 2	QC 2									
C	Tube 5	Tube 1	Sample									
D	Tube 5	Tube 1	Sample									
E	Tube 4	Reconstituted Standard	Sample									
F	Tube 4	Reconstituted Standard	Sample									
G	Tube 3	QC 1	Sample									
H	Tube 3	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.

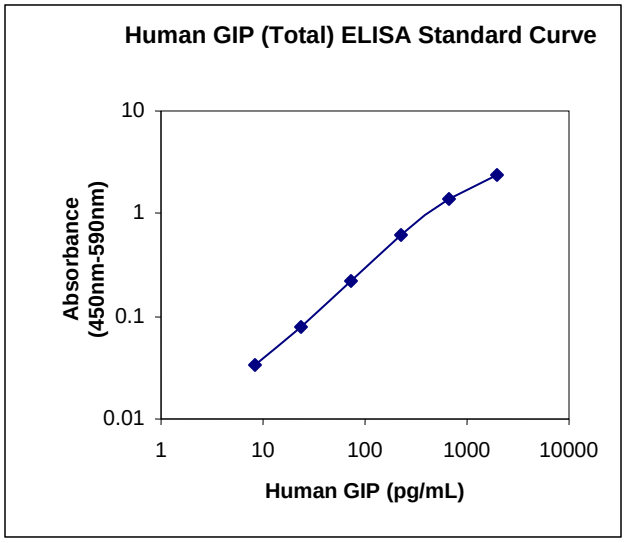
Note: When sample volumes assayed differ from 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µL, compensate the volume deficit 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 4.2 pg/mL human GIP (20 µL sample size).
4. The appropriate range of this assay is 4.2 pg/mL to 2000 pg/mL human GIP (20 µL sample size). Any result greater than 2000 pg/mL in a 20 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 20µL 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 Human GIP that can be detected by this assay is 4.2 pg/mL when using a 20 µL sample size.

B. Specificity

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

C. Precision

Intra-Assay Variation

Sample No.	Mean GIP Levels (pg/mL)	Intra-Assay %CV
1	15	6.7
2	21	7.3
3	279	3.0
4	185	8.8

Inter-Assay Variation

Sample No.	Mean GIP Levels (pg/mL)	Inter-Assay %CV
1	26	6.1
2	50	3.3
3	134	2.3
4	166	1.8

The assay variations of EMD Millipore Human GIP (Total) ELISA kits were studied on 8 human 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 were calculated from results of four separate assays with duplicate samples in each assay.

XV. ASSAY CHARACTERISTICS (continued)

D. Recovery

Spike & Recovery of Human GIP in Human Serum Samples

Sample No.	GIP Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	% of Recovery
1	0	76	76	100
	49.4	125.4	111	89
	148.9	224.9	200	89
	444.4	520.4	481	90
2	0	58	58	100
	49.4	107.4	93	87
	148.9	206.9	167	81
	444.4	502.4	418	83
3	0	54	54	100
	49.4	103.4	89	86
	148.9	202.9	176	87
	444.4	498.4	441	88

Varying amounts of human GIP were added to three human serum samples and the GIP content was determined in three separate assays. 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 (pg/mL)	Observed (pg/mL)	% Of Expected
1	20	396	396	100
	10	198	200	101
	5	99	102	103
	2.5	49.5	50	101
2	20	363	363	100
	10	181.5	176	97
	5	90.75	89	98
	2.5	45.4	45	99
3	20	648	648	100
	10	324	310	96
	5	162	162	100
	2.5	81	84	104

Three human serum samples with the indicated sample volumes were assayed. Required amounts of matrix were added to compensate for lost volumes below 20 µL. The resulting dilution factors of 1.0, 2.0, 4.0, and 8.0 representing 20 µL, 10 µL, 5 µL, and 2.5µL 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 EMD Millipore website emdmillipore.com using the catalog number as the keyword.

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
Human GIP Standard	E8054-K
Human GIP Quality Controls 1 and 2	E6054-K
Assay Buffer	EABGLP
Matrix Solution	EMTX
GIP Detection Antibody	E1054
Enzyme Solution	EHRP-3
Substrate	ESS-TMB
Stop Solution	ET-TMB
10-pack of Human GIP (Total) ELISA kits	EZHGIP-54BK

XIX. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.