Total GIP ELISA

RUO

AL-1013

INTENDED USE

The Total GIP enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of GIP (1-42aa, 3-42aa and 1-30aa) in human serum, plasma, and other biological fluids. This assay is intended for *in vitro research use only*.

SUMMARY AND EXPLANATION

Glucose-dependent insulinotropic polypeptide (GIP) (also known as gastric inhibitory polypeptide) is an incretin hormone produced in the upper gut and secreted to the circulation in response to the ingestion of foods, especially fatty foods[1]. It is a peptide hormone consisting of 42 amino acids and derives from posttranslational processing of pre-pro-GIP, a protein consisting of 153 amino acids. It is structurally similar to members of the secretin/glucagon family that include secretin, glucagon, vasoactive intestinal peptide, and growth hormone-releasing factor[2]. Growing evidence supports the physiological and pharmacological relevance of GIP in development of obesity and the pathogenesis of cardiovascular disease in addition to its involvement in type 2 diabetic pathophysiology[1, 3]. GIP acts in the entero-insular axis as an anabolic hormone that increases insulin levels, which in return increases the glycogen and fatty acid synthesis and inhibits the breakdown of fat. GIP also has extra pancreatic functions as well as roles in the stomach to reduce acid secretion by the parietal cells. On the bone, GIP has a dual effect as it causes proliferation of osteoblasts as well as inhibits osteoclastic bone resorption. The widespread expression of GIP-R in the brain suggests that GIP might play an essential function in neuro-signaling mechanisms[2].

PRINCIPLE OF THE TEST

The Total GIP ELISA is a quantitative two-step sandwich type immunoassay. In the first step Calibrators, Controls and unknown samples are added to GIP antibody coated microtiter wells and incubated with biotinylated GIP antibody. After the first incubation and washing, the wells are incubated with streptavidin horseradish peroxidase conjugate (SHRP). After the second incubation and washing step, the wells are incubated with substrate solution (TMB). After TMB incubation, an acidic stopping solution is added. In principle, the antibody-biotin conjugate binds to the solid phase antibody-antigen complex which in turn binds to the streptavidin-enzyme conjugate. The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme-substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as reference filter. The absorbance measured is directly proportional to the concentration of GIP in the samples and calibrators.

MATERIALS SUPPLIED

CAL-1013A **GIP Calibrator A**

One vial, 2 mL, labeled A, containing concentration of approximately 0 pg/mL GIP in heat treated serum with non-mercury preservative. Store unopened at 2 to 8°C until the expiration date.

CAL-1013F **GIP Calibrator F (Lyophilized)**

One vial, labeled F, containing concentrations of approximately 1000 pg/mL GIP in heat treated serum with non-mercury preservative. Refer to **calibration card** for exact concentration. Store unopened at 2 to 8°C until the expiration date. Reconstitute calibrator F with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze in eppendorf vials for multiple use. Avoid repeated freeze-thaws.

Note: *The GIP concentration in the calibrator is traceable to a synthetic preparation of GIP 3-42aa peptide.*

CTR-1013-I & CTR-1013-II **GIP Controls I & II (Lyophilized)**

Two vials, labeled Levels I and II containing low and high GIP concentrations in heat treated serum with non-mercury preservative. Refer to **calibration card** for exact control ranges. Store unopened at 2 to 8°C until the expiration date. Reconstitute control Levels I and II with 1 mL deionized water. Solubilize, mix well, and use after reconstitution. Aliquot and freeze in Eppendorf vials for multiple use. Avoid repeated freeze-thaws.

PLT-1013 **GIP Coated Microtitration Strips**

One strip-holder, containing 12 strips and 96 microtitration wells with GIP antibody immobilized to the inside wall of each well. Store at 2-8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

BCR-1013 **Total GIP Biotin Conjugate Ready-To-Use (RTU)**

One bottle, 12 mL, containing biotinylated anti-GIP antibody in protein-based buffer with a non-mercury preservative. Store at 2-8°C until expiration date.

SAR-1013 **Total GIP Streptavidin-Enzyme Conjugate—Ready-to-Use**

One amber bottle, 12 mL, containing streptavidin-HRP (horseradish peroxidase) in a protein-based buffer and a non-mercury preservative. Store at 2-8°C until expiration date.

TMB-100 **TMB Chromogen Solution**

One bottle, 12 mL, containing a solution of tetramethylbenzidine (TMB) in buffer with hydrogen peroxide. Store at 2 to 8°C until expiration date.

STP-100 **Stopping Solution**

One bottle, 12 mL, containing 0.2 M sulfuric acid. Store at 2 to 30°C until expiration date.

WSH-100 **Wash Concentrate A**

One bottle, 60 mL, containing phosphate buffer saline solution with a nonionic detergent. Store at 2 to 30°C until expiration date. Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of absorbance measurement at 450 nm, 405 nm and 630 nm.
2. Microplate orbital shaker.
3. Microplate washer.
4. Semi-automated/manual precision pipette to deliver 10–250 µL.
5. Vortex mixer.
6. Deionized water.
7. Disposable 12 x 75 mm culture tubes.
8. Tight fitting 12 x 75 mm tube racks.

WARNINGS AND PRECAUTIONS

For *in vitro* research use only.

The following precautions should be observed:

a) Follow good laboratory practice.

b) Use personal protective equipment. Wear lab coats and disposable gloves when handling immunoassay materials.

c) Handle and dispose of all reagents and material in compliance with applicable regulations.

d) If external package is damaged, inspect the components inside for any other damage. Do not use if the components are damaged.

WARNING: Potential Biohazardous Material

This reagent may contain heat treated serum or materials used in conjunction with human source materials. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 6th Edition, June 2020.[4]

WARNING: Potential Chemical Hazard

Some reagents in this kit contain Proclin 300 and Sodium azide[5] as a preservative. ProClin 300 and Sodium azide in concentrated amounts are irritants to skin and mucous membranes.

For further information regarding hazardous substances in the kit, please refer to the MSDS, either at AnshLabs.com or by request.

SAMPLE COLLECTION AND PREPARATION

a) K_2-EDTA Plasma and serum are the recommended sample types.

b) Sample handling, processing, and storage requirements depend on the brand of blood collection tube that you use. Please reference the manufacturer's instructions for guidance. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products.

c) Avoid storing samples at room temperature or 2-8°C for longer than 8 hours.

d) Samples should be stored at -80°C to avoid loss of bioactivity and contamination.

e) Avoid assaying lipemic, hemolyzed or icteric samples.

f) Avoid repeated freezing and thawing of samples.

g) For shipping, place specimens in leak proof containers in biohazard specimen bags with appropriate specimen identification and test requisition information in the outside pocket of the biohazard specimen bag. Follow DOT and IATA requirements when shipping specimens.[6]

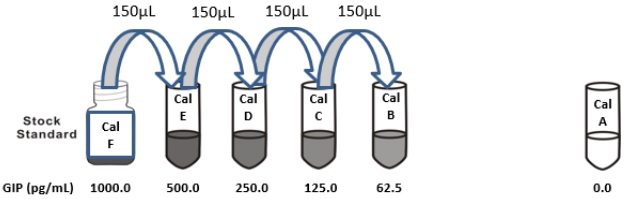
PROCEDURAL NOTES

1. A thorough understanding of this package insert is necessary for successful use of the GIP ELISA assay. It is the user's responsibility to validate the assay for their purpose. Accurate results will only be obtained by using precise laboratory techniques and following the package insert.
2. A calibration curve must be included with each assay.
3. Bring all kit reagents to room temperature (23 ± 2°C) before use. Thoroughly mix the reagents before use by gentle inversion. Do not mix various lots of any kit components and do not use any component beyond the expiration date.
4. Use a clean disposable pipette tip for each reagent, calibrator, control, or sample. Avoid microbial contamination of reagents, contamination of the substrate solutions with the HRP conjugates. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use deionized water.
5. Incomplete washing will adversely affect the outcome and assay precision. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the substrate solution into the wells. Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

PREPARATION OF REAGENTS

1. **GIP Calibrator F and GIP Controls I & II:** Tap and reconstitute GIP Calibrator F and GIP Controls I & II with 1.0 mL deionized water. Solubilize for 10 minutes, mix well, and use after reconstitution.
2. **Preparation of Calibrators B/2, B-E**:
   a. Prepare five tubes and label them as Cal. B/2, Cal. B, Cal. C, Cal. D, and Cal. E.
   b. Add 150 µL of GIP Calibrator A to each tube labeled Cal B-E.
   c. **Cal. E:** Add 150 µL of reconstituted GIP Calibrator F (from step 1) to the tube labeled Cal. E. Vortex and mix the content in the tube thoroughly before the next dilution transfer.
   d. **Cal. D:** Add 150 µL of Cal E (from step c) to the tube labeled Cal. D. Vortex and mix the content in the tube thoroughly before the next dilution transfer.
   e. **Cal. C:** Add 150 µL of Cal. D (from step d) to the tube labeled Cal. C. Vortex and mix the content in the tube thoroughly before the next dilution transfer.
   f. **Cal. B:** Add 150 µL of Cal. C (from step e) to the tube labeled Cal. B. Vortex and mix the content in the tube thoroughly before use.
   g. In case sensitivity below calibrator B level is desired, dilute calibrator B (from step f) as below.
   Cal. B/2: Mix 100 µL of Cal B (from step f) with 100 µL of calibrator A.

A schematic representation of the dilutions is shown below. Refer to the calibration card provided in the kit for exact concentration.



3. **Wash Solution:** Dilute wash concentrate 25-fold with deionized water. The wash solution is stable for one month at room temperature (23 ± 2°C) when stored in a tightly sealed bottle.
4. **Microtitration Wells:** Select the number of coated wells required for the assay. The remaining unused wells should be placed in a resealable pouch with a desiccant. The pouch must be resealed to protect it from moisture.

ASSAY PROCEDURE

Allow all specimens and reagents to reach room temperature (23 ± 2°C) and mix thoroughly by gentle inversion before use. Calibrators, controls, and unknowns should be assayed in duplicates.

NOTE: *All plasma samples reading higher than the highest calibrator should be mixed and diluted in the 0 pg/mL reconstituted Calibrator A prior to assay.*

1. Label the microtitration strips to be used.
2. Pipette **25 µL** of the Calibrators, Controls and Unknowns to the appropriate wells.
3. Add **100 µL** of the Antibody-Biotin Conjugate RTU to each well using a repeater pipette.
4. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **3 hours** at room temperature (23 ± 2°C).
5. Aspirate and wash each strip **5 times** with Washing Solution (**350 µL/per well**) using an automatic microplate washer.
6. Add **100 µL** of the Streptavidin-Enzyme Conjugate-RTU to each well using a repeater pipette.
7. Incubate the plate, shaking at a fast speed (**600-800 rpm**) on an orbital microplate shaker, for **30 minutes** at room temperature (23 ± 2°C).
8. Aspirate and wash each strip **5 times** with the Wash Solution (**350 µL/per well**) using an automatic microplate washer.
9. Add **100 µL** of the TMB chromogen solution to each well using a repeater pipette. Avoid exposure to direct sunlight.
10. Incubate the wells, shaking at **600–800 rpm** on an orbital microplate shaker, for **8-12 minutes** at room temperature (23 ± 2°C).
    NOTE: *Visually monitor the color development to optimize the incubation time*.
11. Add **100 µL** of the stopping solution to each well using a repeater pipette. Read the absorbance of the solution in the wells within 20 minutes, using a microplate reader set to **450 nm**.
    NOTE: *Zero calibrator should be programmed as "Blank" while reading the optical density. If an instrument has a wavelength correction, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction at 630 nm.*

RESULTS

1. Optimum results can be obtained at incubation temperature of 23 ± 2°C.
2. Calculate the mean OD for each calibrator, Control, or Unknown.
3. Plot the log of the mean OD readings for each of the Calibrators along the y-axis versus log of the GIP concentrations in pg/mL along the x-axis, using a cubic regression curve fit.
4. Determine the GIP concentrations of the Controls and Unknowns from the calibration curve by matching their mean OD readings with the corresponding GIP concentrations.
5. Any sample reading higher than the highest Calibrator should be appropriately diluted with the 0 pg/mL (CAL A) and re-assayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor, if required.

LIMITATIONS

The reagents supplied in this kit are optimized to measure GIP levels in plasma samples. If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the samples.[7]

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- Total GIP ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for GIP controls are printed on the **Calibration card**.
- A full calibration curve, low and high-level controls, should be included in each assay.
- TMB should be colorless. Development of any color may indicate reagent contamination or instability.

REPRESENTATIVE CALIBRATION CURVE DATA

Well Number	Well Contents Calibrators	Mean OD	Conc (pg/mL)
A1, A2	A	0.020 (Blank)	0
B1, B2	B	0.054	62.5
C1, C2	C	0.159	125.0
D1, D2	D	0.513	250.0
E1, E2	E	1.529	500.0
F1, F2	F	3.497	1000.0

CAUTION: *The above data must not be employed in lieu of data obtained by the user in the laboratory.*

ANALYTICAL CHARACTERISTICS

All concentrations listed are in pg/mL (1 pg/mL = 0.22 pmol/L).

Analytical Sensitivity:

The analytical sensitivity in the Total GIP ELISA assay, as calculated by the interpolation of mean plus two standard deviations of 16 replicates of calibrator A (0 pg/mL) and calibrator B (62.5 pg/mL), is 8.0 pg/mL.

Imprecision:

Reproducibility of the Total GIP assay was determined in a study using samples in the low, mid, and high range. The study included a total of 5 assays, 3 replicates of each per assay (n=15). Representative data were calculated based on EP10A-3 guidelines and are presented in the following table.

Sample	Mean Conc. (pg/mL)	Total SD (pg/mL)	Total CV %
Low	68.3	5.85	8.57
Mid	199.4	6.36	3.19
High	563.24	166.36	3.73

Analytical Specificity:

The monoclonal antibody pair used in the assay detects GIP and does not cross-react to other closely related analytes.

Cross-Reactant	Concentration	% Cross-reactivity
Oxyntomodulin (1-37)	1000 ng/mL	Non-Detectable
Glucagon (19-29)	1000 pg/mL	Non-Detectable
GLP-1 (7-36)	1000 ng/mL	Non-Detectable
GLP-1 (9-36)	1000 pg/mL	Non-Detectable
GLP-2 (1-34)	1000 ng/mL	Non-Detectable
GRPP	1000 ng/mL	Non-Detectable
MPGF-1	1000 ng/mL	Non-Detectable
MPGF-2	100 ng/mL	Non-Detectable
Insulin	10 ng/mL	Non-Detectable
C-peptide	10 ng/mL	Non-Detectable
Thyroglobulin	10 ng/mL	Non-Detectable
GIP (1-30)	5 ng/mL	47.6%
GIP (1-42)	5 ng/mL	100%
GIP (3-42)	5 ng/mL	100%

Species Immunoreactivity:

The antibody pair used in the assay detects rabbit, Goat, Bovine, Canine, Equine, Feline, Ovine, Porcine, and Squirrel Monkey samples as represented in table below.

Sample#	Species	Type	O.D.	Conc. (pg/mL)
1	Rabbit	Serum	0.018	<5.524
2	Rabbit	Serum	0.022	7.001
3	Goat	Serum	0.046	38.845
4	Goat	Serum	0.064	53.998
5	Bovine	Serum	0.044	36.932
6	Bovine	Serum	0.109	83.363
7	Canine	Tissue Extract	0.066	55.51
8	Canine	Serum	0.036	28.556
9	Equine	Cyst Fluid	0.031 (1:50)	22.481
10	Equine	Serum	0.195	125.938
11	Equine	Serum	0.171	115.107
12	Equine	Serum	0.349	185.422
13	Feline	Serum	0.691	290.217
14	Feline	Serum	0.012	ND
15	Ovine	Serum	0.032	23.767
16	Ovine	Serum	0.023	9.638
17	Porcine	Serum	0.039	31.854
18	Squirrel Monkey	Serum	1.3	445.706

ND: Non-Detectable

Interference:

When potential interferents (Hemoglobin, Biotin, Intralipids and Bilirubin) were added at least at two times their physiological concentration to control sample, GIP concentrations were within ±20% of the control as represented in the table below.

Interferent	Interferent Dose	Sample GIP (pg/mL)	Dosed Sample GIP (pg/mL)	% Difference
Hemoglobin	1mg/mL	623.84	610.97	-2.1
	0.5mg/mL	624.18	636.92	2
Biotin	1200ng/mL	611.89	621.31	1.5
	600ng/mL	663.66	650.69	-2
Intralipids	20mg/mL	603.73	565.35	-6.4
	10mg/mL	633.13	617.06	-2.5
Bilirubin	0.66mg/mL	478.51	404.84	-15.4
	0.2mg/mL	607.41	587.04	-3.4

Dilution Recovery:

Multiple dilutions of GIP 3-42 spiked serum containing various GIP levels were diluted with calibrator A. The % recovery on individual samples is represented in the following table.

Sample ID	Dilution Factor	Expected Conc (pg/mL)	Observed Conc (pg/mL)	%Recovery
3-42aa GIP Spiked Serum	Neat Value	1000.00		
	1:2	500.00	521.514	104%
	1:4	250.00	244.760	98%
	1:8	125.00	129.335	103%
	1:16	62.50	66.082	106%
	1:32	31.25	33.189	106%

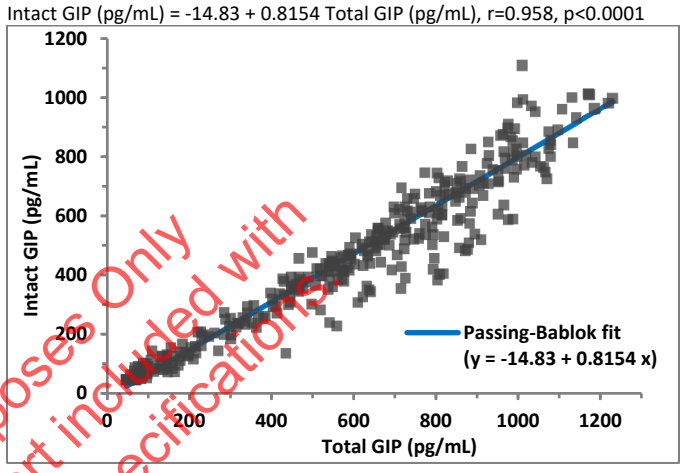
Spike Recovery:

Three serum samples containing endogenous value of GIP were spiked with antigen containing high level of GIP (1000.0 pg/mL) at three levels (5%, 10%, and 15%). The concentration of GIP was measured before and after the addition of exogenous GIP and the percentage recovery was calculated.

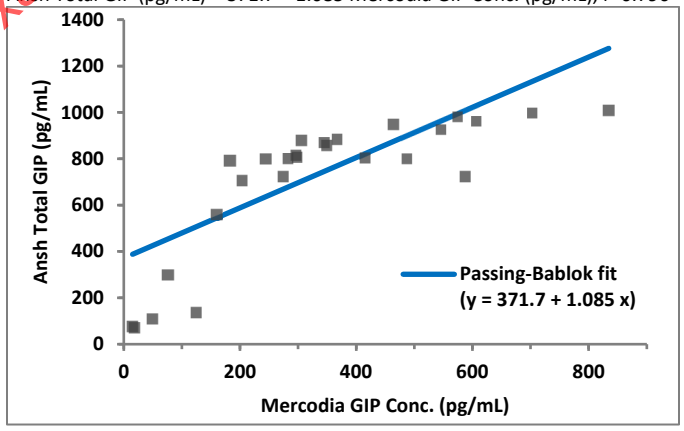
Sample ID	Endogenous Value in pg/mL	Expected in pg/mL	Observed in pg/mL	%Recovery
S1	158.981	201.03	216.30	108%
		243.08	273.37	112%
		285.13	326.74	115%
S2	46.566	94.24	95.33	101%
		141.91	150.54	106%
		189.58	194.14	102%
S3	108.059	152.66	168.12	110%
		197.25	216.07	110%
		241.85	284.40	118%

Method Comparison:

The Ansh Labs Total GIP ELISA (AL-1013) has been compared to Ansh Labs Intact GIP ELISA (AL-1022) using 338 serum samples.
Passing Bablok analysis of the results yielded the following Regression:
Intact GIP (pg/mL) = -14.83 + 0.8154 Total GIP (pg/mL), r=0.958, p<0.0001

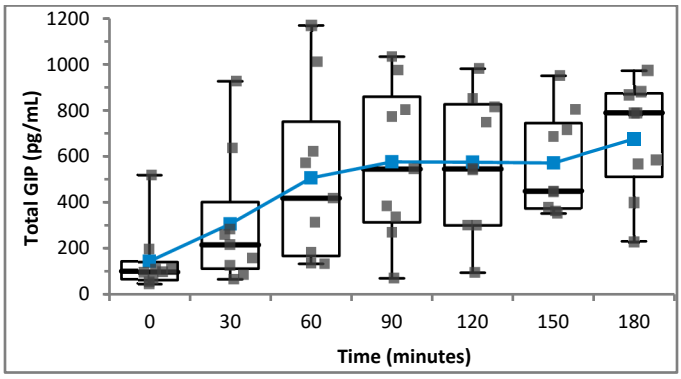


The Ansh Labs Total GIP ELISA (AL-1013) has been compared to Mercodia Total GIP ELISA 10-1258-01) using 26 serum samples.
Passing Bablok analysis of the results yielded the following Regression:
Ansh Total GIP (pg/mL) = 371.7 + 1.085 Mercodia GIP Conc. (pg/mL), r=0.796



Expected values:

Total GIP concentrations were measured in subjects, fasting (0 minutes) and between 30-180 minutes after meals at 30 minutes intervals. The change in Total GIP concentration with time after meal was analyzed and is presented below.



Time after meal (minutes)	N	Mean (pg/mL)	Median (pg/mL)	Range (pg/mL)	95% CI (pg/mL)
0	9	146.1	99.8	47.4 - 518.9	33.4 - 258.9
30	9	307.9	217.4	68.8 - 925.5	87.0 - 528.7
60	9	507.2	419.5	135.1 - 1170.1	217.9 - 796.5
90	9	576.8	545.7	72.3 - 1032.2	319.4 - 834.2
120	9	575.8	546.2	96.7 - 980.3	347.2 - 804.3
150	9	571.8	449.8	353.6 - 949.2	402.6 - 741.1
180	9	676.0	789.6	230.3 - 972.3	485.7 - 866.4

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This assay is intended for *in-vitro research* use only.

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