Mercodia
Total GIP NL-ELISA

Directions for Use

10-1258-01
Reagents for 96 determinations

For Research Use Only
Not for Use in Diagnostic Procedures

Manufactured by

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distributed in the US/Canada by:
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Explanation of symbols used on labels

Symbol	Meaning
Σ = 96	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
LOT	Lot No.

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Intended use

Mercodia Total GIP NL-ELISA provides a method for the quantitative determination of total GIP in human samples.

Summary and explanation of the test

Glucose dependent insulinotropic polypeptide (GIP) is an incretin, a gut-derived circulating peptide hormone that potentiates glucose-dependent insulin secretion following ingestion of a meal[1]. GIP(1-42) (active GIP) is rapidly cleaved in circulation at the N-terminus by DPP-4 to yield GIP(3-42), regarded as a non-active form of GIP[2]. Because of this quick proteolytic degradation, not only intact but also total (i.e. intact plus DPP-4-metabolized) forms of GIP must be measured to study its secretion and processing in vivo.

Principle of the procedure

Mercodia Total GIP NL-ELISA is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the GIP molecule.
GIP in the sample reacts with anti-GIP antibodies bound to microtitration wells and peroxidase-conjugated anti-GIP antibodies in the solution. A simple washing step removes unbound enzyme-labelled antibody. The bound conjugate is detected by reaction with the chemiluminescent substrate. A luminescence plate reader is used to read the intensity of light generated.

Warnings and precautions

- For research use only. Not for use in diagnostic procedures.
- Instrument settings should be optimized according to the manufacturer's instructions.
- Not for internal or external use in humans or animals.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, 5, 6 and Wash Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

  The Enzyme Conjugate Buffer, the Calibrators and Wash Buffer are labeled:

Warning

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

Material required but not provided

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution and substrate working solution)
- Tubes, beakers and cylinders for reagent preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader for chemiluminescence
- Microplate washing device with overflow function (recommended but not required)

Reagents 1 X 96

Each Mercodia Total GIP NL-ELISA kit (10-1258-01) contains reagents for 96 wells, sufficient for 41 samples and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2-8°C.

Reagent	Quantity	Volume	Preparation
Coated Plate Mouse monoclonal anti-GIP For unused microplate wells completely reseal the bag using adhesive tape, store at 2–8°C and use within 6 weeks.	1 plate	96 wells 8-well strips	Ready for Use
Calibrators 1, 2, 3, 4, 5, 6 Synthetic human GIP(3-42) Color-coded yellow Concentration indicated on vial label. Reconstituted Calibrators are stable for 6 weeks at 2-8°C.	6 vials	1000 µL	Lyophilized Reconstitute with 1000 µL redistilled water and vortex thoroughly.
Calibrator 0 Color coded yellow	1 vial	5 mL	Ready for Use
Enzyme Conjugate 11X Mouse monoclonal anti-GIP	1 vial	0.6 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	6 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2-8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash **buffer** 1X solution
Substrate Reagent A Colorless solution The mixture of the two components is stable for several hours at room temperature when protected from light.	1 vial	7 mL	Mix 1:1 with Substrate Reagent B to make substrate working solution e.g. 5 mL + 5 mL
Substrate Reagent B Colorless solution	1 vial	7 mL	Preparation, see Substrate Reagent A

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below.

When preparing enzyme conjugate 1X solution for the whole plate, pour all of the blue Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently. Store at 2-8°C and use within 6 weeks.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	0.36 mL	3.6 mL
4 strips	0.18 mL	1.8 mL

Specimen collection and handling

GIP in plasma samples will be sensitive to storage condition and freeze-thaw cycles. It is recommended to keep samples on ice when thawing them and while preparing the assay. Return to freezer as soon as possible.

Serum

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

EDTA plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant and separate the plasma fraction by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

Preparation of samples

No dilution is normally required, however, samples above the obtained value of Calibrator 6 should be diluted with Calibrator 0. *Note!* Buffers containing sodium azide (NaN_3) cannot be used for sample dilution.

Test procedure

Assay a calibrator curve in each run. All reagents and samples must be brought to room temperature before use.

1. Dilute Wash Buffer 21X with 1000 mL redistilled water to make wash buffer 1X solution.
2. Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer.
3. Reconstitute Calibrators with 1000 µL redistilled water.
4. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
5. Pipette 50 µL of enzyme conjugate 1X solution into appropriate wells.
6. Pipette 25 µL each of Calibrators and samples into appropriate wells and attach the plate sealer. Make sure that the Calibrators and samples are added to the plate within 20 minutes.
7. Incubate overnight (18-22 h) in refrigerator (2-8°C), without shaking.
8. Prepare substrate working solution by mixing equal volumes of Substrate Reagent A and Substrate Reagent B.
9. Before wash, incubate the plate on the bench for 10 min at room temperature (18-25°C).
10. Wash 6 x 700 µL using an automatic plate washer in plate mode combined with overflow-wash function. Ensure that the wells are never left without wash buffer (include soaking of the wells in each cycle). Invert and tap the plate firmly against absorbent paper after the final wash.
    Or manually:
    Discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1X solution to each well. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times for a total of 6 cycles.
11. Add 25 µL substrate working solution into each well.
12. Incubate in the dark for 15 min at room temperature (18-25°C), without shaking.
13. Use a microplate reader for chemiluminescence. Measure all visible light (glow) with an integration time of 1 second. No filter is needed. Use settings for a 96 well plate with flat bottom. Instrument settings should be used according to the manufacturer's instructions. Read within 5 minutes.
14. Apply curve fitting directly on raw data (RLU). Preferably use 5-parametric logistic regression with weighing using $1/Y^2$.

    Note! Be extra careful not to contaminate the substrate working solution with enzyme conjugate solution.

Internal quality controls

Internal plasma pools with low, intermediate and high GIP concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution and/or reconstitution dates of kit components, relative light units (RLU) values for the blank and Calibrators and concentration values for the controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

Calculation of results

The concentration of GIP is obtained by plotting the relative light units (RLU) of the Calibrators, except for Calibrator 0, versus their concentration. It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. It is every laboratory's responsibility to try out the functionality of the chosen curve fitting model and used software. Note that weighting of the curve fit is important to get a proper fit at the low range of the standard curve, especially when the measuring range is wide.

The Mercodia Total GIP NL-ELISA is validated using MARS (BMG Labtech) with Five Parameter Logistic (5PL) and automatic weighting using $1/Y^2$.

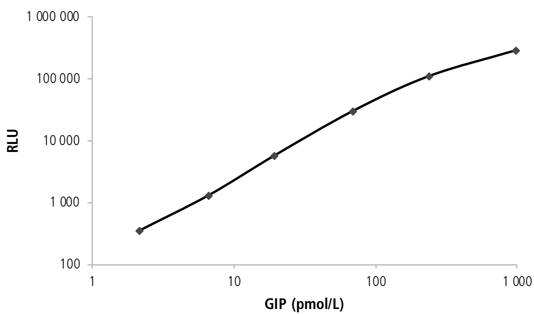
Example of results

Wells	Identity	RLU	Mean conc. pmol/L
1A-B	Calibrator 0	83/89	
1C-D	Calibrator 1*	364/334	
1E-F	Calibrator 2*	1377/1212	
1G-H	Calibrator 3*	5762/5597	
2A-B	Calibrator 4*	29342/30702	
2C-D	Calibrator 5*	111478/105371	
2E-F	Calibrator 6*	285122/284303	
2G-H	Sample 1	603/591	3.55
3A-B	Sample 2	137759/142606	326
3C-D	Sample 3	223167/219898	625

*Concentration stated on vial label

Example of calibrator curve

A typical calibrator curve is shown below. Do not use this curve to determine actual assay results.



Conversion factor

1 pg/mL corresponds to 0.21 pmol/L
1 pmol/L corresponds to 4.75 pg/mL
These conversion factors are used for GIP(3-42).

Limitations of the procedure

Grossly lipemic, icteric or hemolyzed samples do not interfere with the assay.

Expected values

Good laboratory practice dictates that each laboratory establishes its own expected range of values. The following results may serve as a guide until the laboratory has gathered sufficient data of its own. Fasting levels (≥ 9 h) for 122 tested, apparently healthy individuals (18-60 years) yielded a mean of 5.4 pmol/L, a median of 4.0 pmol/L and a central 95% reference range of 0-21 pmol/L analyzed in EDTA-plasma[3].

Performance characteristics

Selected studies are presented here. Additional data can be obtained from Mercodia.

Validation of curve fit

The curve fitting was validated with Five Parameter Logistics with $1/Y^2$ weighting. Five Parameter Logistics without weighting will also give acceptable results.

Detection limit

Detection limit is 1.62 pmol/L as determined by the methodology described in ISO11843-Part 4[4].

Sensitivity and range of quantification

Lower Limit of Quantification, LLOQ, is 2.7 pmol/L.

The Upper Limit of Quantification, ULOQ, is 1000 pmol/L.

Precision and accuracy

Serum controls were analyzed in 4 replicates over 13 different occasions with one kit lot and one instrument system by five different laboratory technicians.

			Coefficient of variation	
Sample	Mean value pmol/L	Between run accuracy %	Repeatability %	Within laboratory precision %
Control LLOQ	3.10	104	5.9	13
Control 1	4.16	104	6.6	13
Control 2	353	101	2.9	8.6
Control 3	703	96	2.7	8.5
Control ULOQ	812	93	4.1	14

Recovery upon addition

Recovery upon addition for five EDTA-plasma samples is 63%-88% (mean 74%).

Recovery upon dilution

Recovery upon dilution for 10 EDTA-plasma samples diluted 1/2 and 1/4 with Calibrator 0 is 87%-121% (mean 104%).

Dilution factor	Mean %	Min %	Max %
2	107	94	121
4	103	87	119

High Dose Hook Effect

Samples with a concentration up to at least 30400 pmol/L can be measured without giving falsely low results.

Cross-reactivity

The following cross-reactions were studied:

Substance	Concentrations tested pmol/L	Cross-reaction
GIP(1-42)	62.6-501	114%-124%
GIP(3-42)	*	100%
GIP(1-30)	62.4-998	n.d.
GIP(3-30)	4373-69969	n.d.
Glucagon	15.2-243	n.d.
GLP-1(9-36)amide	37.2-600	n.d.
GLP-2	63.1-1009	n.d.
Oxyntomodulin	37.3-597	n.d.
Glicentin	37.4-598	n.d.
Miniglucagon	37.4-599	n.d.
Proglucagon 1-61	62.0-992	n.d.
MPGF	125-2000	n.d.
PACAP-38	62.3-997	n.d.

* = Calibrator material
n.d. = not detected

Interference

Interference was tested at GIP(3-42) conc. 236 pmol/L at the following concentrations of selected substance:

Substance	Concentration tested pmol/L	Recovery %
GIP(1-30)	499	103
GIP(3-30)	17492	81
Glucagon	122	104
GLP-1(9-36)amide	300	102
GLP-2	505	104
Oxyntomodulin	299	92
Glicentin	300	92
Miniglucagon	300	97
Proglucagon 1-61	496	94
MPGF	1000	98
PACAP-38	499	98

Calibration

Mercodia Total GIP NL-ELISA is calibrated against a highly purified, commercial GIP(3-42) preparation, validated with amino acid analysis and HPLC-UV LC-MS/MS.

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect of consequential.

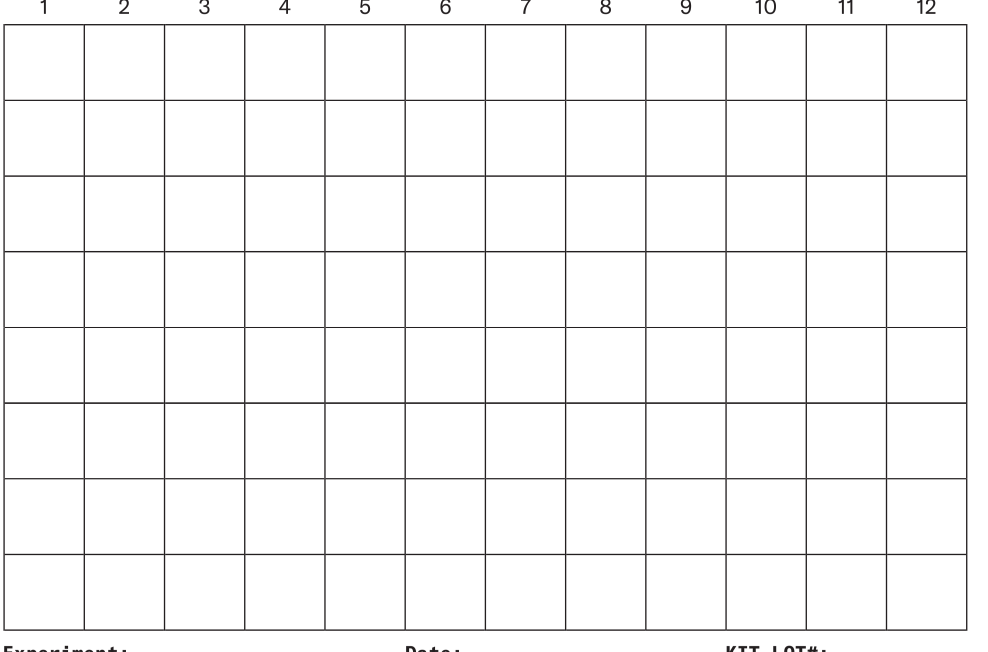
References

1. Reimann and Gribble, Mechanisms underlying glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 secretion. Journal of Diabetes Investigation, Volume 7, Issue S1, April 2016
2. Seino et al., GIP and GLP-1, the two incretin hormones: Similarities and differences. Journal of Diabetes Investigation Volume 1 Issue 1/2 2010
3. C28-A3 Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
4. International Organization for Standardization, www.iso.org/standard, ISO11843-Part 4

Further references can be found on our website: **www.mercodia.com**

Notes

Notes



	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment:

Date:

KIT LOT#:

Summary of protocol sheet
Mercodia Total GIP NL-ELISA

Add enzyme conjugate 1X solution	50 µL
Add Calibrators, controls* and samples and attach a plate sealer	25 µL
Incubate	Overnight (18-22 h) at 2-8°C without shaking
Incubate	10 minutes at 18-25°C on the bench
Wash plate with wash buffer 1X solution	700 µL, 6 times with a soak step between each cycle
Add substrate working solution	25 µL
Incubate	15 minutes in the dark at 18-25°C without shaking
Measure chemiluminescence	1 s integration time (glow)

*not included

For full details see page 7

For technical support please contact: support@mercodia.com

31-3195
Version 3.0
2020-01-28

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