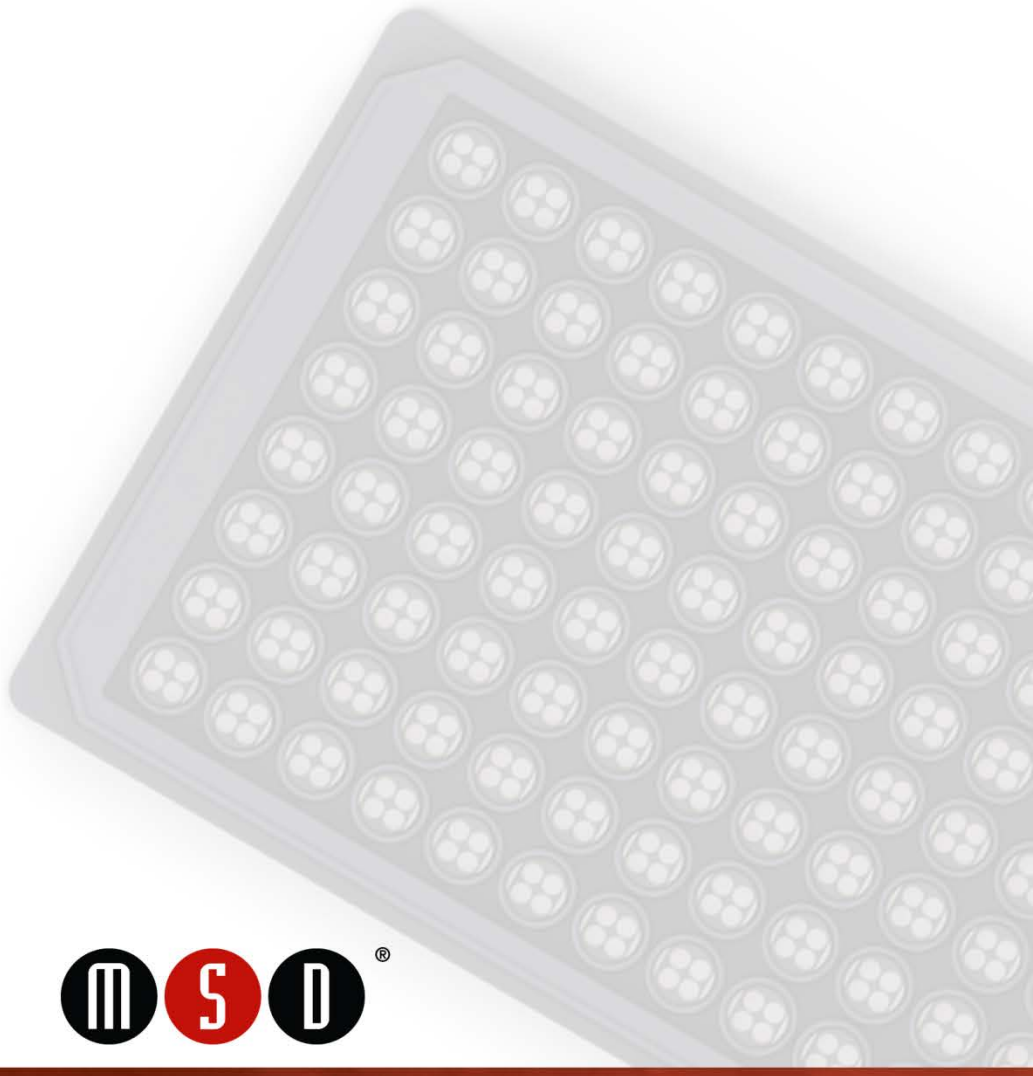
MSD® MULTI-SPOT Assay System

Human Total GIP Kit

1-Plate Kit	K151RPD-1
5-Plate Kit	K151RPD-2
25-Plate Kit	K151RPD-4



MSD Metabolic Assays

Human Total GIP Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Ordering Information

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Introduction

Glucose-dependent insulinotropic polypeptide or gastric inhibitory polypeptide (GIP) is a glucose-dependent, insulinotropic peptide that belongs to the incretin family. GIP is synthesized by enteroendocrine K cells in the duodenal and jejunal mucosa and is secreted into plasma in response to nutrient flow through the small intestine.[1] GIP exerts its effects through binding to glycosylated G-protein coupled receptors in the gut, pancreatic islets, adipose tissue, several regions of the brain, testis, pituitary, lung, heart, vascular endothelium, and bone.[1] GIP protects the small intestine from acid damage by reducing gastric acid secretion, inducing neutralization of stomach acid, and inhibiting gastrointestinal motility.[2] GIP also induces insulin secretion from pancreatic β-cells.[3] Additional functions of GIP have recently been proposed: modulation of fatty acid metabolism by stimulating lipoprotein lipase activity in adipocytes, regulation of bone turnover, and regulation of bloodflow.[4] It has been reported that Type 2 diabetics are resistant to GIP. Clinical applications that exploit GIP activity to regulate glucose homeostasis are under investigation.[5]

Principle of the Assay

MSD metabolic assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human Total GIP is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

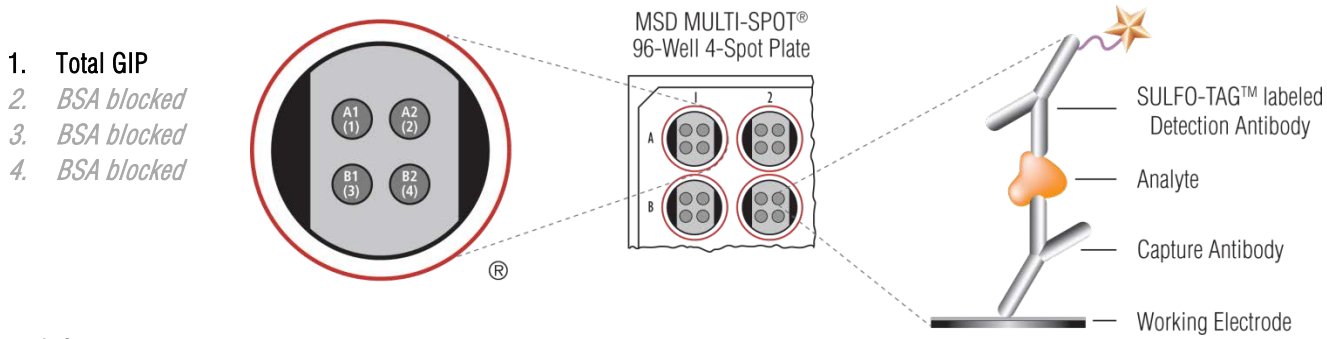


Figure 1. *Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

Reagents Supplied

Product Description	Storage	Quantity per Kit K151RPD-1	K151RPD-2	K151RPD-4
MULTI-SPOT® 96-Well, 4-Spot Human Total GIP Plate N451RPA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Total GIP Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Total GIP Calibrator (50 ng/mL)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 12 R50JA-4(10 mL), R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL ea)	5 bottles (50 mL ea)
Diluent 13 R56BB-4 (10 mL), R56BB-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Aprotinin A (200,000 KIU/mL)	2–8°C	1 vial (250 µL)	1 vial (250 µL)	1 vial (250 µL)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit	1 kit	5 kits
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle	1 bottle	5 bottles

Required Material and Equipment (not supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional safety information is available in the product Material Safety Data Sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate diluents 12 and 13 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the instructions included in the Blocker A kit.

Prepare Metabolic Assay Working Solution

For 1 plate, combine:

- ❑ 35 µL of Aprotinin
- ❑ 6965 µL of Diluent 13

Note: Addition of a final concentration of 0.1 mM DPP-IV inhibitor* in the metabolic assay working solution is strongly recommended (not included in kit).

Important: Aprotinin & DPP-IV Inhibitor should be added prior to use. The metabolic assay working solution should be kept on ice. Do not freeze the metabolic assay working solution for later use.

*Addition of DPP-IV Inhibitor to the metabolic assay working solution will aid in limiting enzymatic action of DPP-IV present in serum/plasma and provides the most accurate measurement of GIP.

Prepare Standards

MSD supplies calibrator for the Human Total GIP Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	Human Total GIP (pg/mL)	Dilution Factor
Stock Calibrator	50 000	
STD-01	2500	20
STD-02	833	3
STD-03	277	3
STD-04	93	3
STD-05	31	3
STD-06	10	3
STD-07	3.3	3
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1) Prepare the highest standard by adding 25 µL of stock calibrator to 475 µL of metabolic assay working solution. Mix well.
2) Prepare the next standard by transferring 150 µL of the highest standard to 300 µL of metabolic assay working solution. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use metabolic assay working solution as the blank.

Dilute Samples

For human EDTA plasma samples, MSD recommends 2-fold dilution in metabolic assay working solution; however, you may need to adjust the dilution factor for the sample set under investigation. To dilute sample 2-fold, add 75 µL of sample to 75 µL of metabolic assay working solution.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Total GIP Antibody
- ❑ 2.94 mL of Diluent 12

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample:** Wash the plate 3 times with 150–300 µL/well of PBS-T. Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 150–300 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

   You may prepare diluted read buffer during incubation.

   Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

4. **Wash and Read:** Wash the plate 3 times with 150–300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

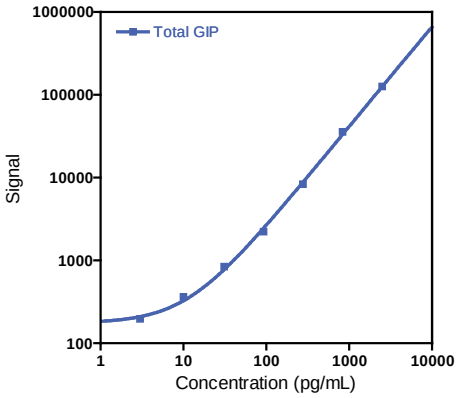
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of the standards.



Total GIP		
Conc. (pg/mL)	Average Signal	%CV
0	125	11.3
3	197	0.7
10	363	0.2
31	841	2.3
93	2 223	2.0
278	8 360	2.7
833	35 729	4.5
2500	126 338	7.2

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank).

	Total GIP
LLOD (pg/mL)	5.0

Tested Samples

Normal human EDTA plasma samples were diluted 2-fold and tested with the Human Total GIP Kit. Median and range of concentrations are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	Total GIP
EDTA Plasma (N=7)	Median (pg/mL)	146
	Range (pg/mL)	52–368
	Samples in Quantitative Range	7

Specificity

GIP is conserved across many species. The Human Total GIP assay is expected to react with human, mouse, rat, and non-human primate samples.

Assay Components

Calibrator

The assay calibrator uses synthetic human Total GIP protein, residues 1-42.

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
Total GIP	Mouse Monoclonal	Rabbit Polyclonal	A

References

1. Holst JJ. On the physiology of GIP and GLP-1. Horm Metab Res. 2440;36:747-754.
2. Pederson RA, et al. Inhibition of histamine-, pentagastrin-, and insulin-stimulated canine gastric secretion by pure "gastric inhibitory polypeptide". Gastroenterology, 1972 62:393-400.
3. Dupre J, et al. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab. 1973;37:826-8.
4. Ugleholdt R. Glucose-dependent Insulinotropic Polypeptide(GIP): From prohormone to actions in endocrine pancreas and adipose tissue. Dan Med Bull. 2011 Dec;58(12):B4368.
5. Diab DL. The contribution of enteroinsular hormones to the pathogenesis of type 2 diabetes mellitus. Curr Diab Rep. 2010 Jun;10(3):192-8.

Summary Protocol

Human Total GIP Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Total GIP assay.

Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare metabolic assay working solution.

Prepare standard solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in metabolic assay working solution.
- Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 2-fold in metabolic assay working solution before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 12.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2: Wash and Add Sample

Wash plate 3 times with 150–300 µL/well of PBS-T.

Add 50 µL/well of sample (standards, controls, or unknowns).

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 150–300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with 150–300 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.

Plate Diagram