Metabolic Panel 1 Kits

C-Peptide, GIP (active), GLP-1 (active), Glucagon, Insulin, Leptin, PP
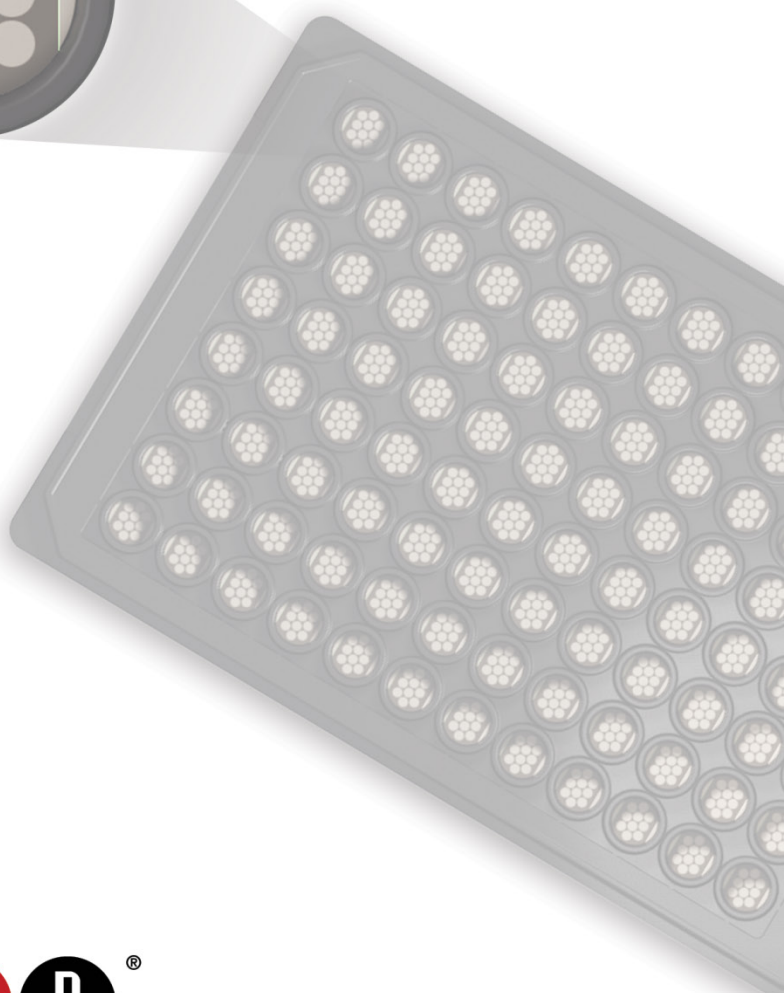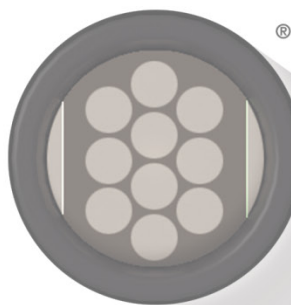
V-PLEX®

	V-PLEX	V-PLEX Plus
Metabolic Panel 1		
Human	K15325D	K15325G
NHP	K15332D	K15332G
Mouse	K15333D	K15333G
Rat	K15334D	K15334G
Canine	K15335D	K15335G
Individual Assay Kits		
Human C-Peptide	K151X5D	K151X5G
GIP (active)	K150T5D	K150T5G
GLP-1 (active)	K1503OD	K1503OG
Glucagon	K150U5D	K150U5G
Human Insulin	K151S5D	K151S5G
Human Leptin	K151V5D	K151V5G
Human PP	K151Y5D	K151Y5G

MSD®

MSD Metabolic Assays

Metabolic Panel 1 (human) Kit

C-Peptide, GIP (active), GLP-1 (active), Glucagon, Insulin, Leptin, and PP

Metabolic Panel 1 (NHP) Kit

C-Peptide, GIP (active), GLP-1 (active), Glucagon, Insulin, and PP

Metabolic Panel 1 (mouse) Kit

GLP-1 (active) and Glucagon

Metabolic Panel 1 (rat) Kit

GIP (active), GLP-1 (active), and Glucagon

Metabolic Panel 1 (canine) Kit

GIP (active), GLP-1 (active), Glucagon, and Insulin

For use with human, NHP, mouse, rat, and canine serum, plasma, and cell culture supernatants.

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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Introduction

MSD offers V-PLEX® assays for customers who require unsurpassed performance and quality. V-PLEX products are developed under rigorous design control and are fully validated according to fit-for-purpose principles[1] in accordance with MSD's Quality Management System. They offer exceptional sensitivity, simple protocols, reproducible results, and lot-to-lot consistency. In addition to the analytical validation, robustness of the assay protocol is assessed during development along with the stability and durability of the assay components and kits. V-PLEX assays are available in both single-assay and multiplex formats.

The V-PLEX assay menu is organized by panels. Grouping the assays into panels by species, analytical compatibility, clinical range, and expected use ensures optimal and consistent performance from each assay while still providing the benefits and efficiencies of multiplexing. V-PLEX panels are provided in MSD's MULTI-SPOT® 96-well plate format as multiplex assays. The composition of the panel and the location of each assay (i.e., its position within the well) are maintained from lot to lot. Most individual V-PLEX assays are provided on MSD's single-spot, 96-well plates. The remaining are provided on the multiplex panel plate.

Metabolic Panel 1 consists of seven of the many biomarkers that play significant roles in the physiological regulation of appetite, body fat and carbohydrate and lipid metabolism, and the pathogenesis of the metabolic syndrome. Metabolic syndrome consists of a group of complex but related metabolic disorders, which include insulin resistance, visceral obesity, atherogenic dyslipidemia, hyperglycemia, and hypertension. Although the syndrome is poorly understood, chronic inflammation associated with obesity, dysregulated lipid metabolism, and insulin or leptin resistance is considered the central players.[2-8] The biomarkers constituting the **Metabolic Panel 1** kits are **C-Peptide**, active form of **Glucose-dependent Insulinotropic polypeptide (GIP)**, active form of **Glucagon-like peptide-1 (GLP-1)**, **Glucagon**, **Insulin**, **Leptin** and **Pancreatic Polypeptide (PP).** Metabolic Panel 1 kits are validated for sample types across five species (human, mouse, NHP, rat, and canine). Metabolic assays that have shown cross-reactivity across different species are available as species-specific Metabolic Panels.

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 across five species (human, mouse, NHP, rat, and canine). The assays in the Metabolic Panel 1 are sandwich immunoassays. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots, as shown in the layout below. Multiplex assays and the individual C-Peptide, GIP (active), and PP assays are provided on 10-spot MULTI-SPOT plates (Figure 1); the individual insulin, GLP-1 (active), glucagon, and leptin assays are provided on Small Spot plates (Figure 2). For information regarding the individual GLP-1 (active) assay (Catalog # K1503OD /G), refer to the product-specific page at mesoscale.com. 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 creates the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample. V-PLEX assay kits have been validated according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by J. W. Lee, et al.[1]

1. Insulin
2. C-Peptide
3. GLP-1 (active)
4. *BSA coated*
5. *BSA coated*
6. *BSA coated*
7. GIP (active)
8. Glucagon
9. PP
10. Leptin
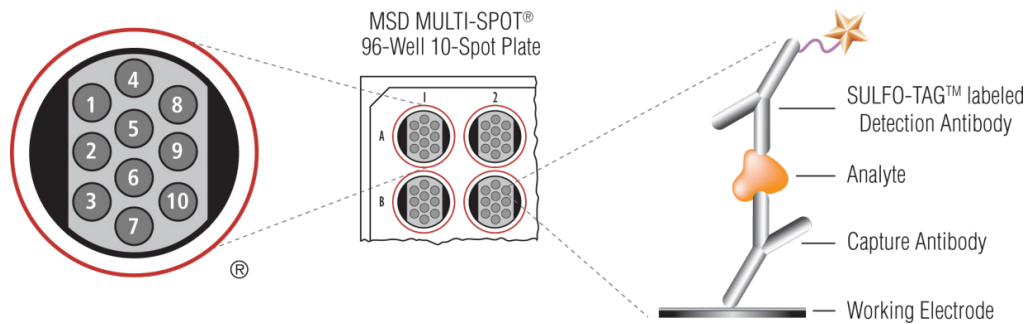
Figure 1. Multiplex plate spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.
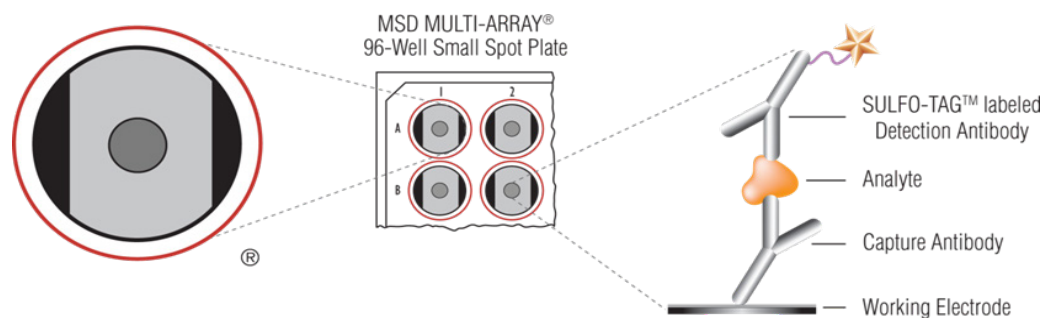
Figure 2. Small Spot plate diagram showing placement of analyte capture antibodies.

Kit Components

Metabolic Panel 1 assays are available as a multiplex kit, as individual assay kits, or as custom V-PLEX kits with subsets of assays selected from the full panel. All V-PLEX kits are provided with pre-coated plates, calibrators, detection antibodies, and reagents. V-PLEX Plus kits include additional items (controls, wash buffer, and plate seals). See below for details (Table 1; Table 2; Table 3; Table 4).

See the **Catalog Numbers** section for a comprehensive list of all kits.

Reagents Supplied With All Kits

Table 1. Reagents supplied with V-PLEX and V-PLEX Plus assay kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Metabolic Panel 1 Calibrator Blend	2–8 °C	C0325-2	1 vial	1 vial	5 vials	25 vials	Lyophilized recombinant and synthetic proteins; Individual analyte concentration provided in the lot-specific COA
Diluent 56‡	≤−10 °C	R50BR-1	10 mL	1 bottle	NA	NA	Diluent for samples and calibrator; contains blockers, protein, and preservatives
		R50BR-2	50 mL	NA	1 bottle	5 bottles	
Diluent 11‡	≤−10 °C	R55BA-5	10 mL	1 bottle	NA	NA	Diluent for detection antibody; contains blockers and preservatives
		R55BA-3	50 mL	NA	1 bottle	5 bottles	
MSD GOLD Read Buffer B	RT**	R60AM-1	18 mL	1 bottle	NA	NA	Buffer to catalyze the electro-chemiluminescence reaction
		R60AM-2	90 mL	NA	1 bottle	5 bottles	

‡Provided in Diluent Assembly 25 and Diluent Assembly 26

**RT = room temperature

NA = not applicable

V-PLEX Plus Kits: Additional Components

Table 2. Additional components supplied with V-PLEX Plus assay kits

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Metabolic Panel 1 Control 1*	2–8 °C	C4325-1	1 vial	1 vial	5 vials	25 vials	Lyophilized recombinant and synthetic proteins; individual concentrations of the controls provided in the lot-specific COA
Metabolic Panel 1 Control 2*	2–8 °C	C4325-1	1 vial	1 vial	5 vials	25 vials	
Metabolic Panel 1 Control 3*	2–8 °C	C4325-1	1 vial	1 vial	5 vials	25 vials	
Wash Buffer (20X)	RT	R61AA-1	100 mL	1 bottle	1 bottle	5 bottles	20-fold concentrated phosphate buffered solution with surfactant
Plate Seals	NA	NA	NA	3	15	75	Adhesive seals for sealing plates during incubations

*Provided as components in the Metabolic Panel 1 Control Pack (catalog no. C4325-1)

NA = not applicable

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Kit-Specific Components

Table 3. *Components supplied with specific kits*

Plates	Storage	Catalog No.	Size	Quantity Supplied			Description
				1-Plate Kit	5-Plate Kit	25-Plate Kit	
Metabolic Panel 1 SECTOR® Plate	2–8 °C	N05325A-1	10-spot	1	5	25	96-well plate, foil sealed, with desiccant
Human Insulin SECTOR Plate	2–8 °C	L451S5A-1	Small Spot	1	5	25	
Glucagon SECTOR Plate	2–8 °C	L451U5A-1	Small Spot	1	5	25	
Human Leptin SECTOR Plate	2–8 °C	L451V5A-1	Small Spot	1	5	25	

Table 4. *Kits are supplied with individual detection antibodies for each assay ordered*

SULFO-TAG Detection Antibody	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	
Anti-hu Insulin Antibody (50X)	2–8 °C	D21S5-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D21S5-3	375 µL	NA	1	5	
Anti-hu C-Peptide Antibody (50X)	2–8 °C	D21X5-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D21X5-3	375 µL	NA	1	5	
GLP-1 (active) Antibody (50X)	2–8 °C	D2O3O-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D2O3O-3	375 µL	NA	1	5	
GIP (active) Antibody (50X)	2–8 °C	D20T5-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D20T5-3	375 µL	NA	1	5	
Glucagon Antibody (50X)	2–8 °C	D20U5-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D20U5-3	375 µL	NA	1	5	
Anti-hu PP Antibody (50X)	2–8 °C	D21Y5-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D21Y5-3	375 µL	NA	1	5	
Anti-hu Leptin Antibody (50X)	2–8 °C	D21V5-2	75 µL	1	NA	NA	SULFO-TAG conjugated antibody
		D21V5-3	375 µL	NA	1	5	

NA = not applicable

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Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ 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
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1000 rpm
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 for plate washing or MSD Wash Buffer catalog no. R61AA-1 (included in V-PLEX Plus kit)
- ❑ Adhesive plate seals (3 per plate included in V-PLEX Plus kits)
- ❑ Deionized water
- ❑ Vortex mixer
- ❑ DPP-IV Inhibitor. Formulate and store as recommended by the manufacturer.

Optional Materials and Equipment

- ❑ Metabolic Panel 1 Control Pack, available for separate purchase from MSD, catalog no. C4325-1 (included in V-PLEX Plus kit)
- ❑ Centrifuge (for sample preparation)
- ❑ Blocker A Kit (R93AA-2; 250 mL)

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 product-specific safety information is available in the applicable safety data sheet(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

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Best Practices

- Mixing and substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific certificate of analysis (COA).

- Assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs.

- Bring frozen diluent to room temperature in a 20–25 °C water bath. Thaw other reagents on wet ice and use as directed immediately.

- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution. Vortex after each dilution before proceeding.

- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.

- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.

- Do not touch the pipette tip on the bottom of the wells when pipetting into the MSD plate.

- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette to the bottom corner.

- Plate shaking should be vigorous, with a rotary motion between 500–1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking in the middle of this range (~700 rpm) or above.

- When using an automated plate washer, use individual wash cycles, and rotate the plate 180 degrees between wash steps to improve assay precision and reduce potential assay issues due to washing.

- Gently tap the plate on a paper towel to remove residual fluid after washing.

- Avoid excessive drying of the plate during washing step. Add solutions to the plate immediately after washing.

- Read buffer should be at room temperature when added to the plate.

- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.

- Do not shake the plate after adding read buffer.

- Ensure that the reagents for the next step are prepared before washing the plates in order to avoid the plates from drying out.

- Remove the plate seals before reading the plate.

- If assay results are above the top of the calibration curve, dilute the samples and repeat the assay.

- We do not recommend attempting to use a partial plate when running this panel.

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Reagent Preparation

Bring all reagents to room temperature.

Note: Upon first thaw, separate Diluent 56 and Diluent 11 into suitably sized aliquots before refreezing.

Prepare Calibrator Dilutions

MSD supplies a multi-analyte lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted in 1,000 µL of Diluent 56. Keep reconstituted calibrator and calibrator solutions on wet ice until use.

For individual assays that do not saturate at the highest calibrator concentration, the calibration curve can be extended by creating an even more concentrated highest calibrator. In this case, follow the steps below using 250 µL instead of 1,000 µL of Diluent 56 when reconstituting the lyophilized calibrator (Figure 3).

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

1) Prepare the highest calibrator solution (Calibrator 1) by adding 1,000 µL of Diluent 56 to the lyophilized calibrator vial. After reconstituting, invert at least 3 times (do not vortex). Let the reconstituted solution equilibrate at room temperature for 15–30 minutes and then vortex briefly using short pulses.

2) Prepare the next calibrator by transferring 100 µL of Calibrator 1 to 300 µL of Diluent 56. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.

3) Use Diluent 56 as the zero calibrator.

Note: Reconstituted calibrator is stable when stored at 2–8 °C for up to seven days. However, it may be stored frozen at ≤−70 °C and is stable through three freeze-thaw cycles. For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.

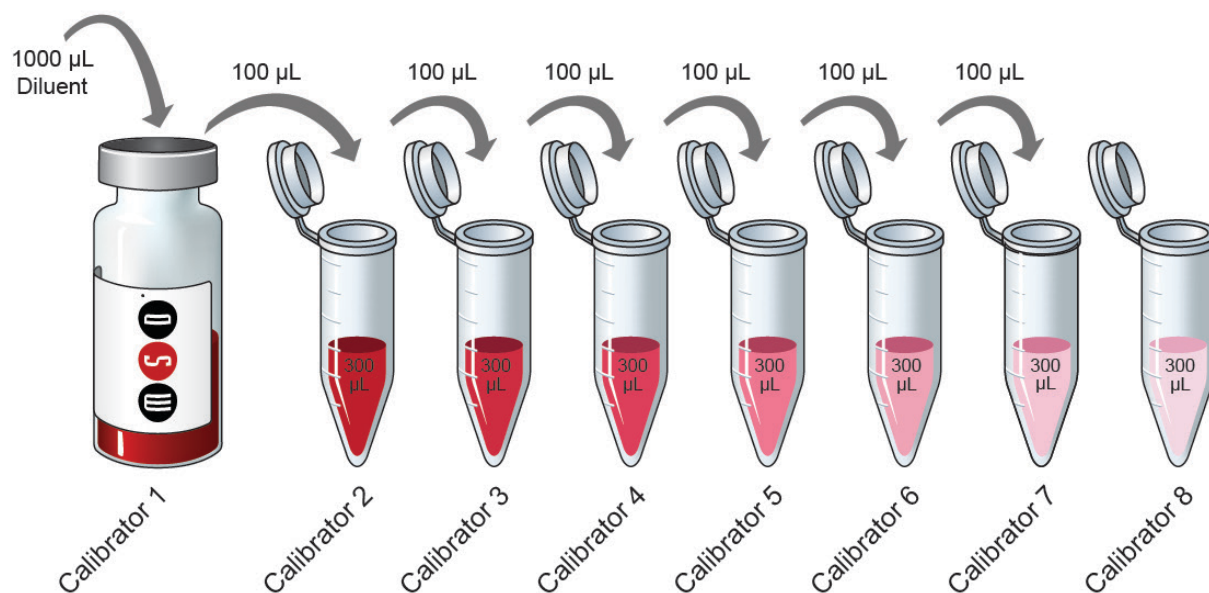*Figure 3.* Dilution schema for preparation of calibrator standards.

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Sample Collection and Handling

General guidelines for sample collection, storage, and handling of metabolic markers, including glucagon and the active forms of GLP-1, and GIP are provided below. If possible, use published guidelines.[9-12] Evaluate sample stability under the selected methods as needed.

The assay requires up to 50 µL/well of a sample. An adequate volume of each sample should be prepared depending upon the desired number of replicates.

Human, NHP, Rat, and Canine Plasma: Samples should be collected using the BD P800 Blood Collection System for Plasma Metabolic Biomarker Preservation[47], which contains DPP-IV and other protease inhibitors (product numbers 366420 or 366421). An alternative collection method described below with K2 EDTA tubes can also be used.

Mouse Plasma: P800 tubes are not recommended. Use the alternative collection method described below.

Alternative collection method: Collect blood in BD Vacutainer EDTA Tubes (Product Number 367841 or 366643). <u>Immediately</u> add DPP-IV inhibitor (0.1 mM final concentration, not provided with the kit) and aprotinin (1,000 kIU/mL final concentration) and mix to avoid cleavage/degradation of GLP-1.

For BD tubes, process as follows.

1) In Swing-out rotor centrifuge, spin the blood collection tubes as follows.

   - For 2 mL tubes: 10 minutes at $1,000 \times g$ (4–8 °C)

   - For 8.5 and 10 mL tubes: 20 minutes at $1,300 \times g$ (4–8 °C)

2) Use the plasma immediately, or the samples can be stored at 2–8 °C if used within 3 hours. For future use, aliquot the plasma and freeze in suitably sized aliquots at ≤ -70 °C.

For samples other than plasma: <u>Immediately</u> add DPP-IV inhibitor (0.1 mM final concentration, not provided with the kit) and aprotinin (1,000 kIU/mL final concentration), and use immediately or freeze at ≤ -70 °C.

Samples with hemolysis or significant lipemia may hinder accurate measurements.

Repeated freezing and thawing of samples is not recommended. After thawing, centrifuge samples at $2,000 \times g$ for 3 minutes to remove particulates prior to performing the assay. If the samples are clear and no particulates are visible, you may not need to centrifuge. Hold on wet ice or at 4–8 °C until processed and used in the assay.

Dilute Samples

Dilute samples with Diluent 56. For human, mouse, rat, and canine serum and plasma samples, MSD recommends a minimum 2-fold dilution. For example, when running samples in duplicate, add 60 µL of sample to 60 µL of Diluent 56. For NHP samples, MSD recommends a minimum 4-fold dilution. For example, when running samples in duplicate, add 30 µL of sample to 90 µL of Diluent 56. We recommend running at least two replicates per sample. You may conserve sample volume by using a higher dilution. Nonfasted or tissue culture supernatants may require additional dilutions based on stimulation and analyte concentrations in the sample. Additional diluent can be purchased at www.mesoscale.com.

Prepare Controls

Three levels of multi-analyte, lyophilized controls are available for separate purchase from MSD in the Metabolic Panel 1 Control Pack (Catalog number C4325-1).

Note: Controls are included only in the V-PLEX Plus kits.

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Reconstitute the lyophilized controls in 250 µL of Diluent 56. Do not invert or vortex the vials. Wait for a minimum of 15–30 minutes at room temperature before diluting controls 2-fold in Diluent 56. Vortex briefly using short pulses.

Note: Reconstituted controls are stable when stored at 2–8 °C for up to seven days. However, it may be stored at ≤−70 °C and is stable through three freeze-thaw cycles. For the lot-specific concentration of each analyte in the control pack, refer to the supplied COA. You can also find a copy of the COA at www.mesoscale.com.

Prepare Detection Antibody Solution

MSD provides each detection antibody separately as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use. Follow the preparation as per the kit ordered.

Note: Avoid prolonged exposure of the detection antibody vials and detection solution to light.

Metabolic Panel 1 (human) kit
For one plate, combine the following detection antibodies and add to 2,580 µL of Diluent 11:

- ❑ 60 µL of SULFO-TAG Anti-hu Insulin Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu C-Peptide Antibody
- ❑ 60 µL of SULFO-TAG Anti-GLP-1 (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-GIP (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-Glucagon Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu PP Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu Leptin Antibody

Metabolic Panel 1 (NHP) kit
For one plate, combine the following detection antibodies and add to 2,640 µL of Diluent 11:

- ❑ 60 µL of SULFO-TAG Anti-hu Insulin Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu C-Peptide Antibody
- ❑ 60 µL of SULFO-TAG Anti-GLP-1 (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-GIP (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-Glucagon Antibody
- ❑ 60 µL of SULFO-TAG Anti-hu PP Antibody

Metabolic Panel 1 (mouse) kit
For one plate, combine the following detection antibodies and add to 2,880 µL of Diluent 11:

- ❑ 60 µL of SULFO-TAG Anti-GLP-1 (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-Glucagon Antibody

Metabolic Panel 1 (rat) kit
For one plate, combine the following detection antibodies and add to 2,820 µL of Diluent 11:

- ❑ 60 µL of SULFO-TAG Anti-GLP-1 (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-GIP (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-Glucagon Antibody

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Metabolic Panel 1 (canine) kit

For one plate, combine the following detection antibodies and add to 2,820 µL of Diluent 11:

- ❑ 60 µL of SULFO-TAG Anti-GLP-1 (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-GIP (active) Antibody
- ❑ 60 µL of SULFO-TAG Anti-Glucagon Antibody

Custom multiplex kits

For one plate, combine 60 µL of each supplied detection antibody with enough Diluent 11 to bring the final volume to 3,000 µL.

Individual assay kits

For one plate, add 60 µL of the supplied detection antibody to 2,940 µL of Diluent 11.

Prepare Wash Buffer

MSD provides 100 mL of Wash Buffer as a 20X stock solution in the V-PLEX Plus kit. Dilute the stock solution before use. PBS + 0.05% Tween-20 can be used instead.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

Prepare MSD GOLD Read Buffer B

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

Note: Unlike Read Buffer T, which is provided at a 4X concentration, MSD GOLD Read Buffer B is provided at the working concentration for the assay. Diluting MSD GOLD Read Buffer B may compromise assay results.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1; Figure 2) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Prewash plates before use as recommended in the assay protocol.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Wash and Add Sample

- ❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer.
- ❑ Add 50 μL of prepared samples, calibrators, or controls per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 2 hours.
  - ❑ **Note:** Washing the plate prior to sample addition is an optional step that may provide greater uniformity of results for certain assays. Analytical parameters including limits of quantification, recovery of controls, and sample quantification are not affected by washing the plate prior to sample addition.
  - ❑ **Optional Blocking step**: A pre-assay blocking step may be incorporated if consistency with older protocols is required. Prepare Blocker A according to the instructions provided in the Blocker A Kit (Catalog no: R93AA-2). Dispense 150 μL of Blocker A solution into each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with shaking (~700 rpm) at room temperature, then proceed to step 1 above.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer.
- ❑ Add 25 μL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking (~700 rpm) for 1 hour.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 μL/well of 1X MSD Wash Buffer.
- ❑ Add 150 μL of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument. Incubation in MSD GOLD Read Buffer B is not required before reading the plate.

  Important: Unlike Read Buffer T, which is provided at a 4X concentration, MSD GOLD Read Buffer B is provided at the working concentration for the assay. Diluting MSD GOLD Read Buffer B may compromise assay results.

Alternate Protocols

The suggestions below may be useful as alternate protocols; however, not all were tested using multiple kit lots.

- **Alternate Protocol 1, Reduced Wash:** For tissue culture samples, you may simplify the protocol by eliminating one of the wash steps. After incubating diluted sample, calibrator, or control, add detection antibody solution to the plate without decanting or washing the plate. See **Appendix A** for assay performance using this protocol.

- **Alternate Protocol 2, Extended Sample Incubation:** Incubating samples overnight at 2–8 °C may improve sensitivity for some assays. See **Appendix A** for specific assays that may benefit from this alternate protocol.

- **Alternate Protocol 3, Dilute In-plate:** To limit sample handling, you may dilute samples and controls directly in the plate. For 2-fold dilution, add 25 μL of assay diluent to each sample or control well, and then add 25 μL of neat control or sample. Calibrators should not be diluted in the plate; add 50 μL of each calibrator directly into empty wells. Tests conducted according to this alternate protocol produced results that were similar to the recommended protocol (data not shown).

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Validation

MSD's V-PLEX products are validated following fit-for-purpose principles[1] and MSD design control procedures. V-PLEX assay components go through an extensive, critical reagents program to ensure that the reagents are controlled and well characterized. Prior to the release of each V-PLEX panel, at least three independent kit lots are produced. Using results from multiple runs (typically greater than 50) and multiple operators, these lots are used to establish product specifications for sensitivity, specificity, accuracy, and precision. During validation, each individual assay is analytically validated as a singleplex and is also independently evaluated as a multiplex component by running the full multiplex plate using only the single detection antibody for that assay. These results are compared with the results from the multiplex panel when using all detection antibodies. This approach demonstrates that each assay is specific and independent, allowing them to be multiplexed in any combination. The COA provided with each kit outlines the kit release specifications for sensitivity, specificity, accuracy, and precision.

➢ **Dynamic Range**

Calibration curve concentrations for each assay are optimized for a maximum dynamic range while maintaining enough calibration points near the bottom of the curve to ensure a proper fit for accurate quantification of samples that require high sensitivity.

➢ **Sensitivity**

The lower limit of detection (LLOD) is a calculated concentration corresponding to the average signal 2.5 standard deviations above the background (zero calibrator). The LLOD is calculated using results from multiple plates for each lot, and the median and range of calculated LLODs for a representative kit lot are reported in this product insert. The upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) are established for each lot by measuring multiple levels near the expected LLOQ and ULOQ levels. The final LLOQ and ULOQ specifications for the product are established after the assessment of all validation lots.

➢ **Accuracy and Precision**

Accuracy and precision are evaluated by measuring calibrators and matrix-based validation samples or controls across multiple runs and multiple lots. For most assays, the results of control measurements fall within 20% of the expected concentration for each run. Precision is reported as the coefficient of variation (CV). Intra-run %CVs are typically below 7%, and inter-run %CVs are typically below 7%. Rigorous management of Inter-lot reagent consistency and calibrator production results in typical Inter-lot %CVs below 20%. Validation lots are compared using controls and at least 25 samples in various sample matrices. Samples are well correlated with an Inter-lot bias, typically below 15%.

➢ **Matrix Effects and Samples**

Matrix effects from serum, plasma, and cell culture media are measured as part of development and validation. Parallelism, dilution linearity, and spike recovery studies are performed on individual samples to assess the variability of results due to matrix effects. The sample dilution suggested in the protocol gives an appropriate dilution factor for all assays in the multiplex. Some assays may benefit from lower or higher dilution factors, depending on the samples and application (data are provided in this product insert). In addition to the matrices listed above, urine, CSF, saliva, and cell lysate samples were tested, but dilution linearity and spike recovery studies were not performed.

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➢ Specificity

The specificity of both capture and detection antibodies is measured during assay development. Antibody specificity is assessed by first running each assay using the multiplex plate with an assay-specific detection antibody and an assay-specific calibrator. These results are compared to the assay's performance when the plate is run 1) with the multi-analyte calibrator and assay-specific detection antibodies and 2) with assay-specific calibrator and all detection antibodies. For each validation lot and for product release, assay specificity is measured using a multi-analyte calibrator and individual detection antibodies. The calibrator concentration used for specificity testing is chosen to ensure that the specific signal is greater than 50,000 counts.

In addition to measuring the specificity of antibodies to analytes in the multiplex kit, specificity and interference from other related markers are tested during development. This includes evaluation of selected related proteins and receptors or binding partners.

➢ Assay Robustness and Stability

The robustness of the assay protocol is assessed by examining the boundaries of the selected incubation times, evaluating the stability of assay components during the experiment, and the stability of reconstituted lyophilized components during storage. For example, the stability of a reconstituted calibrator is assessed in real-time over 30 days. Assay component (calibrator, antibody, control) stability is assessed via freeze-thaw testing and accelerated stability studies.

Representative data from verification and validation studies are presented in the following sections. The calibration curve and measured limits of detection for each lot can be found in the lot-specific COA that is included with each kit and available for download at www.mesoscale.com.

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Analysis of Results

The calibration curves used to calculate analyte concentrations were established by fitting the signals from the calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The weighting function provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve. Analyte concentrations were determined from the electrochemiluminescence (ECL) signals by back fitting to the calibration curve. These assays have a wide dynamic range (4 logs), which allows accurate quantification of samples without the need for multiple dilutions or repeated testing. The calculations to establish calibration curves and determine concentrations were carried out using the MSD DISCOVERY WORKBENCH® analysis software.

Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of two replicates at each calibrator level.

Typical Data

Data from the Metabolic Panel 1 were collected over two months of testing by ten operators (51 runs in total). Calibration curve accuracy and precision were assessed for two kit lots. Representative data from one lot are presented below (Figure 4). Data from individual assays are presented in **Appendix B**. The multiplex panel was tested with individual detection antibodies to demonstrate that the assays are independent of one another. **Appendix C** compares results for each assay in the kit when the panel is run using the individual detection antibody versus all detection antibodies. The calibration curves were comparable. Calibration curves for each lot are presented in the lot-specific COA.
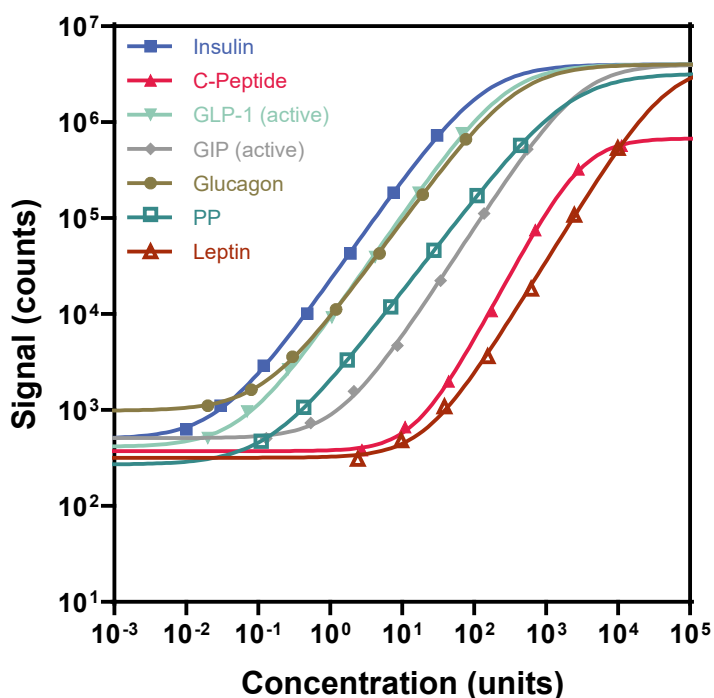
Figure 4. Typical calibration curves for the Metabolic Panel 1 assay.

Sensitivity

The LLOD is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). The LLOD shown below was calculated based on 88 runs.

The ULOQ is the highest concentration at which the %CV (%CV = coefficient of variation) of the calculated concentration is <20%, and the recovery of each analyte is within 80% to 120% of the known value.

The LLOQ is the lowest concentration at which the %CV of the calculated concentration is <20%, and the recovery of each analyte is within 80% to 120% of the known value.

The quantitative range of the assay lies between the LLOQ and ULOQ.

The LLOQ and ULOQ are verified for each kit lot, and the results are provided in the lot-specific COA that is included with each kit and available at www.mesoscale.com. See Table 5.

Table 5. LLOD, LLOQ, and ULOQ for each analyte in the Metabolic Panel 1 Kit

Analyte	Assay Units	Median LLOD	LLOD Range	LLOQ	ULOQ
Insulin	µIU/mL	0.006	0.002–0.052	0.07	19.5
C-Peptide	pg/mL	4.72	2.07–10.2	19	3,150
GLP-1 (active)	pM	0.011	0.005–0.027	0.1	39
GIP (active)	pg/mL	0.233	0.106–0.691	3.18	390
Glucagon	pM	0.015	0.007–0.083	0.33	52
PP	pg/mL	0.043	0.022–0.212	0.41	325
Leptin	pg/mL	5.78	3.10–14.3	37.7	7,150

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Precision

Controls were made by spiking calibrator into non-human matrix at three levels within the quantitative range of the assay. Analyte levels were measured by four operators using a minimum of three replicates on 48 runs over 22 days. Results are shown below (Table 6). While a typical specification for precision is a concentration %CV of less than 20% for controls on both intra- and inter-day runs, for this panel, the data shows most assays are below 10%.

Average intra-run %CV is the average %CV of the control replicates within an individual run across 48 runs (three kit lots).

Inter-run %CV is the variability of controls across 12 runs within a single kit lot (across three kit lots).

Inter-lot %CV is the variability of controls across 3 kit lots (total of 48 runs).

Table 6. Inter-run and Intra-run %CVs for the Metabolic Panel 1 Kit

Analyte	Control	Assay Units	Average Conc.	Average Intra-run %CVs	Inter-run %CV	Inter-lot %CV
Insulin	Control 1	µIU/mL	12.5	2.8%	2.5%	6.2%
	Control 2	µIU/mL	1.72	3.0%	2.7%	8.5%
	Control 3	µIU/mL	0.283	6.1%	4.9%	12.4%
C-Peptide	Control 1	pg/mL	5,470	3.1%	2.9%	6.5%
	Control 2	pg/mL	598	2.1%	1.9%	6.8%
	Control 3	pg/mL	78.6	3.9%	3.6%	12.9%
GLP-1 (active)	Control 1	pM	23.1	4.0%	3.8%	6.3%
	Control 2	pM	2.74	4.5%	4.2%	9.2%
	Control 3	pM	0.323	5.8%	5.2%	12.7%
GIP (active)	Control 1	pg/mL	234	3.8%	4.0%	6.9%
	Control 2	pg/mL	21.9	4.0%	3.8%	10.5%
	Control 3	pg/mL	9.93	4.3%	4.7%	13.3%
Glucagon	Control 1	pM	30.7	3.5%	3.1%	5.8%
	Control 2	pM	3.88	3.5%	2.8%	6.7%
	Control 3	pM	1.07	5.6%	4.4%	11.2%
PP	Control 1	pg/mL	163	2.8%	2.9%	6.2%
	Control 2	pg/mL	22.2	2.4%	2.3%	8.4%
	Control 3	pg/mL	1.35	6.2%	5.4%	15.2%
Leptin	Control 1	pg/mL	3,410	3.0%	2.7%	7.0%
	Control 2	pg/mL	466	3.4%	3.1%	13.8%
	Control 3	pg/mL	107	6.7%	6.3%	19.9%

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Parallelism (Human)

To assess linearity, normal serum and P800 EDTA plasma from a commercial source containing only endogenous analyte were diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (Table 7). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \: recovery = \frac{measured \: concentration}{expected \: concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 7. Analyte percent recovery at various dilutions in human serum and P800 EDTA plasma samples

Sample Type	Fold Dilution	Insulin		C-Peptide		GLP-1 (active)		GIP (active)	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (n = 12)	4	107	100–114	114	85–174	131	124–137	149	127–182
	8	105	96–113	118	98–179	<LLOQ	NA	215	172–257
	16	107	98–117	119	96–181	<LLOQ	NA	<LLOQ	NA
P800 EDTA Plasma (n = 17)	4	112	103–140	108	97–119	107	98–123	110	93–122
	8	116	103–170	112	95–129	100	90–114	113	85–151
	16	119	104–184	119	99–138	100	89–116	103	78–122

<LLOQ = below the lower limit of quantification
NA = not applicable

Table 7 (continued). Analyte percent recovery at various dilutions in human serum and P800 EDTA plasma samples

Sample Type	Fold Dilution	Glucagon		PP		Leptin	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (n = 12)	4	109	97–124	103	90–119	119	82–189
	8	106	94–118	100	86–121	139	59–231
	16	112	93–128	100	84–118	184	59–337
P800 EDTA Plasma (n = 17)	4	111	99–117	99	92–112	127	95–170
	8	113	100–126	98	89–119	161	105–297
	16	115	104–127	99	82–128	177	97–421

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Dilution Linearity (Human)

To assess linearity, normal human serum and P800 EDTA plasma from a commercial source, as well as cell culture supernatants were spiked with calibrators and diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (Table 8). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \ recovery = \frac{measured \ concentration}{expected \ concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 8. Analyte percent recovery at various dilutions in human serum, P800 EDTA plasma, and cell culture supernatant samples

Sample Type	Fold Dilution	Insulin		C-Peptide		GLP-1 (active)		GIP (active)	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (n = 12)	4	107	100–115	109	97–126	120	109–131	119	106–141
	8	105	100–112	109	91–128	115	103–127	132	118–165
	16	106	99–115	113	92–140	109	98–120	139	120–184
P800 EDTA Plasma (n = 12)	4	108	104–114	112	102–123	101	95–111	106	92–121
	8	108	100–124	113	89–130	91	84–99	106	85–148
	16	108	98–126	119	104–140	85	80–94	105	80–147
RPMI (media only)	4	97	NA	108	NA	90	NA	94	NA
	8	93	NA	107	NA	81	NA	101	NA
	16	93	NA	104	NA	82	NA	100	NA
RPMI (10% FBS, 1% Pen-Strep)	4	98	NA	111	NA	90	NA	104	NA
	8	86	NA	110	NA	80	NA	119	NA
	16	93	NA	106	NA	82	NA	124	NA
DMEM (media only)	4	99	NA	109	NA	86	NA	120	NA
	8	95	NA	106	NA	81	NA	148	NA
	16	93	NA	105	NA	75	NA	146	NA
DMEM (10% FBS, 1% Pen-Strep)	4	102	NA	114	NA	91	NA	131	NA
	8	96	NA	116	NA	87	NA	184	NA
	16	95	NA	113	NA	82	NA	193	NA
Krebs Ringer	4	97	NA	105	NA	86	NA	83	NA
	8	96	NA	107	NA	77	NA	82	NA
	16	94	NA	105	NA	77	NA	74	NA

NA = not applicable

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Table 8 (continued). Analyte percent recovery at various dilutions in human serum, P800 EDTA plasma, and cell culture supernatant samples

Sample Type	Fold Dilution	Glucagon		PP		Leptin	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (n = 12)	4	104	95–121	99	85–110	114	90–129
	8	105	95–118	96	83–104	128	74–167
	16	107	91–124	94	80–105	142	68–246
P800 EDTA Plasma (n = 12)	4	111	102–120	101	97–111	128	100–174
	8	109	91–122	99	89–116	156	97–249
	16	114	95–128	101	91–123	166	89–276
RPMI (media only)	4	94	NA	90	NA	85	NA
	8	88	NA	87	NA	83	NA
	16	84	NA	85	NA	83	NA
RPMI (10% FBS, 1% Pen-Strep)	4	92	NA	96	NA	94	NA
	8	87	NA	95	NA	98	NA
	16	81	NA	89	NA	99	NA
DMEM (media only)	4	92	NA	94	NA	86	NA
	8	92	NA	90	NA	82	NA
	16	87	NA	87	NA	79	NA
DMEM (10% FBS, 1% Pen-Strep)	4	98	NA	100	NA	96	NA
	8	97	NA	100	NA	100	NA
	16	93	NA	93	NA	102	NA
Krebs Ringer	4	91	NA	88	NA	86	NA
	8	80	NA	81	NA	82	NA
	16	76	NA	76	NA	83	NA

NA = not applicable

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Spike Recovery (Human)

Spike recovery measurements were evaluated for different sample types throughout the quantitative range of the assays. Multiple individual human samples (serum and P800 EDTA plasma) were obtained from a commercial source. These samples, along with cell culture supernatants, were spiked with calibrators at three levels (high, mid, and low) then diluted 2-fold (Table 9;Table 10). The average percent recovery for each sample type is reported along with %CV and percent recovery range.

$$\% \, recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 9. Spike and Recovery measurements of human serum and P800 EDTA plasma samples

Analyte	Serum ($n = 12$)			P800 EDTA Plasma ($n = 12$)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
Insulin	101	4.8	96–106	91	7.1	77–98
C-Peptide	86	3.5	82–93	78	12.3	56–97
GLP-1 (active)	65	15.8	44–93	113	12.6	89–134
GIP (active)	40	10.9	28–64	86	18.7	69–108
Glucagon	96	11.5	80–109	93	9.3	77–104
PP	98	15.5	78–125	92	12.1	74–109
Leptin	58	34.4	35–118	65	18.1	45–101

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Table 10. Spike and Recovery measurements of cell culture supernatant samples

Analyte	RPMI (media only)			RPMI (10% FBS, 1% Pen-Strep)			DMEM (media only)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
Insulin	96	4.0	92–99	101	5.7	96–107	103	7.2	95–110
C-Peptide	95	11.3	83–104	94	10.7	83–102	97	12.1	85–109
GLP-1 (active)	106	6.9	98–112	112	10.5	102–125	109	9.4	100–120
GIP (active)	86	5.1	83–91	68	9.0	64–75	58	3.4	56–59
Glucagon	94	1.2	93–95	101	0.7	100–102	102	3.2	99–105
PP	111	4.7	105–115	104	2.8	102–107	116	1.2	115–118
Leptin	98	6.1	92–104	88	9.5	81–98	103	9.1	95–113

Table 10 (continued). Spike and Recovery measurements of cell culture supernatant samples

Analyte	DMEM (10% FBS, 1% Pen-Strep)			Krebs Ringer		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
Insulin	102	6.1	95–107	106	9.1	96–116
C-Peptide	95	8.8	85–101	96	13.2	82–107
GLP-1 (active)	115	9.4	107–127	115	10.6	106–129
GIP (active)	51	3.8	50–53	111	13.6	100–128
Glucagon	103	4.6	98–108	103	11.7	96–117
PP	116	3.2	113–120	118	2.5	116–121
Leptin	75	5.8	73–80	105	9.3	98–116

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Parallelism (NHP)

To assess linearity, normal non-human primate (rhesus macaque and cynomolgus macaque) serum and P800 EDTA plasma from a commercial source containing only endogenous analyte were diluted 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration (Table 11). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \ recovery = \frac{measured \ concentration}{expected \ concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 11. Analyte percent recovery at various dilutions in non-human primate serum and P800 EDTA plasma samples

Species	Sample Type	Fold Dilution	Insulin		C-Peptide		GLP-1 (active)	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Rhesus macaque	Serum (*n* = 6)	8	100	95–104	125	117–147	< LLOQ	NA
		16	100	91–104	152	142–173	< LLOQ	NA
		32	102	96–107	176	157–200	< LLOQ	NA
	P800 EDTA Plasma (*n* = 6)	8	104	100–107	116	105–125	106	106–106
		16	105	101–109	142	133–151	101	101–101
		32	106	101–113	156	136–168	< LLOQ	NA
Cynomolgus macaque	Serum (*n* = 10)	8	99	92–106	115	111–121	< LLOQ	NA
		16	95	82–104	135	128–148	< LLOQ	NA
		32	92	73–109	153	139–162	< LLOQ	NA
	P800 EDTA Plasma (*n* = 6)	8	108	105–110	115	111–119	100	91–104
		16	106	102–110	130	124–137	< LLOQ	NA
		32	104	99–109	141	134–149	< LLOQ	NA

<LLOQ = below the lower limit of quantification
NA = not applicable

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Table 11 (continued). Analyte percent recovery at various dilutions in non-human primate serum and P800 EDTA plasma samples

Species	Sample Type	Fold Dilution	GIP (active)		Glucagon		PP	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Rhesus macaque	Serum (*n* = 6)	8	< LLOQ	NA	109	109–109	105	100–109
		16	< LLOQ	NA	< LLOQ	NA	109	102–113
		32	< LLOQ	NA	< LLOQ	NA	109	81–123
	P800 EDTA Plasma (*n* = 6)	8	122	122–122	103	94–108	106	103–111
		16	148	148–148	109	98–118	116	104–150
		32	< LLOQ	NA	110	99–120	125	101–190
Cynomolgus macaque	Serum (*n* = 10)	8	< LLOQ	NA	107	100–116	102	94–115
		16	< LLOQ	NA	107	96–135	105	95–122
		32	< LLOQ	NA	117	105–140	105	95–127
	P800 EDTA Plasma (*n* = 6)	8	118	111–123	105	101–107	102	97–105
		16	136	117–144	105	99–109	103	101–105
		32	127	127–127	110	105–117	106	103–113

<LLOQ = below the lower limit of quantification
NA = not applicable

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Dilution Linearity (NHP)

To assess linearity, normal non-human primate (rhesus macaque and cynomolgus macaque) serum and P800 EDTA plasma from a commercial source were spiked with calibrators and diluted 4-fold, 8-fold, 16-fold, and 32-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration (Table 12). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \; recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 12. Analyte percent recovery at various dilutions in non-human primate serum and P800 EDTA plasma samples

Species	Sample Type	Fold Dilution	Insulin		C-Peptide		GLP-1 (active)	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Rhesus macaque	Serum (n = 6)	8	100	99–102	109	105–112	115	105–125
		16	95	91–99	113	108–123	114	103–128
		32	89	87–92	114	103–124	115	100–133
	P800 EDTA Plasma (n = 6)	8	99	95–102	107	99–111	94	90–99
		16	94	86–99	114	104–122	88	82–97
		32	87	76–93	113	105–120	84	77–96
Cynomolgus macaque	Serum (n = 6)	8	99	96–107	104	101–109	110	101–122
		16	94	87–107	110	106–113	109	97–124
		32	87	77–105	109	103–118	101	87–122
	P800 EDTA Plasma (n = 6)	8	108	107–109	114	112–116	94	92–96
		16	104	100–109	122	120–126	90	87–92
		32	101	96–106	126	120–129	87	84–91

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Table 12 (continued). *Analyte percent recovery at various dilutions in non-human primate serum and P800 EDTA plasma samples*

Species	Sample Type	Fold Dilution	GIP (active)		Glucagon		PP	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Rhesus macaque	Serum (*n* = 6)	8	129	115–160	114	98–129	102	98–108
		16	160	128–196	115	97–127	102	95–107
		32	169	169–169	110	96–134	95	82–102
	P800 EDTA Plasma (*n* = 6)	8	109	99–118	100	98–105	106	94–127
		16	128	120–139	98	92–104	106	90–147
		32	136	120–149	96	89–102	108	90–167
Cynomolgus macaque	Serum (*n* = 6)	8	117	102–122	102	96–110	107	97–112
		16	139	118–151	101	90–118	104	94–115
		32	<LLOQ	NA	98	84–125	101	88–117
	P800 EDTA Plasma (*n* = 6)	8	108	101–113	102	98–104	101	94–110
		16	121	109–132	103	100–106	100	91–108
		32	122	108–140	103	94–114	96	82–108

<LLOQ = below the lower limit of quantification
NA = not applicable

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Spike Recovery (NHP)

Spike recovery measurements were evaluated for different sample types throughout the quantitative range of the assays. Multiple individual non-human primate (rhesus macaque and cynomolgus macaque) samples (serum and P800 EDTA plasma) were obtained from a commercial source. These samples were spiked with calibrators at three levels (high, mid, and low) then diluted 4-fold (Table 13). The average percent recovery for each sample type is reported along with %CV and percent recovery range.

$$\% \, recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 13. *Spike and Recovery measurements of non-human primate serum, and P800 EDTA plasma samples*

Species	Analyte	Serum (n = 6)			P800 EDTA Plasma (n = 6)		
		Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
Rhesus macaque	Insulin	99	8.2	89–112	N/A	N/A	N/A
	C-Peptide	88	4.4	83–94	91	5.6	87–98
	GLP-1 (active)	77	13.8	60–96	107	7.1	93–118
	GIP (active)	47	13.7	34–55	66	7.1	60–76
	Glucagon	69	27.1	48–105	90	NA	90–90
	PP	89	11.7	76–104	77	17.4	52–97
Cynomolgus macaque	Insulin	97	3.8	93–101	N/A	N/A	N/A
	C-Peptide	86	3.8	81–91	N/A	N/A	N/A
	GLP-1 (active)	96	6.8	89–112	104	4.6	98–115
	GIP (active)	59	14.2	47–76	75	9.3	62–84
	Glucagon	89	9	74–101	152	48.1	91–244
	PP	85	13.1	64–98	90	20.4	74–136

\>LOQ = above the limit of quantification
NA = not applicable
N/A = not available

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Parallelism (Rodent)

To assess linearity, normal rat P800 EDTA plasma and mouse EDTA plasma (with Inhibitors) from a commercial source containing only endogenous analyte were diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (Table 14; Table 15). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \; recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Mouse samples were tested using EDTA plasma spiked with aprotinin & the DPP-IV inhibitor (P800 tubes were not used for mouse samples).

Table 14. *Analyte percent recovery at various dilutions in P800 EDTA Plasma for rat sample*

Species	Sample Type	Fold Dilution	GLP-1 (active)		Glucagon		GIP (active)	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Rat	P800 EDTA Plasma (n = 6)	4	117	108–129	<LLOQ	NA	104	98–116
		8	<LLOQ	NA	<LLOQ	NA	109	99–125
		16	<LLOQ	NA	<LLOQ	NA	122	111–141

<LLOQ = below the lower limit of quantification
NA = not applicable

Table 15. *Analyte percent recovery at various dilutions in EDTA plasma with Inhibitors for mouse sample*

Species	Sample Type	Fold Dilution	GLP-1 (active)		Glucagon	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Mouse	EDTA Plasma with Inhibitors (n = 6)	4	105	95–114	110	101–117
		8	<LLOQ	NA	113	107–119
		16	<LLOQ	NA	110	92–118

<LLOQ = below the lower limit of quantification
NA = not applicable

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Dilution Linearity (Rodent)

To assess linearity, normal rat P800 EDTA plasma and mouse EDTA plasma (with Inhibitors) from a commercial source were spiked with calibrators and diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 4-fold concentration (Table 16; Table 17). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \ recovery = \frac{measured \ concentration}{expected \ concentration} \times 100$$

Mouse samples were tested using EDTA plasma spiked with aprotinin & the DPP-IV inhibitor (P800 tubes were not used for mouse samples).

Table 16. Analyte percent recovery at various dilutions in P800 EDTA Plasma for rat samples

Species	Sample Type	Fold Dilution	GLP-1 (active)		GIP (active)		Glucagon	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Rat	P800 EDTA Plasma (n = 5)	4	99	95–104	118	114–125	104	96–118
		8	90	82–93	149	145–158	108	101–119
		16	88	86–94	162	157–175	117	106–131

Table 17. Analyte percent recovery at various dilutions in EDTA plasma with Inhibitors for mouse samples

Species	Sample Type	Fold Dilution	GLP-1 (active)		Glucagon	
			Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Mouse	EDTA Plasma with Inhibitors (n = 6)	4	109	104–112	110	105–113
		8	107	97–115	110	108–115
		16	110	101–117	114	110–117

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Spike Recovery (Rodent)

Spike recovery measurements were evaluated for different sample types throughout the quantitative range of the assays. Multiple individual rat samples (P800 EDTA plasma) and mouse sample (EDTA plasma with Inhibitor) were obtained from a commercial source. These samples were spiked with calibrators at three levels (high, mid, and low) then diluted 2-fold (Table 18). The average percent recovery for each sample type is reported along with %CV and percent recovery range.

$$\% \, recovery = \frac{measured \ concentration}{expected \ concentration} \times 100$$

Mouse samples were tested using EDTA plasma spiked with aprotinin & the DPP-IV inhibitor (P800 tubes were not used for mouse samples).

Table 18. Spike and Recovery measurements of rat P800 EDTA plasma and mouse EDTA plasma with Inhibitors samples

Analyte	Rat			Mouse		
	P800 EDTA Plasma ($n = 6$)			EDTA Plasma with Inhibitors ($n = 6$)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
GLP-1 (active)	120	4.8	110–131	125	5.7	110–137
GIP (active)	47	9.7	40–55	NC	NC	NC
Glucagon	85	8.4	72–94	79	11.5	58–92

NC = not cross-reactive

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Parallelism (Canine)

To assess linearity, normal canine serum, and P800 EDTA plasma from a commercial source containing only endogenous analyte were diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (Table 19). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \; recovery = \frac{measured \; concentration}{expected \; concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 19. Analyte percent recovery at various dilutions in canine serum and P800 EDTA plasma samples

Sample Type	Fold Dilution	Insulin		GLP-1 (active)		GIP (active)		Glucagon	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (n = 6)	4	89	84–100	128	128–128	<LLOQ	NA	104	98–108
	8	69	62–83	<LLOQ	NA	<LLOQ	NA	103	93–112
	16	55	49–65	<LLOQ	NA	<LLOQ	NA	105	96–120
P800 EDTA Plasma (n = 6)	4	88	79–104	111	108–113	120	112–126	113	106–119
	8	74	59–88	111	106–119	<LLOQ	NA	117	104–130
	16	66	60–73	109	108–109	<LLOQ	NA	129	118–150

<LLOQ = below the lower limit of quantification
NA = not applicable

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Dilution Linearity (Canine)

To assess linearity, normal canine serum, and P800 EDTA plasma from a commercial source were spiked with calibrators and diluted 2-fold, 4-fold, 8-fold, and 16-fold before testing. Percent recovery at each dilution level was normalized to the dilution-adjusted, 2-fold concentration (Table 20). The average percent recovery is based on samples within the quantitative range of the assay.

$$\% \ recovery = \frac{measured \ concentration}{expected \ concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 20. Analyte percent recovery at various dilutions in canine serum, and P800 EDTA plasma samples

Sample Type	Fold Dilution	Insulin		GLP-1 (active)		GIP (active)		Glucagon	
		Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Serum (n = 6)	4	97	94–101	127	120–141	114	105–124	106	100–124
	8	88	83–95	131	120–151	132	106–182	105	94–127
	16	80	68–89	130	104–163	150	107–301	109	95–156
P800 EDTA Plasma (n = 6)	4	105	103–108	108	104–112	99	94–104	109	103–119
	8	95	85–100	98	95–105	94	90–99	109	98–121
	16	94	83–101	100	95–108	87	79–94	122	110–148

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Spike Recovery (Canine)

Spike recovery measurements were evaluated for different sample types throughout the quantitative range of the assays. Multiple individual canine samples (serum and P800 EDTA plasma) were obtained from a commercial source. These samples, along with cell culture supernatants, were spiked with calibrators at three levels (high, mid, and low) then diluted 2-fold (Table 21). The average percent recovery for each sample type is reported along with %CV and percent recovery range.

$$\% \ recovery = \frac{measured \ concentration}{expected \ concentration} \times 100$$

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 21. Spike and Recovery measurements of canine serum and P800 EDTA plasma samples

	Serum ($n = 6$)			P800 EDTA Plasma ($n = 6$)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
Insulin	98	7.7	87–111	93	6.0	84–102
GLP-1 (active)	77	17.4	51–90	91	14.3	66–105
GIP (active)	65	34.6	16–96	72	14.2	54–91
Glucagon	89	9.3	75–96	86	10.8	68–94

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Specificity

To assess specificity, the assays were tested for nonspecific binding to numerous proteins, including proglucagon metabolites and hormones, human cytokines, and chemokines (Table 22). All analytes were tested at concentrations that approximate those found in circulation. Each assay in the panel was also tested using blended calibrator and single detection antibodies. Nonspecific binding was less than 0.5% for all assays in the kit, except Glucagon assay. Glucagon detect showed 0.8% nonspecific binding (1422 counts) with GLP-1 (active) assay.

$$\% \, Nonspecificity = \frac{nonspecific \, signal}{specific \, signal} \times 100$$

Table 22. Assay % cross-reactivity to selected metabolites and hormones

Analyte	Metabolite/Hormone	Conc. (pg/mL)	Insulin	C-Peptide	GLP-1 (active)	GIP (active)	Glucagon	PP	Leptin
GLP-1 Fragments	GLP-1 (1-36)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	GLP-1 (1-37)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	GLP-1 (7-37)	1,000	< LLOQ	< LLOQ	73.5%	< LLOQ		< LLOQ	< LLOQ
	GLP-1 (9-36)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
GIP Fragments and Mutants	GIP (1-30)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	GIP (3-42)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
GLP-1 Receptor Agonists	Liraglutide*	1,000	< LLOQ	< LLOQ	0.16%	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Exenatide	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Proglucagon Metabolites	Major Proglucagon Fragment (MPGF)	1,000	< LLOQ	< LLOQ	0.43%	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	GRPP	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Glicentin (1-61)‡	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	30%	< LLOQ	< LLOQ
	Glicentin (1-69)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	GLP-2	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Oxyntomodulin	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Miniglucagon	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Proinsulin Fragments and Receptors	Proinsulin**	1,000	0.03%	32.4%	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Soluble Insulin receptor	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	IGF-1	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	IGF-2	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Lispro Insulin	1,000	0.10%	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Aspart Insulin	1,000	0.72%	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Glargine Insulin	1,000	0.76%	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
Non-human Metabolic Analytes	Mouse leptin	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Rat leptin	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Canine leptin	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ

*Liraglutide and GLP-1 have nearly identical sequences and have the same homology at the N-terminus (7-cleavage site), therefore the assay is able to capture both molecules. The detection antibody is not expected to specifically bind to liraglutide, but there may be some weak affinity that leads to elevated apparent levels of active GLP-1 when concentrations of active GLP-1 are low. When levels of liraglutide are much higher than 50 pM, the active GLP-1 concentrations may be suppressed.
**Proinsulin cross-reacts with the Insulin and C-peptide assay (0.03% and 32.4% respectively).
‡Cross-reactivity of Glucagon and circulating glicentin (1-61) is expected due to sequence similarities.
<LLOQ = below the lower limit of quantification

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Analyte	Metabolite/Hormone	Conc. (pg/mL)	Insulin	C-Peptide	GLP-1 (active)	GIP (active)	Glucagon	PP	Leptin
General Metabolic Analytes	PYY (1-36)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	PYY (3-36)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	PYY (3-34)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	NPY	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Resistin	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	IL-6	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Leptin receptor	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	ApoJ	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	A2M	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	G-CSF	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	LIF	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Oncostatin M	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	CNTF	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	IL-11	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	IL-12	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Ghrelin (active)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	Ghrelin (inactive)	1,000	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ

<LLOQ = below the lower limit of quantification

Species Cross-Reactivity

Insulin, C-Peptide, GLP-1 (active), GIP (active), glucagon, and PP assays cross-react with non-human primate serum and plasma samples. Insulin, GLP-1 (active), GIP (active), and glucagon assays cross-react with canine serum and plasma samples. GLP-1 (active), GIP (active), and glucagon assays cross-react with rat plasma samples. GLP-1 (active) and glucagon assays cross-react with mouse plasma samples.

Stability

The reconstituted calibrator, reconstituted controls, and diluents were tested for freeze-thaw stability. Results (not shown) demonstrate that reconstituted calibrator, reconstituted controls, and diluents can undergo three freeze-thaw cycles without significantly affecting the performance of the assays. Once reconstituted, the multi-analyte calibrator and controls are stable for seven days at 2–8 °C. Reconstituted calibrator and controls are also stable when stored frozen at ≤−70 °C through three freeze-thaw cycles. The plates cannot be stored after removing from the pouch; hence, we do not recommend testing partial plates when running this panel.

Calibration

All the assays in the panel are calibrated against a reference calibrator generated at MSD.

MSD reference calibrators for the following analytes were evaluated against the NIBSC/WHO International biological reference preparations (Table 23). Where applicable, the ratios of NIBSC standard units relative to MSD calibrator units are shown in the table below. To convert MSD concentrations to biological activity relative to the WHO International Standard, multiply the MSD concentration by the ratio provided.

Table 23. Ratios of NIBSC international units or mass relative to MSD Calibrator units

Analyte	NIBSC Catalog No.*	NIBSC Units:MSD units	NIBSC units	MSD units
Insulin	83/500	2.38×10^{-8}	mg/mL	µIU/mL
C-Peptide	13/146	0.721×10^{-6}	µg/mL	pg/mL
Glucagon	69/194	2.78	IU/mL	pM
Leptin	97/594	1.19×10^{-6}	µg/mL	pg/mL

*The NIBSC catalog numbers are the same as the WHO International biological reference preparations codes.

There are no GLP-1, GIP, or PP reference standards available from NIBSC or other equivalent bodies. Each of the peptide calibrator concentrations was assigned against a reference calibrator generated at MSD. Amino acid-analysis was used to assign the reference calibrator concentrations.

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Tested Samples

Normal Human Samples

Commercially available normal serum, P800 EDTA plasma, urine, CSF, and saliva samples from human donors were diluted 2-fold and tested. The results for each sample set are displayed below. Concentrations are corrected for sample dilution. Medians and ranges are calculated from samples with concentrations at or above the LLOD. Percent detected is the percentage of samples tested with concentrations at or above the LLOD (Table 24).

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 24. Normal human samples tested in the Metabolic Panel 1 Kit

Sample Type	Statistic	Insulin (µIU/mL)	C-Peptide (pg/mL)	GLP-1 (active) (pM)	GIP (active) (pg/mL)	Glucagon (pM)	PP (pg/mL)	Leptin (pg/mL)
Serum (*n* = 13)	Median	12	1,610	0.17	6.18	13.1	140	5,530
	Range	6.73–76.4	834–4,940	0.072–0.536	2.67–23.4	5.06–62.5	49.4–2520	113–44,100
	% Quantified*	85%	100%	38%	46%	100%	92%	77%
	% Detected**	100%	100%	92%	100%	100%	100%	100%
P800 EDTA Plasma (*n* = 17)	Median	21.3	2,820	1.27	38.6	13.6	135	3,300
	Range	4.63–116	905–10,900	0.217–8.26	4.26–776	4.14–33.1	25.8–2,370	425–55,700
	% Quantified	76%	88%	100%	94%	100%	88%	71%
	% Detected	100%	100%	100%	100%	100%	100%	100%
EDTA Plasma (n = 13)	Median	8.46	992	0.04	1.08	0.85	59.1	1,620
	Range	0.276–56.5	73–2,660	0.024–0.106	0.448–2.44	0.044–22.6	4.95–356	53.8–5,510
	% Quantified	85%	100%	0%	0%	46%	100%	85%
	% Detected	100%	100%	92%	85%	85%	100%	100%
Urine (*n* = 5)	Median	10.7	36,400	0.08	0.54	ND	8.23	24.9
	Range	0.51–28.7	798–352,000	0.023–0.093	0.452–0.753	NA	1.98–10.5	24.9–24.9
	% Quantified	100%	20%	0%	0%	0%	100%	0%
	% Detected	100%	100%	100%	80%	0%	100%	20%
CSF (*n* = 5)	Median	0.25	57.6	0.03	1.77	0.15	1.66	80.2
	Range	0.153–0.784	20.5–124	0.023–0.027	0.68–1.99	0.047–0.652	0.359–4.78	24.7–119
	% Quantified	100%	80%	0%	0%	0%	80%	60%
	% Detected	100%	100%	80%	80%	100%	100%	100%
Saliva (*n* = 5)	Median	5.31	14	0.07	0.93	0.18	0.44	38.2
	Range	0.06–108	7.96–44.7	0.037–0.205	0.541–1.93	0.18–0.18	0.181–4.8	18.9–259
	% Quantified	60%	20%	20%	0%	0%	40%	20%
	% Detected	100%	80%	80%	80%	20%	100%	100%

*% Quantified = percent of samples with concentrations within quantitative range (LLOQ to ULOQ)

**% Detected = percent of samples with concentrations at or above the LLOD
ND = not detected, below assay detection limit
NA = not applicable

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Normal non-Human Samples

Normal serum and EDTA plasma from NHP, rat, mouse, and canine donors were diluted and tested. Mouse EDTA plasma samples were collected in the presence of DPP-IV and protease inhibitors. For all other species, plasma was collected in P800 vacutainer tubes. All samples were diluted 2-fold, except NHP samples, which were diluted 4-fold. The results for each sample set are displayed below. Concentrations are corrected for sample dilution. Medians and ranges are calculated from samples with concentrations at or above the LLOD. Percent detected is the percentage of samples tested with concentrations at or above the LLOD (Table 25).

Standard serum collection tubes do not contain protease inhibitors necessary to stabilize metabolic analytes glucagon and the active forms of GLP-1 and GIP. The measurement of these analytes in serum is not recommended. Data generated during the multiplex testing is shown below.

Table 25. Normal non-human primate samples tested in the Metabolic Panel 1 Kit

Species	Sample Type	Statistic	Insulin (μIU/mL)	C-Peptide (pg/mL)	GLP-1 (active) (pM)	GIP (active) (pg/mL)	Glucagon (pM)	PP (pg/mL)
Rhesus macaque	Serum (*n* = 6)	Median	13.7	1,460	0.09	1.40	1.40	34.4
		Range	3.02–90.4	658–3,110	0.045–0.264	0.99–4.24	0.143–2.66	18.5–62.2
		% Quantified*	83%	100%	0%	0%	17%	100%
		% Detected**	100%	100%	67%	100%	33%	100%
	P800 EDTA Plasma (*n* = 6)	Median	33.4	4,370	0.4	13	58.1	69.5
		Range	16.3–226	2,600–11,400	0.18–1.64	4.85–34.4	35.9–151	35.5–201
		% Quantified	83%	100%	50%	50%	100%	100%
		% Detected	100%	100%	100%	100%	100%	100%
Cynomolgus macaque	Serum (*n* = 10)	Median	28.3	3,860	8.41	40	16.3	75.1
		Range	9.31–96.5	2,440–7,060	0.209–9.57	3.58–54.4	6.14–28.4	37.3–98.7
		% Quantified	90%	100%	70%	60%	100%	100%
		% Detected	100%	100%	100%	100%	100%	100%
	P800 EDTA Plasma (*n* = 7)	Median	73.9	7,940	9.73	79.8	70.3	98.9
		Range	64–131	7,630–9,750	1.1–12.2	48.8–111	44.5–222	77.4–137
		% Quantified	57%	100%	100%	100%	86%	100%
		% Detected	100%	100%	100%	100%	100%	100%

*% Quantified = percent of samples with concentrations within quantitative range (LLOQ to ULOQ);
**% Detected = percent of samples with concentrations at or above the LLOD

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Table 25 (continued). Normal rodent samples tested in the Metabolic Panel 1 Kit

Species	Sample Type	Statistic	GLP-1 (active) (pM)	GIP (active) (pg/mL)	Glucagon (pM)
Rat	P800 EDTA Plasma (*n* = 6)	Median	0.338	5.46	14.1
		Range	0.245–0.484	4.71–7.58	11.5–15.7
		% Quantified	100%	33%	100%
		% Detected	100%	100%	100%
Mouse	EDTA Plasma with Inhibitors (*n* = 6)	Median	0.619	NC	13.1
		Range	0.23–0.672	NC	10.9–15.4
		% Quantified	100%	NC	100%
		% Detected	100%	NC	100%

*% Quantified = percent of samples with concentrations within quantitative range (LLOQ to ULOQ);
**% Detected = percent of samples with concentrations at or above the LLOD
NC = not cross-reactive

Table 25 (continued). Normal canine samples tested in the Metabolic Panel 1Kit

Species	Sample Type	Statistic	Insulin (µIU/mL)	GLP-1 (active) (pM)	GIP (active) (pg/mL)	Glucagon (pM)
Canine	Serum (*n* = 6)	Median	6.80	0.133	4.15	19
		Range	2.47–14.2	0.113–0.426	1.96–8.65	10.6–26.8
		% Quantified	100%	17%	17%	100%
		% Detected	100%	100%	100%	100%
	P800 EDTA Plasma (*n* = 6)	Median	1.11	0.6	14.7	5.93
		Range	0.773–5.07	0.139–2.46	9.19–20	4.96–7.6
		% Quantified	100%	83%	100%	100%
		% Detected	100%	100%	100%	100%

*% Quantified = percent of samples with concentrations within quantitative range (LLOQ to ULOQ);
**% Detected = percent of samples with concentrations at or above the LLOD

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Cell Supernatants and Fasted Plasma Samples

Cell culture supernatants

Supernatants from human Pancreatic Islet cell and several different standard cell lines were tested for the presence of V-PLEX Metabolic Panel 1 analytes. Several analytes were expressed at levels higher than the detection limit of the assay. The dilution-adjusted concentrations for each assay are displayed below (Table 26).

Table 26. Dilution-adjusted concentrations of Metabolic Panel 1 analytes in cell supernatants

Cell Type	Insulin	C-Peptide	GLP-1 (active)	GIP (active)	Glucagon	PP	Leptin
	μIU/mL	pg/mL	pM	pg/mL	pM	pg/mL	pg/mL
MCF7 cell supernatant	82	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
NCI-H716 cell supernatant	< LLOQ	< LLOQ	67.5	< LLOQ	64.6	< LLOQ	< LLOQ
HuTu 80 cell supernatant	< LLOQ	< LLOQ	0.32	< LLOQ	8.64	< LLOQ	< LLOQ
STC-1 cell supernatant	< LLOQ	236	491	21.6	1,126	< LLOQ	225
Pancreatic Islet cell supernatant	51,600	67,800	118	36.6	9,840	9,880	1,290

<LLOQ = below the lower limit of quantification

Fasted Plasma samples

Fasted samples from apparently healthy individuals were tested for the presence of V-PLEX Metabolic Panel 1 analytes. P800 EDTA Plasma samples were collected from different individuals at different fasting time points (2.0 – 14.5 hours). The sample concentration prior to dilution factor adjustment are displayed below (Figure 5).
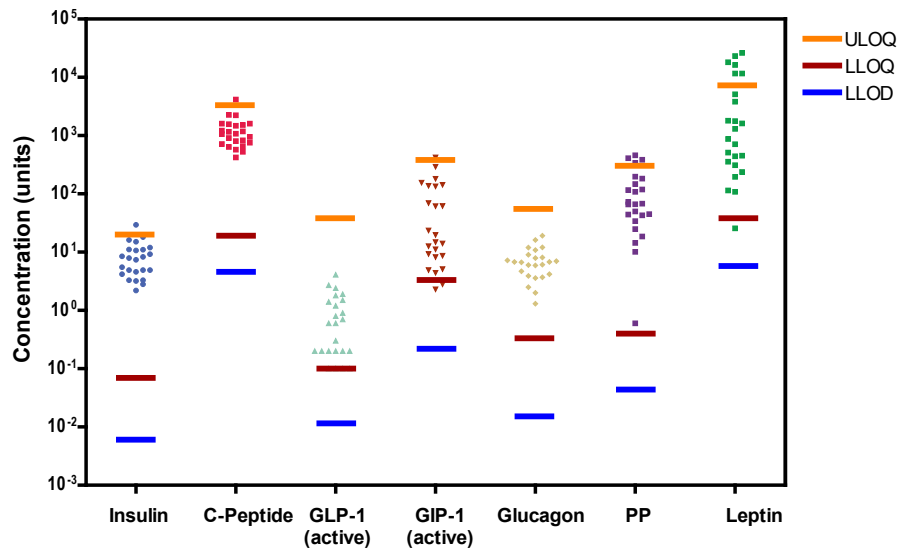
Figure 5. Fasting levels of metabolic analytes in individuals at different time points, measured using the V-PLEX Metabolic Panel 1 Kit.

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Assay Components

Calibrators

The assay calibrator blend uses various recombinant proteins produced in an *E. coli* expression system, as well as peptides (Table 27).

Table 27. *Recombinant proteins (and expression system) and synthetic peptides used as calibrators*

| Calibrator	Expression System
Insulin	*E. coli*
C-Peptide	Synthetic peptide
GLP-1 (7-36)	Synthetic peptide
GIP (1-42)	Synthetic peptide
Glucagon	*E. coli*
PP	Synthetic peptide
Leptin	*E. coli*

Antibodies

The antibody source species are listed in Table 28.

Table 28. *Antibody source species*

Analyte	Source Species MSD Capture Antibody	Source Species MSD Detection Antibody	Assay Generation
Insulin	Mouse monoclonal	Mouse monoclonal	A
C-Peptide	Mouse monoclonal	Mouse monoclonal	A
GLP-1 (active)	Mouse monoclonal	Mouse monoclonal	A
GIP (active)	Mouse monoclonal	Mouse monoclonal	A
Glucagon	Mouse monoclonal	Mouse monoclonal	A
PP	Mouse monoclonal	Mouse monoclonal	A
Leptin	Mouse monoclonal	Mouse monoclonal	A

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Appendix A

Calibration curves below illustrate the relative sensitivity for each assay under **Alternate Protocols**: Reference Protocol (2-hour sample incubation/2 wash steps, blue curve), Alternate Protocol 1 (tissue culture: single wash, red curve), Alternate Protocol 2 (overnight sample incubation, teal curve), and Alternate Protocol 3 (in-well sample dilution, gray curve).
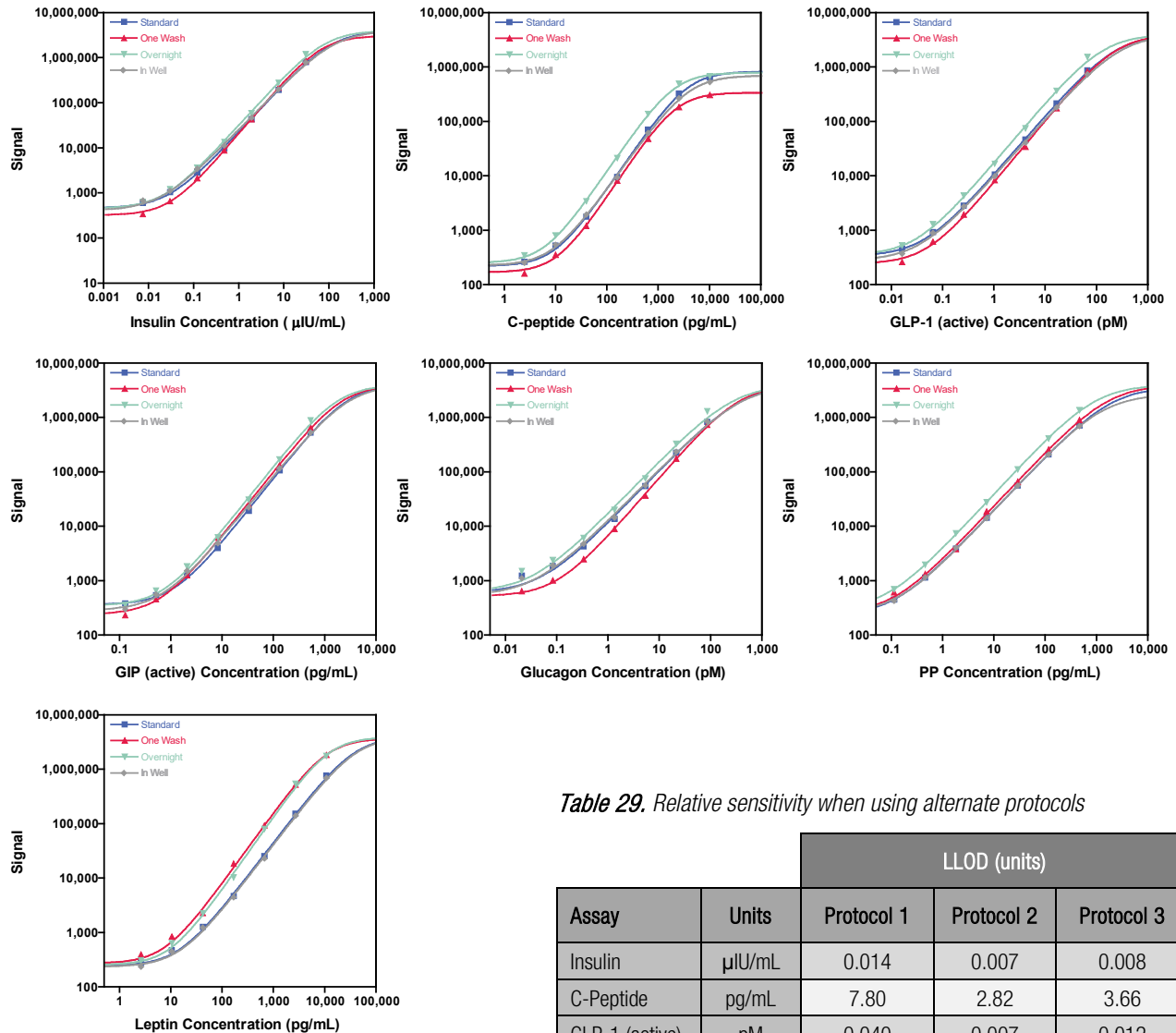

Table 29. Relative sensitivity when using alternate protocols

Assay	Units	LLOD (units)		
		Protocol 1	Protocol 2	Protocol 3
Insulin	µIU/mL	0.014	0.007	0.008
C-Peptide	pg/mL	7.80	2.82	3.66
GLP-1 (active)	pM	0.040	0.007	0.012
GIP (active)	pg/mL	0.683	0.180	0.183
Glucagon	pM	0.039	0.019	0.026
PP	pg/mL	0.516	0.056	0.048
Leptin	pg/mL	19.3	4.31	5.27

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Appendix B

The calibration curves below compare assay performance when the assay is run as an individual assay on a single spot plate (red curve) vs. on the multiplex plate (blue curve).
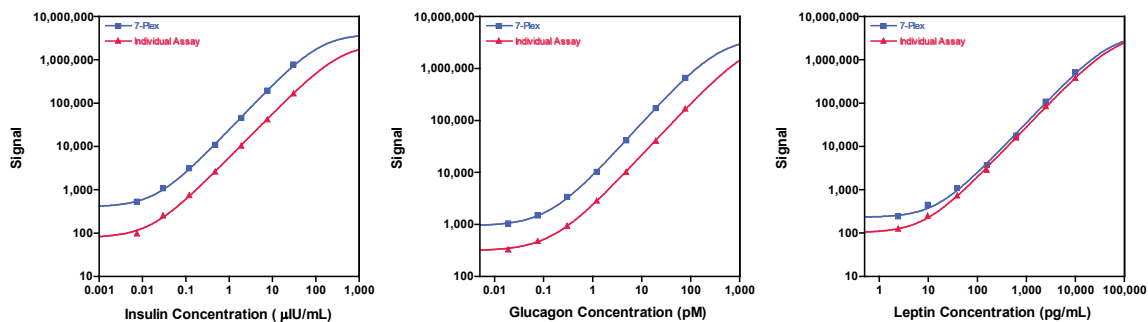
Table 30. *Assay performance for individual and 7-plex assays*

Assay	Units	LLOD (units)	
		Individual	7-plex
Insulin	μIU/mL	0.005	0.004
Glucagon	pM	0.012	0.020
Leptin	pg/mL	2.53	5.32

In general, assays in the single-spot format yielded a lower overall signal compared to the 7-plex format.

Note: Assay performance for C-Peptide, GIP (active), and PP are not included since the individual assays are run on multiplex plates.

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Appendix C

The calibration curves below compare results for each assay in the panel when the assays were run on the 10-spot plate using all detection antibodies as a blend (blue curve) vs. running each assay using a single, assay-specific detection antibody (red curve).
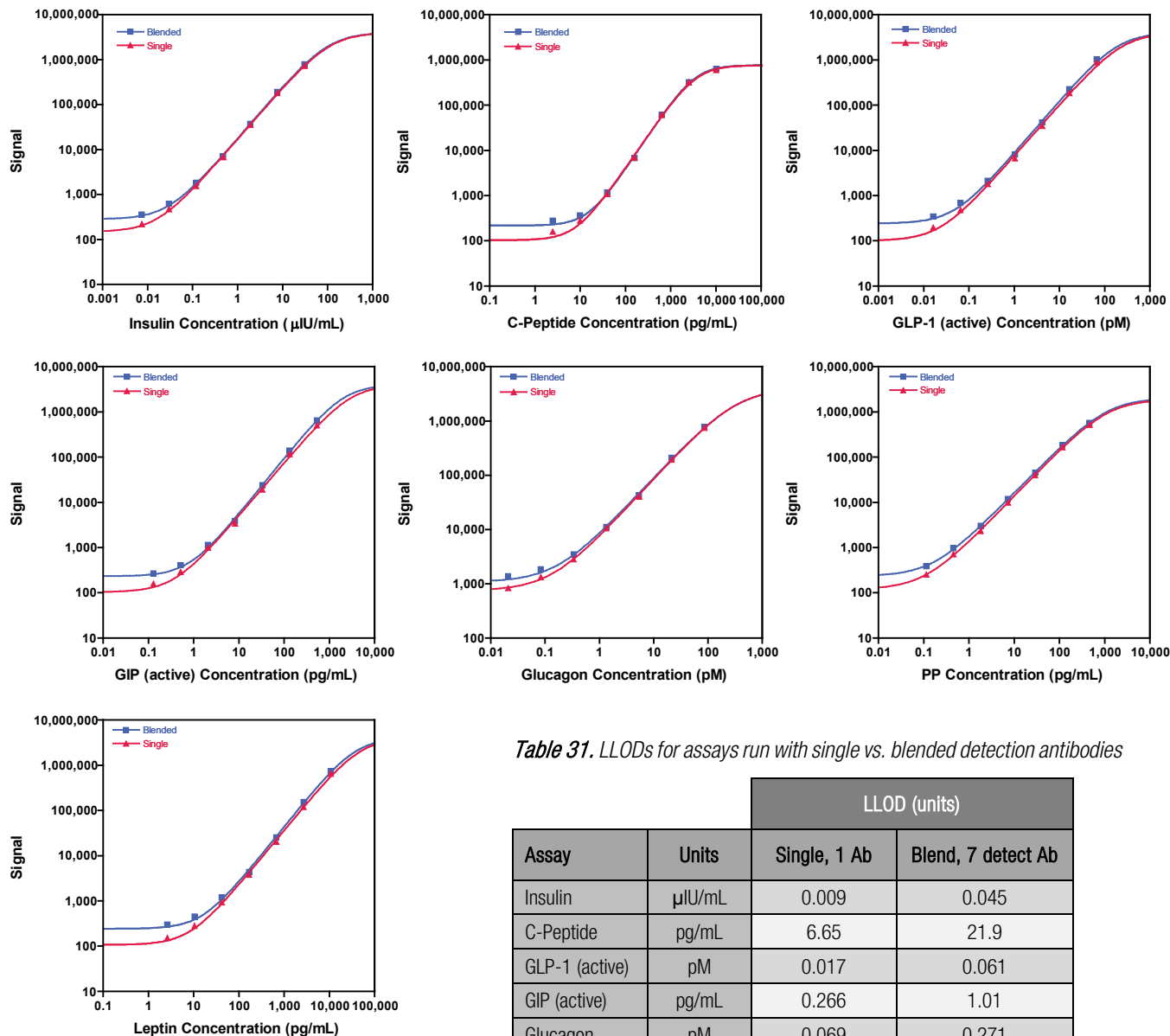
Table 31. LLODs for assays run with single vs. blended detection antibodies

Assay	Units	LLOD (units)	
		Single, 1 Ab	Blend, 7 detect Ab
Insulin	µIU/mL	0.009	0.045
C-Peptide	pg/mL	6.65	21.9
GLP-1 (active)	pM	0.017	0.061
GIP (active)	pg/mL	0.266	1.01
Glucagon	pM	0.069	0.271
PP	pg/mL	0.130	0.216
Leptin	pg/mL	11.4	15.9

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Summary Protocol

Metabolic Panel 1 Kits

MSD provides this summary protocol for your convenience.

Please read the entire detailed protocol prior to performing the Metabolic Panel 1 assays.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature.

- ❑ Prepare calibration solutions in Diluent 56 using the supplied calibrator:

  - o Reconstitute the lyophilized calibrator blend.

  - o Invert 3 times, equilibrate 15–30 minutes at room temperature.

  - o Vortex briefly using short pulses.

  - o Perform a series of 4-fold dilution steps and prepare a zero calibrator.

- ❑ Dilute the samples and controls 2-fold (4-fold for NHP samples) in Diluent 56 before adding to the plate.

- ❑ Prepare combined detection antibody solution by diluting each 50X detection antibody 50-fold in Diluent 11.

STEP 1: Wash and Add Sample*

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.

- ❑ Add 50 µL/well of sample (calibrators, controls, or unknowns).

- ❑ Incubate at room temperature with shaking (~700 rpm) for 2 hours.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.

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

- ❑ Incubate at room temperature with shaking (~700 rpm) for 1 hour.

STEP 3: Wash and Read Plate

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.

- ❑ Add 150 µL/well of MSD GOLD Read Buffer B.

- ❑ Analyze plate on the MSD instrument.

*Note: Washing the plate prior to sample addition is an optional step that may provide greater uniformity of results for certain assays. Analytical parameters, including limits of quantification, recovery of controls, and sample quantification, are not affected by washing the plate prior to sample addition.

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Catalog Numbers

Table 32. *Catalog Numbers for Multiplex and Individual Assay Kits*

Kit Name	V-PLEX			V-PLEX Plus*		
	1-Plate Kit	5-Plate kit	25-Plate Kit	1-Plate Kit	5-Plate Kit	25-Plate Kit
Multiplex Kits						
Metabolic Panel 1 (human)	K15325D-1	K15325D-2	K15325G-4	K15325G-1	K15325G-2	K15325G-4
Metabolic Panel 1 (NHP)	K15332D-1	K15332D-2	K15332G-4	K15332G-1	K15332G-2	K15332G-4
Metabolic Panel 1 (mouse)	K15333D-1	K15333D-2	K15333G-4	K15333G-1	K15333G-2	K15333G-4
Metabolic Panel 1 (rat)	K15334D-1	K15334D-2	K15334G-4	K15334G-1	K15334G-2	K15334G-4
Metabolic Panel 1 (canine)	K15335D-1	K15335D-2	K15335G-4	K15335G-1	K15335G-2	K15335G-4
Individual Assay Kits						
Human C-Peptide	K151X5D-1	K151X5D-2	K151X5G-4	K151X5G-1	K151X5G-2	K151X5G-4
GIP (active)	K150T5D-1	K150T5D-2	K150T5G-4	K150T5G-1	K150T5G-2	K150T5G-4
Glucagon	K150U5D-1	K150U5D-2	K150U5G-4	K150U5G-1	K150U5G-2	K150U5G-4
Human Insulin	K151S5D-1	K151S5D-2	K151S5D-4	K151S5G-1	K151S5G-2	K151S5G-4
Human Leptin	K151V5D-1	K151V5D-2	K151V5G-4	K151V5G-1	K151V5G-2	K151V5G-4
Human PP	K151Y5D-1	K151Y5D-2	K151Y5G-4	K151Y5G-1	K151Y5G-2	K151Y5G-4

*V-PLEX Plus kits include controls, plate seals, and wash buffer. See **Kit Components** (Table 3) for details.

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Plate Diagram