Meso Scale Discovery®

MULTI-SPOT® Assay System

Human Active GLP-1 (7-36)amide, Insulin, Glucagon, Leptin Assay Kit

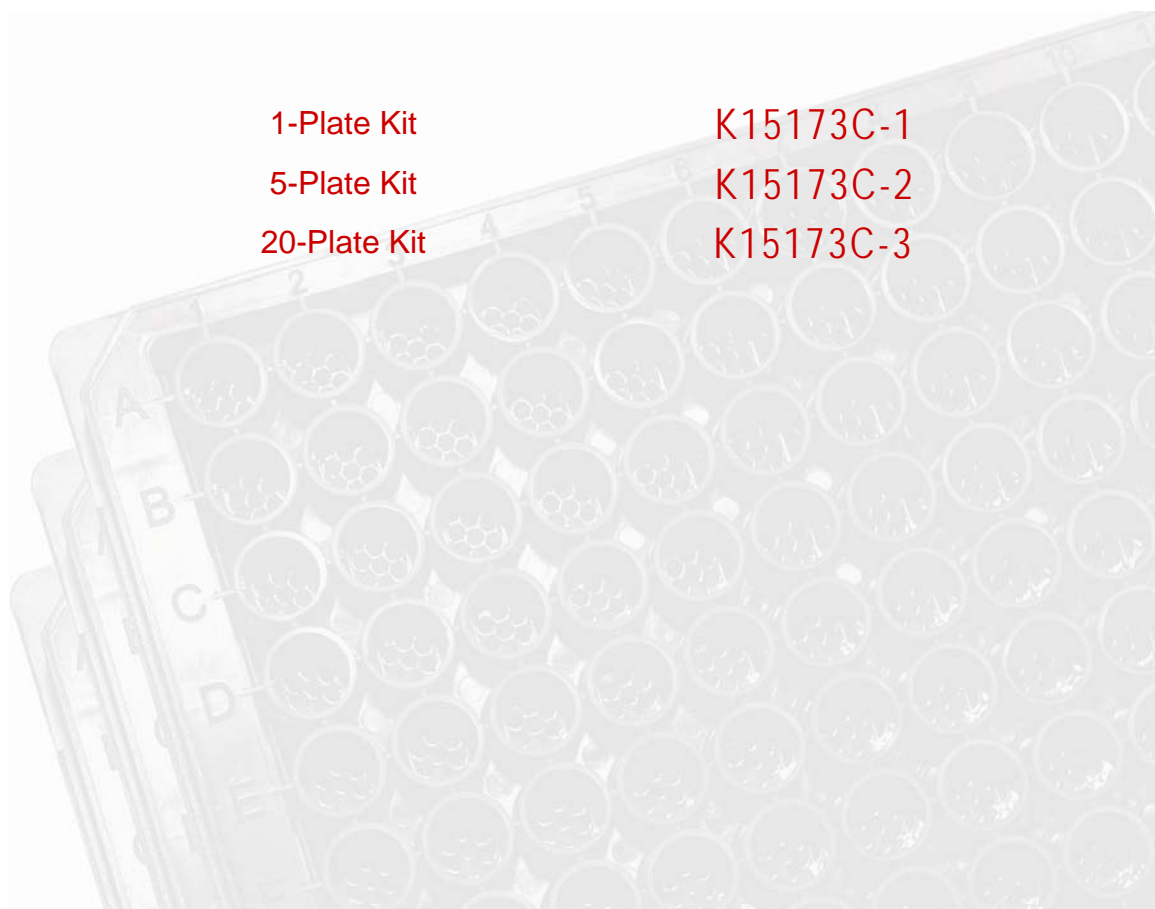|	
1-Plate Kit	K15173C-1
5-Plate Kit	K15173C-2
20-Plate Kit	K15173C-3

MSD Metabolic Assays

Human Active GLP-1 (7-36)amide, Insulin, Glucagon, Leptin Assay Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

Meso Scale Discovery

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Ordering Information

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Introduction

Glucagon-like peptide-1 (GLP-1) is a 3.5 kD protein hormone produced in intestinal L cells and is associated with lowering blood glucose levels. By activation of different physiological systems, it plays roles in gastric emptying upon intake of nutrients, the regulation of short-term feeding behavior, the promotion of glucose-dependent insulin secretion and insulin biosynthesis, and also the inhibition of glucagon secretion. The cleaved peptides, commonly referred to as GLP-1 (7-36)amide and GLP-1 (7-37), are the biologically active forms of GLP-1. *In vivo,* the amidated form is rapidly degraded by dipeptidyl peptidase IV (DPP IV). Since GLP-1, in its bioactive form, plays a crucial role in blood glucose regulation, GLP-1 mimetics and inhibitors of DPP IV are currently being evaluated as potential drug candidates in treatment of diabetes. MSD offers a comprehensive array of GLP-1 assays that measure both the active and total GLP-1 protein using detection antibodies specific for the C-terminal, 36th and/or 37th amino acids.

Insulin is a 51-residue peptide hormone that is produced in the pancreas by β-cells of the islets of Langerhans. Insulin is involved in the regulation of carbohydrate, fat and protein metabolism. Lowered levels of insulin cause liver cells to convert glycogen back to glucose and secrete it into the blood. Insulin also has an effect on small vessel muscle tone, storage and release of (fat) triglycerides and cellular uptake of amino acids and electrolytes. Type 1 diabetes results when the β-cells are destroyed and no longer producing insulin resulting in high glucose levels in the blood. Patients with type 1 diabetes depend on exogenous insulin for their survival because of an absolute deficiency of the hormone; patients with type 2 diabetes have either relatively low insulin production or insulin resistance or both.

Glucagon is a 29-residue polypeptide hormone that is produced in the pancreas by the α-cells of the islets of Langerhans. Glucagon is involved in maintaining normal levels of glucose in the blood by acting on liver glycogen, converting it to glucose. Glucagon is a stimulator of hepatic glycogenolysis, gluconeogenesis, and ketogenesis which are antagonistic effects to those of insulin action, resulting in increased blood glucose levels. Glucagon receptors have been found in liver, kidney, intestinal smooth muscle, brain and adipose tissue.

Leptin is a 16 kD product of the ob gene that is produced and released by adipocytes. Acting via cytokine-like receptors in the CNS, leptin plays a key role in metabolism and regulation of adipose tissue. Leptin is released in amounts mirroring overall body fat stores and acts on neurons and hypothalamic receptors thereby influencing the brain's perception of nutritional energy status and appetite. The absence of functional leptin (or its receptor) leads to uncontrolled food intake and resulting obesity. Fasting reduces circulating insulin and leptin levels in plasma. Leptin may therefore be a critical regulator of obesity often accompanied by insulin resistance and hyperinsulinemia.

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. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Human Active GLP-1 (7-36)amide, Insulin, Glucagon, Leptin Assay detects the GLP-1 (7-36)amide isoform, insulin, glucagon and leptin in a multiplexed sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with Active GLP-1, insulin, glucagon and leptin capture antibodies. The user adds the sample and a solution containing the labeled detection antibodies—anti-GLP-1 (7-36)amide, anti-insulin, anti-glucagon and anti-leptin labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. GLP-1 (7-36)amide, insulin, glucagon and leptin in the sample bind to their specific capture antibody immobilized on the working electrode surface; recruitment of each labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR® instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of GLP-1 (7-36)amide (Figure 2), insulin, glucagon and leptin present in the sample.
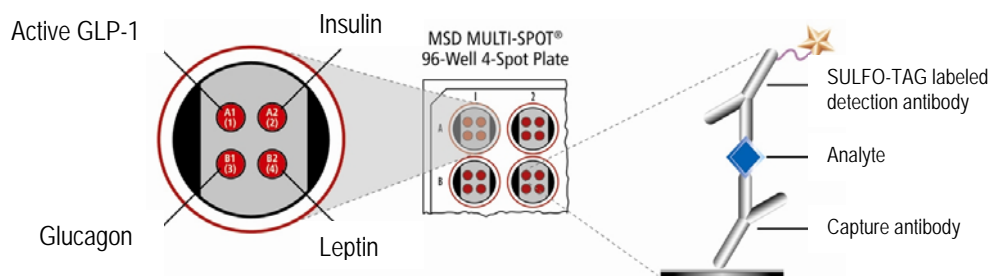

Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding to that spot. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

Figure 2. *Schematic of the antibodies recognition sites for the Active GLP-1 (7-36)amide Assay on GLP-1 protein amino acids 1-37.*

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K15173C-1	K15173C-2	K15173C-3
MULTI-SPOT 96-well Human Active GLP-1, Insulin, Glucagon, Leptin Plate(s) N45173A-1 (K15173C)	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-GLP-1 (7-36)amide Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
SULFO-TAG Anti-hInsulin Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
SULFO-TAG Anti-Glucagon Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
SULFO-TAG Anti-hLeptin Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
GLP-1 (7-36)amide Calibrator 1 µg/mL	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	20 vials (15 µL ea)
Insulin Calibrator 5 µg/mL	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	20 vials (15 µL ea)
Glucagon Calibrator 1 µg/mL	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	20 vials (15 µL ea)
Human Leptin Calibrator 10 µg/mL	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	20 vials (20 µL ea)
Blocker A kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 mL ea)
Aprotinin (200,000 KIU/mL)	2-8°C	1 vial (50 µL)	1 vial (250 µL)	4 vials (250 µL ea)
Blocker E	≤-10°C	1 vial (0.09 mL)	1 vial (0.45 mL)	4 vials (0.45 mL ea)
Blocker D-B (10%)	≤-10°C	1 vial (0.25 mL)	1 vial (1.2 mL)	4 vials (1.2 mL ea)
Blocker D-R (10%)	≤-10°C	1 vial (0.2 mL)	1 vial (1.0 mL)	4 vials (1.0 mL ea)
Diluent 6 R53BB-4 (8 mL) R53BB-3 (40 mL) R53BB-2 (200 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	1 bottle (200 mL)
Diluent 12	≤-10°C	1 bottle (10 mL)	2 bottles (10 mL ea)	2 bottles (50 mL ea)
Read Buffer T (with surfactant), 4X R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)

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[1] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

IV Required Materials and Equipment - not supplied

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

V Safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

VI Reagent Preparation

Bring all plates and diluents to room temperature.
Blockers D-B, D-R and E can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of each blocker can be stored at 2-8°C for up to 1 month.

> **Important:** Upon first thaw, separate Diluent 6 and Diluent 12 into aliquots appropriate to the size of your assay needs. These diluents can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution
Follow instructions included with the Blocker A Kit.

Prepare Metabolic Assay Working Solution
In a 15 mL tube combine (per plate):

- ❑ 35 µL of Aprotinin
- ❑ 70 µL of Blocker E
- ❑ 6895 µL of Diluent 6

> **Important:** Aprotinin 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.

Prepare Calibrator and Control Solutions

The stock Calibrator vials are supplied at 1 µg/mL for GLP-1 (7-36)amide and Glucagon, at 5 µg/mL for Insulin and at 10 µg/mL for Leptin. For the assay, an 8-point standard curve is recommended with 4-fold serial dilution steps and a zero Calibrator.

The table below shows the concentrations of the 8-point standard curve:

Standard	GLP-1 (7-36)amide conc. (pg/mL)	Insulin conc. (pg/mL)	Glucagon conc. (pg/mL)	Leptin conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	1000000	5000000	1000000	10000000	
STD-01	10000	50000	10000	100000	100
STD-02	2500	12500	2500	25000	4
STD-03	625	3125	625	6250	4
STD-04	156	781	156	1563	4
STD-05	39	195	39	391	4
STD-06	9.8	49	9.8	98	4
STD-07	2.4	12.2	2.4	24	4
STD-08	0	0	0	0	n/a

To prepare this 8-point standard curve:

1) Prepare the highest Calibrator by adding 10 µL of 1 µg/mL GLP-1 (7-36)amide, 10 µL of 5 µg/mL Insulin, 10 µL of 1 µg/mL Glucagon and 10 µL of 10 µg/mL Leptin to 960 µL of Metabolic Assay Working Solution.
2) Prepare the next Calibrator by transferring 50 µL of the diluted Calibrator to 150 µL of Metabolic Assay Working Solution. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 150 µL of Metabolic Assay Working Solution to be used as zero calibrator.
4) Diluted Calibrators should be kept on ice prior to addition to the plate.

Preparation of Serum and Plasma Samples

1) The assay format requires 40 µL of sample per well. An adequate volume of each sample should be prepared depending upon desired number of replicates.
2) There are numerous proteases in serum and plasma that may cause degradation of GLP-1 and Glucagon. Blood samples should be drawn into tubes containing 500 KIU Aprotinin per mL of whole blood. Alternately, Aprotinin should be added immediately following blood draw. Invert the blood tube several times to mix the sample.
   a. To obtain serum, tubes containing Aprotinin should be allowed to clot for 30' on a rocker. Spin the tubes for 10 minutes at 1000 x g (4°C) and aliquot serum into separate tubes and store at -80°C until use. Avoid repeated freeze-thaw (> 2) of these aliquots.
   b. Plasma samples should be obtained in vacutainer or syringe containing Na$_2$EDTA (1.25 mg/mL) and 500 KIU Aprotinin per mL of whole blood. Tubes should be spun for 10 minutes at 1000 x g (4°C) and then plasma immediately aliquotted into separate tubes and stored at -80°C until use. Avoid repeated freeze-thaw (> 2) of these aliquots.
3) To preserve the integrity of Active GLP-1, a DPP IV inhibitor should be added to the sample tube prior to collection.
4) Keep isolated or thawed serum/plasma samples on ice or at 4°C prior to subsequent processing or until use in the assay.
5) Samples with hemolysis or significant lipemia may hinder accurate assay measurements.

Prepare Detection Antibody Solution

The Detection Antibodies are provided as a 100X stock solution. The working Detection Antibody Solution should contain 1X as the final concentration of each antibody.
In a 15 mL tube combine (per plate):

- ❑ 90 µL of 10% Blocker D-B
- ❑ 90 µL of 10% Blocker D-R
- ❑ 30 µL of 100X SULFO-TAG Anti-GLP-1 (7-36)amide Antibody
- ❑ 30 µL of 100X SULFO-TAG Anti-hInsulin Antibody
- ❑ 30 µL of 100X SULFO-TAG Anti-Glucagon Antibody
- ❑ 30 µL of 100X SULFO-TAG Anti-hLeptin Antibody
- ❑ 2700 µL of Diluent 12

Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of stock Read Buffer T (4X) to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibodies for the analytes shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

Notes

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

2. **Wash and Addition of Sample or Calibrator:** Wash the plate 3X with PBS-T. First, dispense 20 µL of Metabolic Assay Working Solution into each well of the MSD plate. Then, immediately add 40 µL of sample or Calibrator into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking a 96-well MSD MULTI-SPOT plate typically accelerates capture at the working electrode.

3. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3X with PBS-T. Dispense 25 µL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

4. **Wash and Read:** Wash the plate 3X with PBS-T. Add 150 µL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

   Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII   Analysis of Results

*a n a l y s i s   o f   r e s u l t s*

The calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantitation in many samples without the need for dilution. The MSD Discovery Workbench® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX   Typical Standard Curve

t y p i c a l   s t a n d a r d   c u r v e

The MSD Human Active GLP-1 (7-36)amide, Insulin, Glucagon, Leptin Assay is designed for use with human serum and plasma samples.

The following standard curves are examples of the dynamic ranges of the assay. The actual signals may vary. A standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.
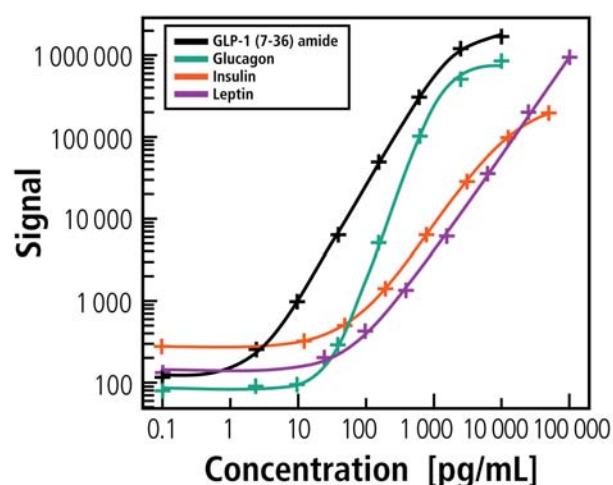
GLP-1 (7-36)amide		
Conc. (pg/mL)	Average Counts	%CV
0	116	10
2.4	256	11
9.8	990	13
39	6344	18
156	49367	20
625	307438	17
2500	1188617	14
10000	1688513	0

Insulin		
Conc. (pg/mL)	Average Counts	%CV
0	276	3
12.2	324	7
49	495	0
195	1424	3
781	6340	4
3125	28848	4
12500	96902	2
50000	193611	2

Glucagon		
Conc. (pg/mL)	Average Counts	%CV
0	79	6
2.4	90	4
9.8	95	12
39	284	1
156	5012	5
625	103421	1
2500	504791	6
10000	859757	1

Leptin		
Conc. (pg/mL)	Average Counts	%CV
0	130	4
24	202	7
98	424	5
391	1349	4
1563	6133	11
6250	35437	9
25000	202941	8
100000	926888	1

X   Sensitivity

s e n s i t i v i t y

The lower limit of detection (LLOD) is the calculated concentration of the signal that is
2.5 standard deviations over the zero calibrator. Values below represent the average LLODs over
multiple kit lots.

	GLP-1 (7-36)amide	Insulin	Glucagon	Leptin
LLOD (pg/mL)	1.0	9.0	20	22

XI   Spike Recovery

s p i k e r e c o v e r y

Serum, heparin plasma, and EDTA plasma were spiked with the calibrators at multiple values
throughout the range of the assay. Measured analyte represents average spike recovery in 4-6 pooled
human serum or plasma. MSD recommends using plasma samples for optimal assay performance.
% Recovery = measured / expected x 100

Sample	Spike level	% Recovery of Spiked Calibrator			
		GLP-1 (7-36)amide	Insulin	Glucagon	Leptin
Serum	**Low**	85	103	67	83
	Medium	88	114	88	89
	High	87	114	99	89
EDTA Plasma	**Low**	99	106	68	96
	Medium	103	127	92	99
	High	103	137	95	98
Heparin Plasma	**Low**	67	92	55	73
	Medium	78	96	84	67
	High	78	100	88	61

XII Linearity

l i n e a r i t y

Measured spiked analyte levels in pooled human serum and plasma followed by subsequent dilution.
% Recovery = (measured x dilution factor) / expected x 100

Sample	Dilution Factor	% Recovery of Dilution Linearity			
		GLP-1 (7-36)amide	Insulin	Glucagon	Leptin
Serum	2	85	103	67	83
	4	88	114	88	89
	8	87	114	99	89
EDTA Plasma	2	99	106	68	96
	4	103	127	92	99
	8	103	137	95	98
Heparin Plasma	2	67	92	55	73
	4	78	96	84	67
	8	78	100	88	61

XIII Cross-Reactivity

c r o s s - r e a c t i v i t y

The cross-reactivity shown below is calculated based on signal generated using different GLP-1 isoforms and other related forms of proteins.

Active GLP-1 (7-36)amide	
Form	Cross-Reactivity
GLP-1 (7-36)amide	100%
GLP-1 (9-36)amide	<0.1%
GLP-1 (1-36)amide	<0.1%
GLP-1 (7-37)	<0.1%
GLP-1 (1-37)	<0.1%

Glucagon	
Analyte	Cross-Reactivity
Glucagon	100%
Insulin	<0.1%
GIP	<0.1%
GLP-1 (7-36)amide	<0.1%
GLP-2	<0.1%
Oxyntomodulin	<0.1%

Insulin	
Analyte	Cross-Reactivity
Insulin	100%
Proinsulin	<0.1%
C-Peptide	<0.1%
GLP-1 (7-36)amide	<0.1%

Kit Components

GLP-1 (7-36)amide

Calibrator source: Synthetic amidated peptide (amino acids 7-36) of human GLP-1

Capture Antibody	
Analyte	Active GLP-1
Source	Mouse monoclonal
Isoforms Recognized	Reacts with GLP-1 (7-36)amide and GLP-1 (7-37), does not react with GLP-1 (9-36) or GLP-1 (9-37)
Species cross-reactivity	Human, mouse, rat (100% conserved in all mammalian species)

Detection Antibody	
Analyte	GLP-1
Source	Mouse monoclonal
Isoforms Recognized	Reacts with the amidated C terminus of GLP-1 (7-36)amide, GLP-1 (9-36)amide and GLP-1 (1-36)amide, does not react with GLP-1 (7-37), GLP-1 (9-37) or GLP-1 (1-37)
Species cross-reactivity	Human, mouse, rat (100% conserved in all mammalian species)

Insulin

Calibrator source: Recombinant human insulin

The Insulin Calibrator has been anchored and referenced to international standards. The table below summarizes the reference information.

Analyte	WHO Standard Reference Number	WHO Standard Units / µg	MSD Calibrator 1µg = WHO Units	WHO Units
Insulin	66/304	0.023	0.023	IU

Capture Antibody	
Analyte	Human insulin
Source	Mouse monoclonal
Isoforms Recognized	Reacts with human insulin, does not react with proinsulin or human C-peptide
Species cross-reactivity	Human, porcine, bovine
Detection Antibody	
Analyte	Human insulin
Source	Mouse monoclonal
Isoforms Recognized	Reacts with human insulin, does not react with proinsulin or human C-peptide
Species cross-reactivity	Human only

Glucagon
Calibrator source: Synthetic human glucagon (amino acids 1-29)

Capture Antibody	
Analyte	Human glucagon
Source	Mouse monoclonal
Isoforms Recognized	n/a
Species cross-reactivity	Human, mouse, rat
Detection Antibody	
Analyte	Human glucagon
Source	Mouse monoclonal, ascites
Isoforms Recognized	Pancreatic glucagon, reacts weakly to gut glucagon
Species cross-reactivity	Human, mouse, rat, sheep, rabbit, pig, canine, pig, guinea pig

Leptin
Calibrator source: Purified, recombinant human leptin expressed in E.coli

Capture Antibody	
Analyte	Human leptin
Source	Mouse monoclonal
Isoforms Recognized	Recognizes recombinant leptin and leptin circulating in human blood
Species cross-reactivity	Human
Detection Antibody	
Analyte	Human leptin
Source	Mouse monoclonal
Isoforms Recognized	n/a
Species cross-reactivity	Human

XV　References

references

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Summary Protocol
MSD 96-well MULTI-ARRAY
Human Active GLP-1 (7-36)amide, Insulin, Glucagon, Leptin Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing
the Human Active GLP-1 (7-36)amide, Insulin, Glucagon, Leptin Assay.

Step 1 : Sample and Reagent Preparation

Bring all diluents and plates to room temperature.

Prepare Blocker A Solution.

Prepare Metabolic Assay Working Solution and keep on ice.

The Calibrator stock solutions should be thawed and kept on ice.

Prepare an 8-point standard curve using supplied calibrator:

- The Calibrator should be diluted in Metabolic Assay Working Solution.
- Dilute the stock Calibrators 1:100 as indicated in Reagent Preparation section, then perform a series of 4-fold dilution steps and a no Calibrator blank.
- Diluted Calibrators should be kept on ice until use.

Prepare serum or plasma samples.

Prepare Detection Antibody Solution by diluting the 100X Detection Antibodies into a 1X final concentration of each antibody. The Detection antibodies should be diluted in 3.0 mL of Diluent 12 containing Blocker D-B and D-R as indicated in Reagent Preparation section.

Prepare 20 mL of 1X Read Buffer T by diluting 4X MSD Read Buffer T (with surfactant) with deionized water.

Step 2 : Add Blocker A Solution

Dispense 150 µL/well Blocker A Solution.

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

Step 3 : Wash and Add Sample or Calibrator

Wash plate 3X with PBS-T.

Dispense 20 µL/well Metabolic Assay Working Solution.

Immediately, dispense 40 µL/well Calibrator or Sample.

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

Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

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

Step 5 : Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 µL/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.