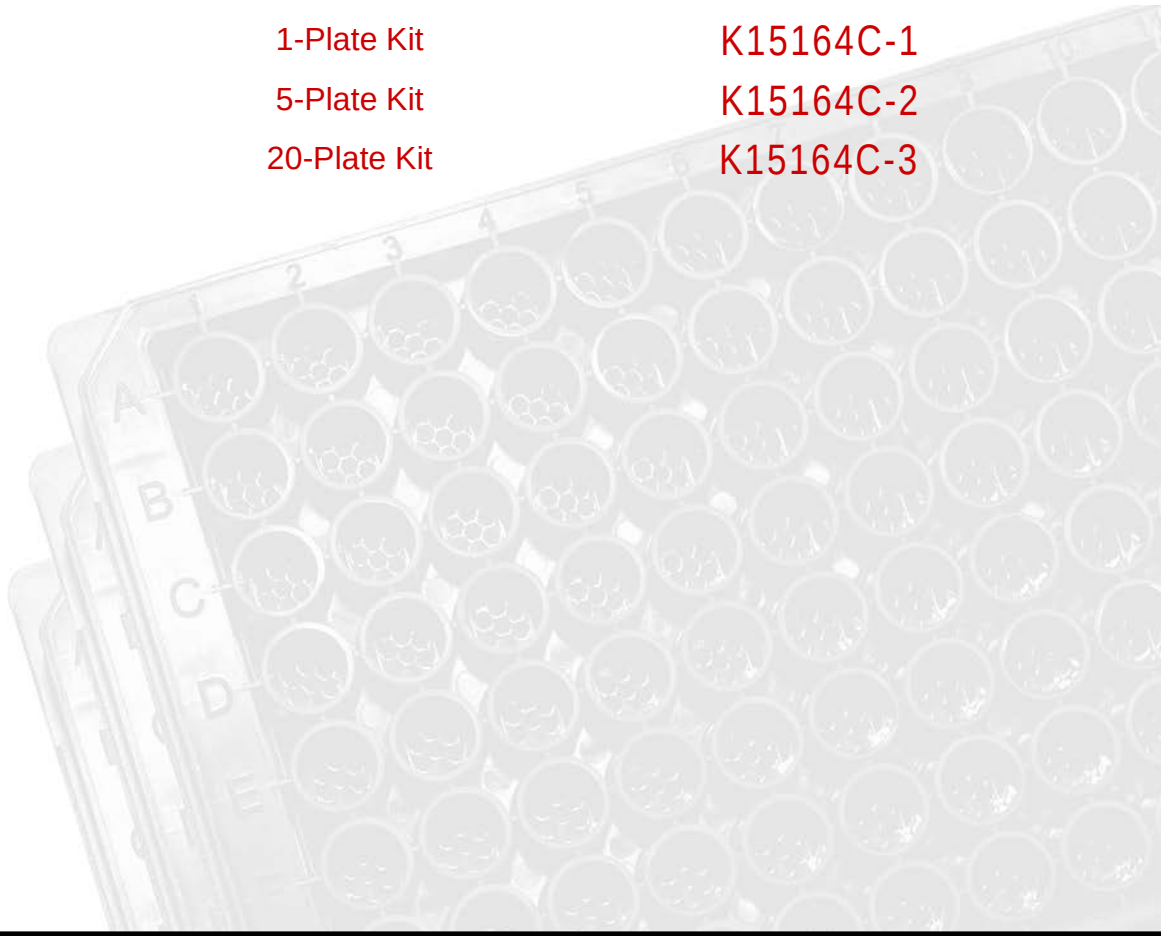
Meso Scale Discovery®

MULTI-SPOT® Assay System

Human Leptin, Insulin Kit

1-Plate Kit	K15164C-1
5-Plate Kit	K15164C-2
20-Plate Kit	K15164C-3



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MSD Metabolic Assays

Human Leptin, Insulin 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.

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Ordering Information

ordering information

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Introduction

introduction

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 obeseity often accompanied by insulin resistance and hyperinsulinemia.

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.

Principle of the Assay

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 Leptin, Insulin Assay detects leptin and insulin in a multiplexed sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with leptin and insulin capture antibodies on spatially distinct spots. The user adds the sample and a solution containing the labeled detection antibodies—anti-leptin and anti-insulin labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Leptin and insulin in the sample binds to capture antibodies immobilized on the working electrode surface; recruitment of the labeled detection antibodies by bound analytes 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 leptin and insulin present in the sample.

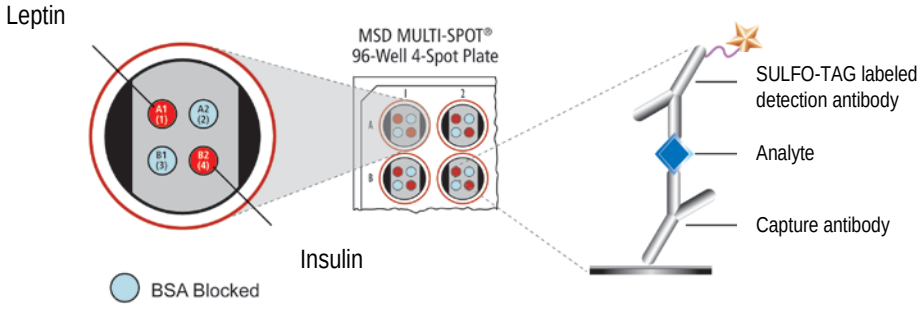


Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding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

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K15164C-1	K15164C-2	K15164C-3
MULTI-SPOT 96-well human Leptin, Insulin Plate(s) N45164A-1	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-hLeptin 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)
Human Leptin Calibrator 10 µg/mL	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	20 vials (20 µL ea)
Insulin Calibrator 5 µg/mL	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	20 vials (15 µL ea)
Blocker A kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 mL ea)
Blocker D-B (10%)	≤-10°C	1 vial (0.25 mL)	1 vial (1.2 mL)	4 vials (1.2 mL ea)
Diluent 13 R56BB-4 (10 mL) R56BB-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	4 bottles (50 mL ea)
Diluent 12	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	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)

IV Required Materials and Equipment - not supplied

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

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

V Safety

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

reagent preparation

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

> **Important:** Upon first thaw, separate Diluent 13 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 Calibrator and Control Solutions

The stock Calibrator vials are supplied at 10 µg/mL for Leptin and at 5 µg/mL for Insulin. For the assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Leptin conc. (pg/mL)	Insulin conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	10000000	5000000	
STD-01	100000	50000	100
STD-02	33333	16667	3
STD-03	11111	5556	3
STD-04	3704	1852	3
STD-05	1235	617	3
STD-06	412	206	3
STD-07	137	69	3
STD-08	0	0	n/a

To prepare this 8-point standard curve:

1) Prepare the highest Calibrator by adding 10 µL of 10 µg/mL Leptin and 10 µL of 5 µg/mL Insulin to 980 µL of Diluent 13.
2) Prepare the next Calibrator by transferring 100 µL of the diluted Calibrator to 200 µL of Diluent 13. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 200 µL of Diluent 13 to be used as zero calibrator.

Preparation of Serum and Plasma Samples

The assay format requires 25 µL of sample per well. An adequate volume of each sample should be prepared depending upon desired number of replicates.

Prepare Detection Antibody Solution

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

- ❑ 90 µL of 10% Blocker D-B
- ❑ 30 µL of 100X SULFO-TAG Anti-hLeptin Antibody
- ❑ 30 µL of 100X SULFO-TAG Anti-hInsulin Antibody
- ❑ 2850 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

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 25 µL of Diluent 13 into each well of the MSD plate. Then, immediately add 25 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.
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.

Notes

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

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

analysis of results

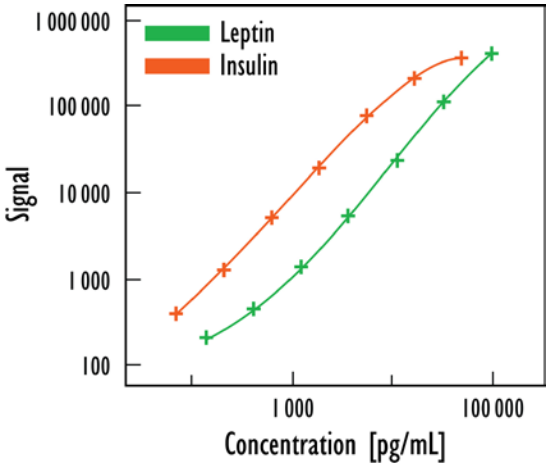
The calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have 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

typical standard curve

The MSD Human Leptin, Insulin Assay is designed for use with human serum and plasma samples.

The following standard curves are examples of the dynamic range 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.



Leptin			Insulin		
Conc. (pg/mL)	**Mean**	**%CV**	**Conc. (pg/mL)**	**Mean**	**%CV**
0	122	23	**0**	113	26
137	213	16	**69**	396	11
412	459	4	**206**	1274	10
1235	1384	4	**617**	5095	10
3704	5250	9	**1852**	18965	4
11111	23801	2	**5556**	76405	7
33333	111784	6	**16667**	208075	6
100000	399540	6	**50000**	360856	2

X Sensitivity

sensitivity

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

	Leptin	Insulin
LLOD (pg/mL)	135	25

XI Endogenous Levels

Endogenous levels

Endogenous levels of human leptin and insulin in five matched individual serum and plasma samples.

	Serum (pg/mL)		EDTA Plasma (pg/mL)		Heparin Plasma (pg/mL)	
Sample ID	**Leptin**	**Insulin**	**Leptin**	**Insulin**	**Leptin**	**Insulin**
1	1468	105	1404	181	1382	153
2	8429	628	9120	785	6526	733
3	783	117	938	228	880	208
4	8846	733	6407	866	7278	561
5	1122	109	1152	191	1154	170

Endogenous levels of human leptin and insulin in four diseased serum samples.

Sample ID	Leptin (pg/mL)	Insulin (pg/mL)	Diabetes	BMI
1	3806	89	Type I	49
2	24368	804	Type II	71
3	18032	7116	Type II	36
4	19980	456	Type II	40

XII Spike Recovery

spike recovery

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 and plasma including high, mid, and low calibrator spikes.
% Recovery = (measured value *100)/expected value

	Average % Recovery	
	Leptin	**Insulin**
Spiked Serum	92	91
Spiked Heparin Plasma	81	76
Spiked EDTA Plasma	102	98

XIII Linearity

linearity

Average % Recovery in pooled normal human EDTA plasma was assayed at several dilution ratios. Recovery is calculated from the measured concentration from the previous dilution.

	Average % Recovery	
	Leptin	Insulin
1/2	118	117
1/4	129	118
1/8	129	111

XIV Kit Components

kit components

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

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

XV References

references

Leptin

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Insulin

4. Bristow AF, Das RE, Bangham DR. World Health Organization International Standards for highly purified human, porcine and bovine insulins. J Biol Stand. 1988 Jul;16(3):165-78
5. Golla R, Seethala R. A sensitive, robust high-throughput electrochemiluminescence assay for rat insulin. J Biomol Screen. 2004 Feb;9(1):62-70
6. Plum L, Belgardt BF, Brüning JC. Central insulin action in energy and glucose homeostasis. J Clin Invest. 2006 Jul;116(7):1761-6
7. Saltiel AR**,** Kahn CR**.** Insulin signalling and the regulation of glucose and lipid metabolism. Nature**.** 2001. Dec 13;414(6865):799-806

Summary Protocol

MSD 96-well MULTI-ARRAY Human Leptin, Insulin Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Human Leptin, Insulin Assay.

Step 1 : Sample and Reagent Preparation

Bring appropriate Diluents and plates to room temperature.

Prepare Blocker A Solution.

Prepare serum or plasma samples.

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

Prepare an 8-point standard curve using supplied calibrators:

- The Calibrators should be diluted in Diluent 13.
- Dilute the stock Calibrators 1:100 as indicated in Reagent Preparation section, then perform a series of 3-fold dilution steps and a no calibrator blank.

Prepare Detection Antibody Solution by diluting the 100X Anti-hLeptin Antibody and the 100X Anti-hInsulin Antibody to 1X and the 10% Blocker D-B to 0.3% in 3.0 mL of Diluent 12 per plate.

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 25 µL/well Diluent 13.

Immediately, dispense 25 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.

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