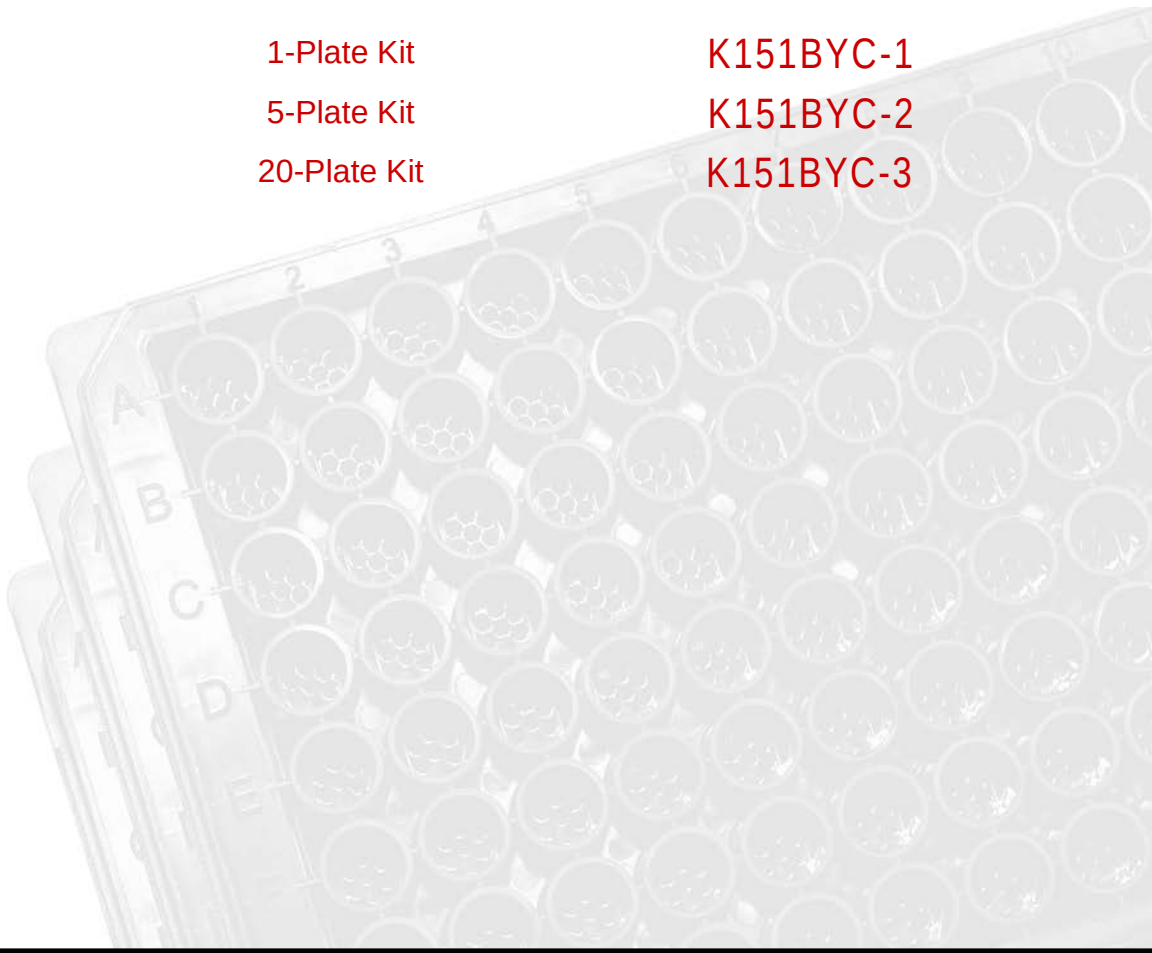
MESO SCALE DISCOVERY

MULTI-ARRAY® Assay System

Human Leptin Kit

1-Plate Kit	K151BYC-1
5-Plate Kit	K151BYC-2
20-Plate Kit	K151BYC-3



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MSD Metabolic Assays

Human Leptin Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

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Ordering Information

ordering information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

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 obesity often accompanied by insulin resistance and hyperinsulinemia.

Principle of the Assay

principle of the assay

MSD® metabolic assays provide rapid and convenient methods for measuring the levels of protein targets within single small-volume samples. 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 Assay detects leptin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with leptin antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-leptin labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Leptin in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the 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 leptin present in the sample.

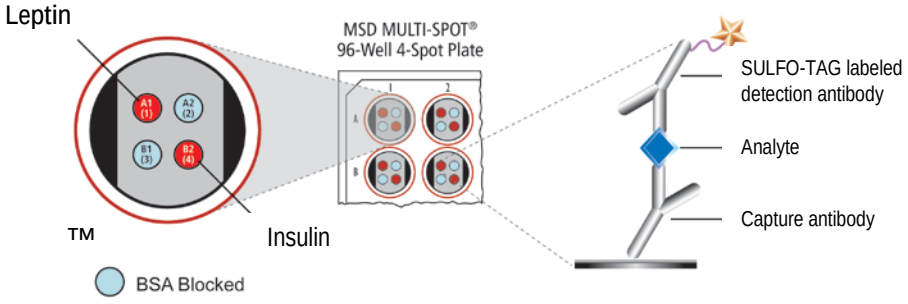


Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding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

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K151BYC-1	K151BYC-2	K151BYC-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)
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)
Blocker E (100X)	≤-10°C	1 vial (0.09 mL)	1 vial (0.45 mL)	4 vials (0.45 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 R50JA-4 (10 mL) R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	2 bottles (50 mL ea)
Read Buffer T (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

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.

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

VI Reagent Preparation

reagent preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.
Blocker E 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 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):

- ❑ 70 µL of Blocker E
- ❑ 6930 µL of Diluent 6

Prepare Calibrator and Control Solutions

Calibrator for the Human Leptin Assay is supplied at 10 µg/mL. 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)	Dilution Factor
Stock Cal. Vial	10000000	
STD-01	100000	100
STD-02	33333	3
STD-03	11111	3
STD-04	3704	3
STD-05	1235	3
STD-06	412	3
STD-07	137	3
STD-08	0	n/a

To prepare this 8-point standard curve:

1) Prepare the highest Calibrator by transferring 10 µL of the Calibrator stock vial to 990 µL of Metabolic Assay Working Solution.
2) Prepare the next Calibrator by transferring 100 µL of the diluted Calibrator to 200 µL of Metabolic Assay Working Solution. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Working Solution (i.e. zero Calibrator).
4) Diluted Calibrators should be kept on ice prior to addition to the plate.

Note: The standard curve can be modified as necessary to meet specific assay requirements.

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 Antibody is provided at 100X stock solution. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 30 µL aliquot of the stock Detection Antibody into 2.97 mL of Diluent 12.

Prepare Read Buffer

The Read Buffer should be diluted 4-fold in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of 4X Read Buffer T 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 3 times with PBS-T. Dispense 25 µL of Metabolic Assay Working Solution into each well of the MSD plate. 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.

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 3 times 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 3 times 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

analysis of results

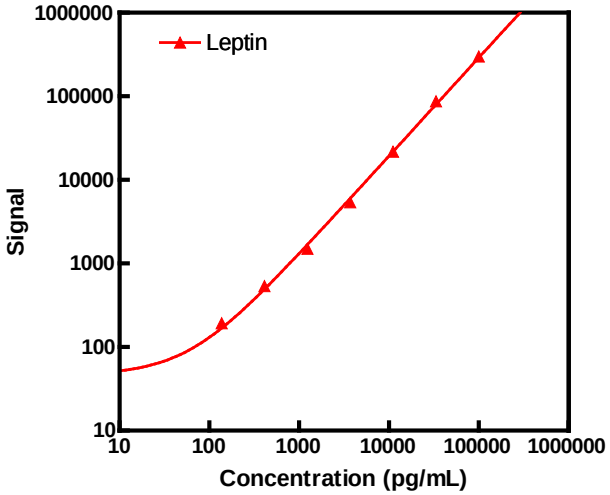
The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification 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 Assay is designed for use with human serum and plasma samples.

The following standard curve is an example 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 quantification of unknown samples.



	Leptin	
Conc. (pg/mL)	Average Signal	%CV
0	45	9.9
137	193	3.6
412	535	1.0
1235	1504	6.9
3704	5389	8.4
11111	21726	1.3
33333	87339	7.5
100000	298952	3.0

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
LLOD (pg/mL)	43

XI Endogenous Levels

Endogenous levels

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

Sample	Serum (pg/mL)	EDTA Plasma (pg/mL)	Heparin Plasma (pg/mL)
1	2127	1951	1988
2	17470	15677	15578
3	1178	1259	1049
4	10629	8817	8925
5	1700	2209	1679

Endogenous levels of human leptin in four diseased serum samples; BMI (Body Mass Index).

Sample	Leptin (pg/mL)	Diabetes	BMI
1	6170	Type I	49
3	37002	Type II	71
4	32709	Type II	36
5	30478	Type II	40

XII Spike Recovery

spike recovery

Serum, EDTA plasma, and heparin 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 samples.
% Recovery = measured /expected x 100

	Spike Conc. (pg/mL)	% Recovery
Spiked Serum	1000	88
	5000	83
	10000	88
Spiked Heparin Plasma	1000	82
	5000	83
	10000	82
Spiked EDTA Plasma	1000	94
	5000	95
	10000	99

XIII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled human plasma followed by subsequent dilution.
Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).
% Recovery = measured x dilution factor / expected x 100

	Fold Dilution	% Recovery
Serum	2	115
	4	123
	8	117
EDTA Plasma	2	111
	4	109
	8	94
Heparin Plasma	2	131
	4	137
	8	131

XIV Assay Components

assay components

Calibrator	
Analyte	Human leptin
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

1. Matares G, Moschos S, Mantzoros CS. Leptin in Immunology. The Journal of Immunology, 2005 173: 3137–3142
2. Coll AP, Farooqi SI, O'Rahilly S. The Hormonal Control of Food Intake. 2007 Cell 129(2):l 252-262, 2007
3. Ahren B, Mansson S, Ginderich RL, Havel P. Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting. American Journal of Physiology. 1997 273(42): R113-R120

Summary Protocol

MSD 96-well MULTI-ARRAY Human Leptin Kit

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

Step 1 : Sample and Reagent Preparation

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

Prepare Blocker A Solution.

Prepare serum or plasma samples.

Prepare Metabolic Assay Working Solution and keep on ice.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Metabolic Assay Working Solution.
- Dilute the stock Calibrator 1:100 as indicated in Reagent Preparation section, then perform a series of 3-fold dilution steps and a no Calibrator blank.
- Diluted Calibrators should be kept on ice until use.

Note: *The standard curve can be modified as necessary to meet specific assay requirements.*

Prepare Detection Antibody Solution by diluting the 100X Anti-hLeptin Antibody to 1X in 3.0 mL of Diluent 12 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T 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 3 times with PBS-T.

Dispense 25 µL/well Metabolic Assay Working Solution.

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 3 times with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

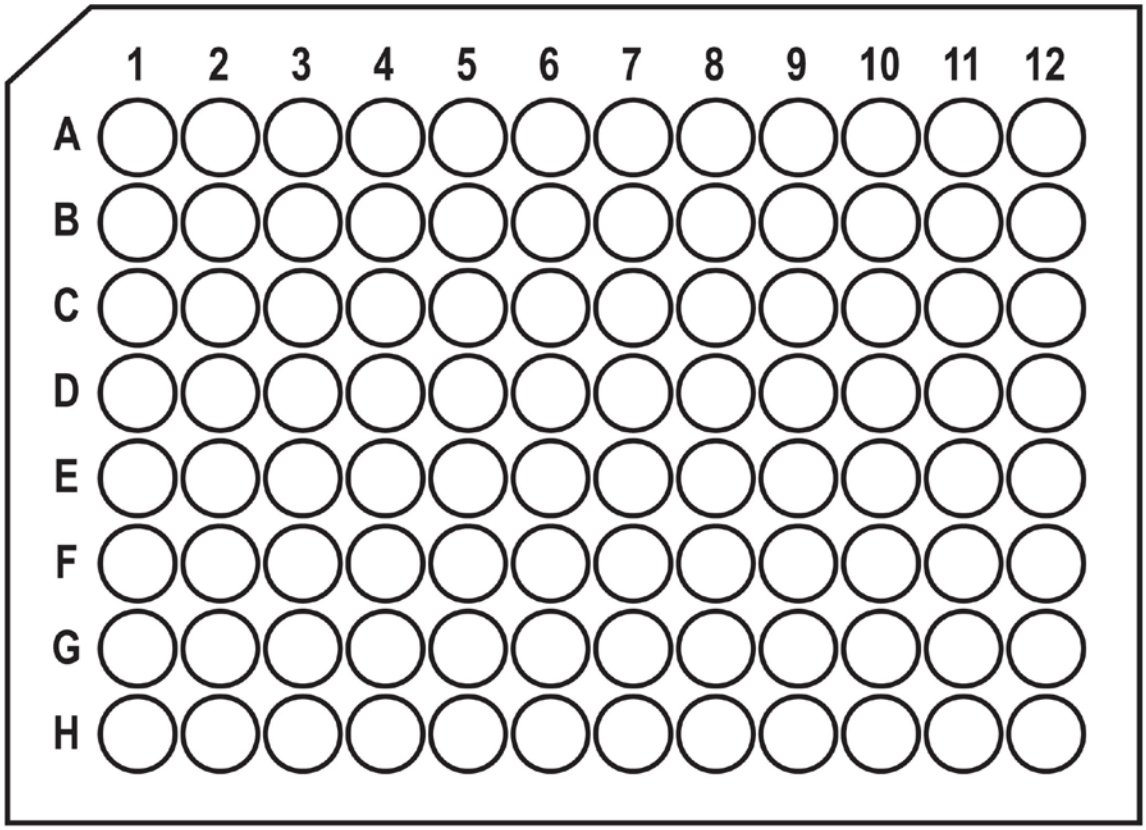
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 5 : Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.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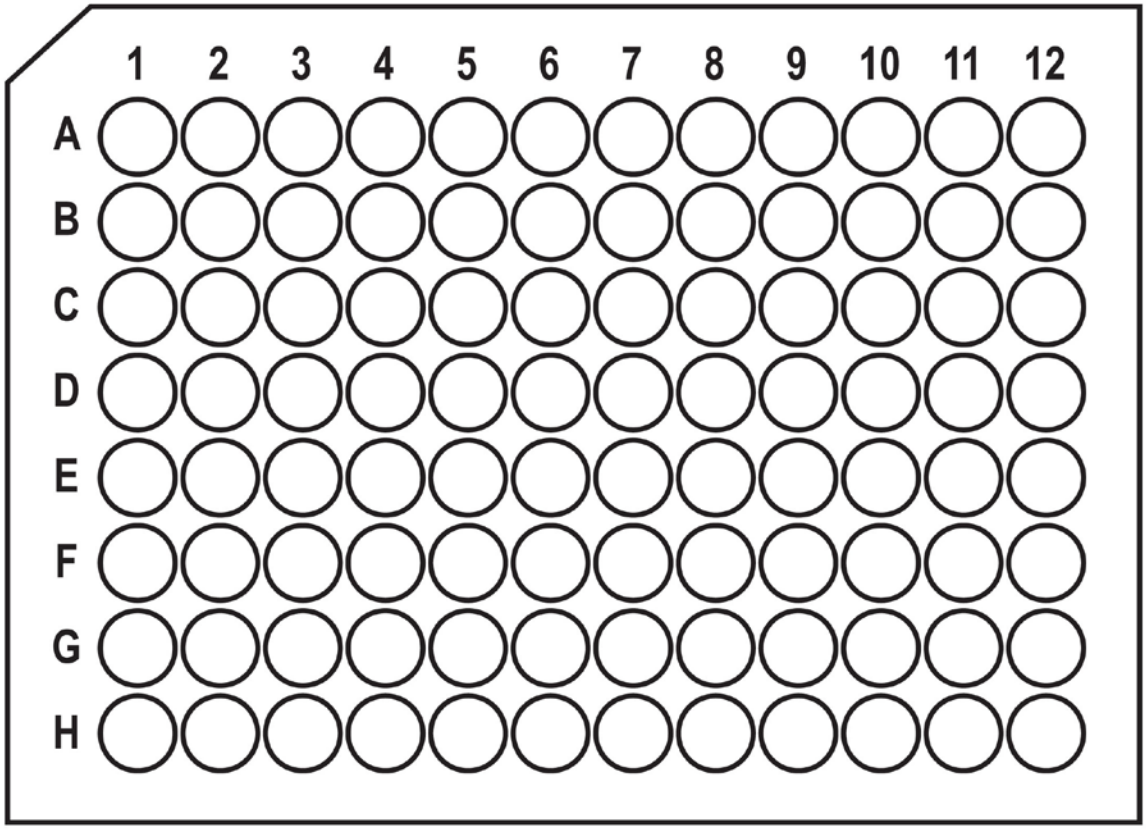
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