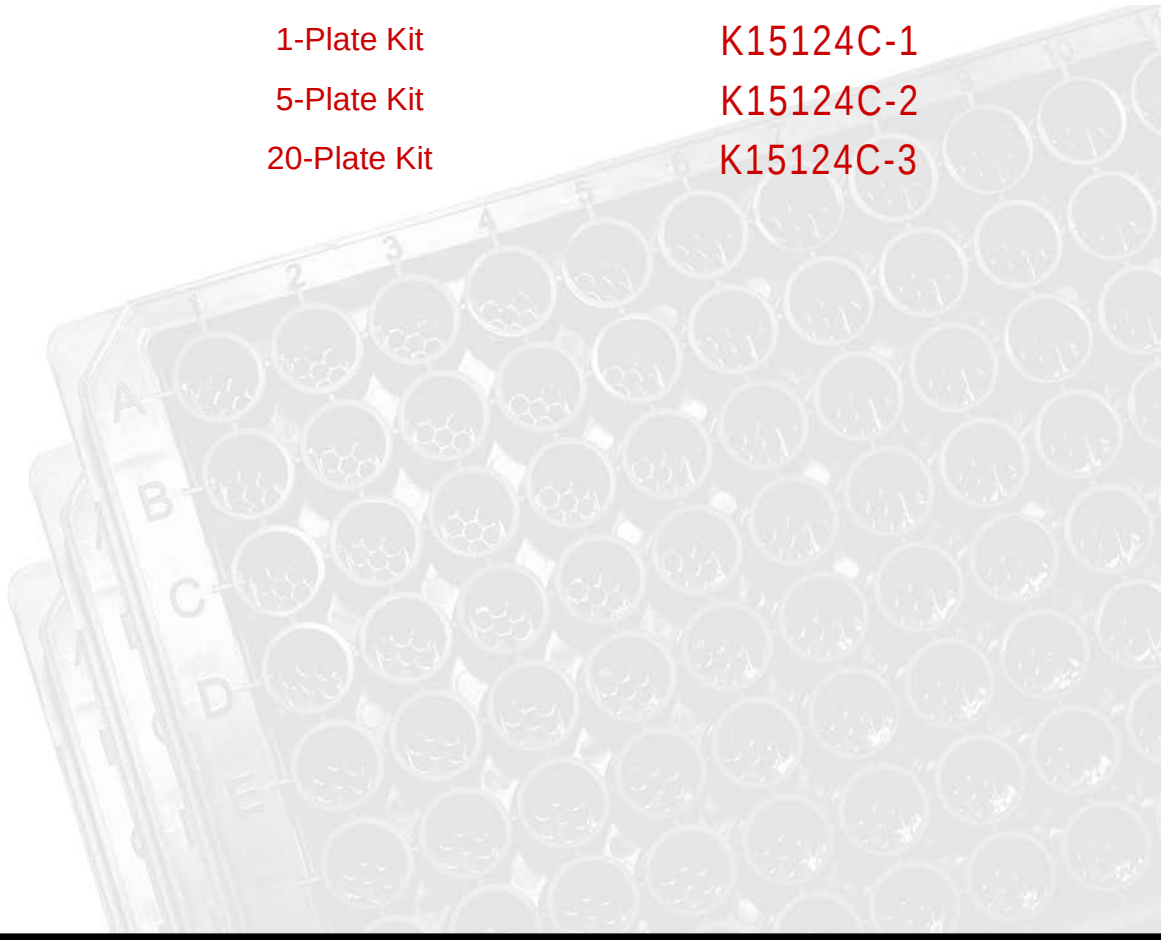
Meso Scale Discovery®

MULTI-SPOT® Assay System

Mouse Metabolic Kit

1-Plate Kit	K15124C-1
5-Plate Kit	K15124C-2
20-Plate Kit	K15124C-3



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MSD Metabolic Assays

Mouse Metabolic Kit

Leptin, Insulin

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC 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 obesity 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 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 Mouse Metabolic 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.

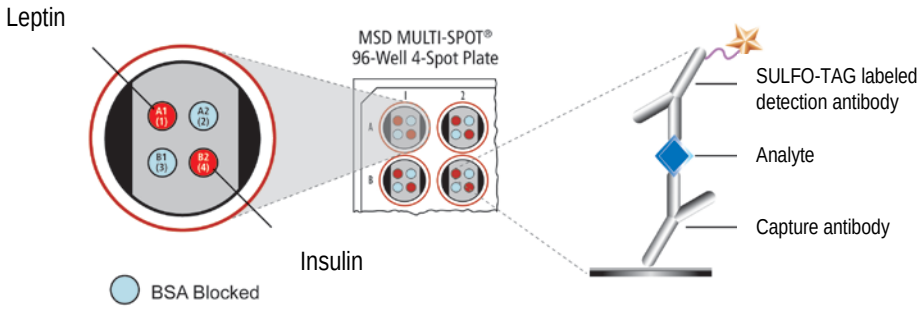


Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding 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 K15124C-1	K15124C-2	K15124C-3
MULTI-SPOT 96-well m/r Metabolic Plate(s) N45124A-1	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-Mouse Metabolic Detection Antibody Blend[1] (100X)	2-8°C	1 vial (60 µL)	1 vial (300 µL)	4 vials (300 µL ea)
Mouse Leptin Calibrator 10 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)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 mL ea)
Diluent 17 R50KA-4 (6 mL) R50KA-3 (30 mL)	≤-10°C	1 bottle (6 mL)	1 bottle (30 mL)	4 bottles (30 mL ea)
Diluent 100 R50AA-4 (50 mL) R50AA-2 (200 mL)	2-8°C	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)
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

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

Important: Upon first thaw, separate Diluent 17 into aliquots appropriate to the size of your assay needs. This diluent 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 transferring 10 µL of 10 µg/mL Leptin and 10 µL of 5 µg/mL Insulin to 980 µL of Diluent 100.
2) Prepare the next Calibrator by transferring 100 µL of the diluted Calibrator to 200 µL of Diluent 100. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 100 (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 10 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 as a 100X stock of Anti-Mouse Metabolic Detection Antibody Blend. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute 50 µL of the Detection Antibody stock into a final volume of 5 mL of Diluent 17.

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 the Detection Antibody Solution followed by Sample or Calibrator:** Wash the plate 3 times with PBS-T. Dispense 40 µL of 1X Detection Antibody Solution into each well of the MSD plate. Immediately add 10 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 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

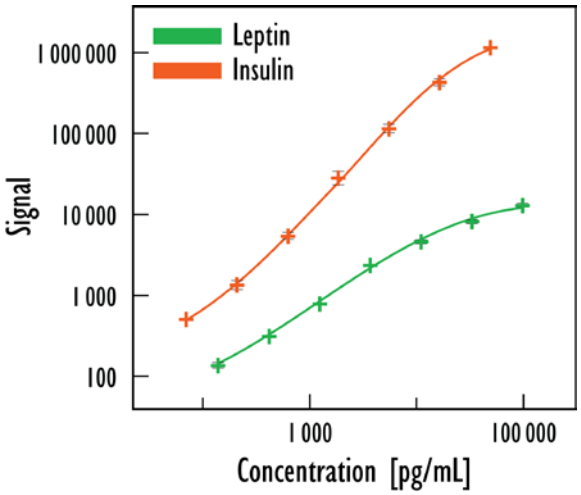
The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have 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 Mouse Metabolic Assay is designed for use with mouse 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	64	14	**0**	277	3
137	135	8	**69**	497	1
412	316	5	**206**	1349	12
1235	785	6	**617**	5663	9
3704	2334	2	**1852**	28144	18
11111	4580	5	**5556**	114310	12
33333	8235	6	**16667**	422598	10
100000	13011	6	**50000**	1121247	6

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)	43	15

XI Endogenous Levels

Endogenous levels

Endogenous levels of leptin and insulin from individual normal mouse serum samples

	Normal	
Sample ID	**Leptin (pg/mL)**	**Insulin (pg/mL)**
1	893	4410
2	947	3219
3	1706	13569
4	969	6376
5	2285	3805
6	5962	8302
7	1787	6103
8	612	1167

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 mouse serum and plasma samples including high, mid, and low Calibrator spikes.
% Recovery = measured /expected x 100

	Average % Recovery	
	Leptin	Insulin
Spiked Serum	85	88
Spiked Heparin Plasma	83	95
Spiked EDTA Plasma	88	95

XIII Linearity

linearity

Linearity was measured by spiking analyte levels in pooled normal mouse EDTA 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

	Average % Recovery	
	Leptin	Insulin
1/2	101	93
1/4	107	109
1/8	112	113

Leptin

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

Capture Antibody	
Analyte	Mouse leptin
Source	Rabbit polyclonal
Isoforms Recognized	Reacts with recombinant and natural mouse leptin
Species cross-reactivity	Mouse, rat

Detection Antibody	
Analyte	Mouse leptin
Source	Goat polyclonal
Isoforms Recognized	n/a
Species cross-reactivity	Mouse

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	Mouse/rat insulin
Source	Mouse monoclonal
Isoforms Recognized	Does not react with human proinsulin, rat or human C-peptide
Species cross-reactivity	Human, mouse, rat, porcine, bovine

Detection Antibody	
Analyte	Mouse/rat insulin
Source	Mouse monoclonal
Isoforms Recognized	Does not react with human proinsulin, rat or human C-peptide
Species cross-reactivity	Human, mouse, rat, porcine, bovine

XV References

references

Leptin

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Insulin

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Summary Protocol

MSD 96-well MULTI-ARRAY Mouse Metabolic Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Mouse Metabolic 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 an 8-point standard curve using supplied Calibrators.

- The Calibrators should be diluted in Diluent 100.
- Dilute the stock Calibrators 1:100 in Diluent 100 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-Mouse Detection Antibody Blend to 1X in a final volume of 5.0 mL of Diluent 17 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 Detection Antibody Solution Followed by Sample or Calibrator

Wash plate 3 times with PBS-T.

Dispense 40 µL/well 1X Detection Antibody Solution.

Immediately, dispense 10 µL/well Calibrator or Sample.

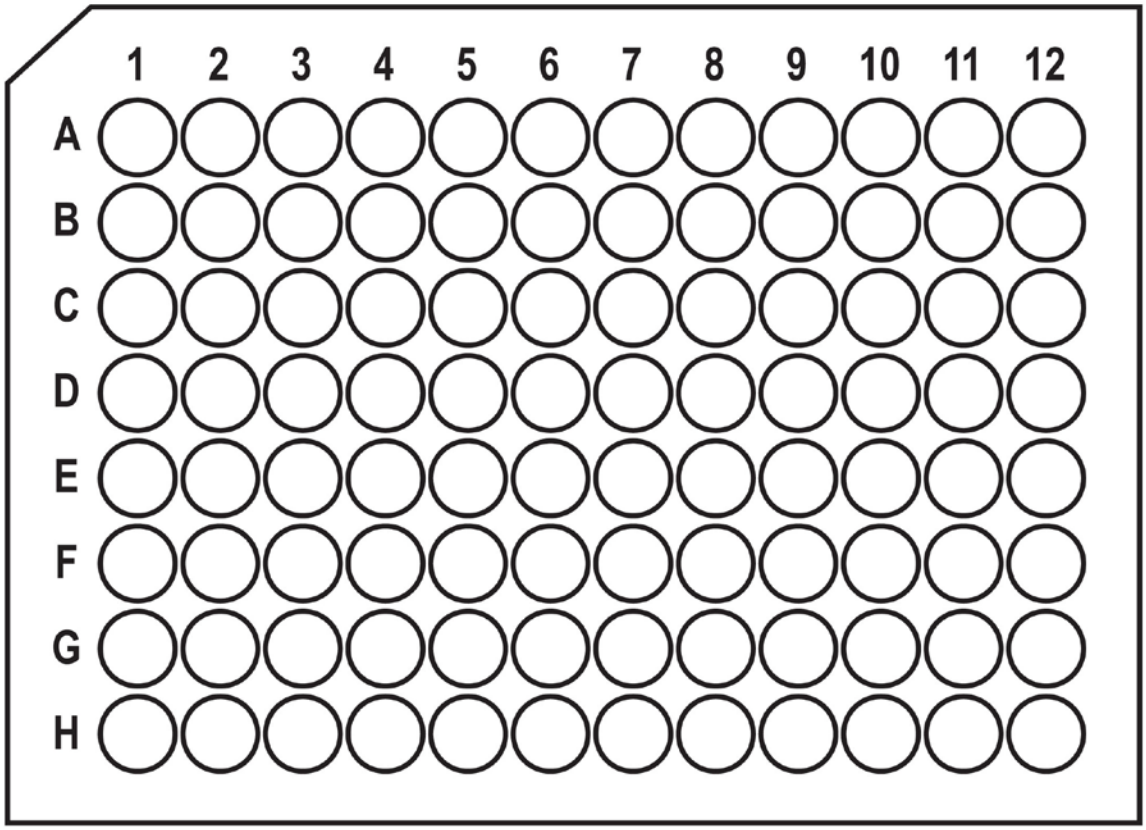
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : 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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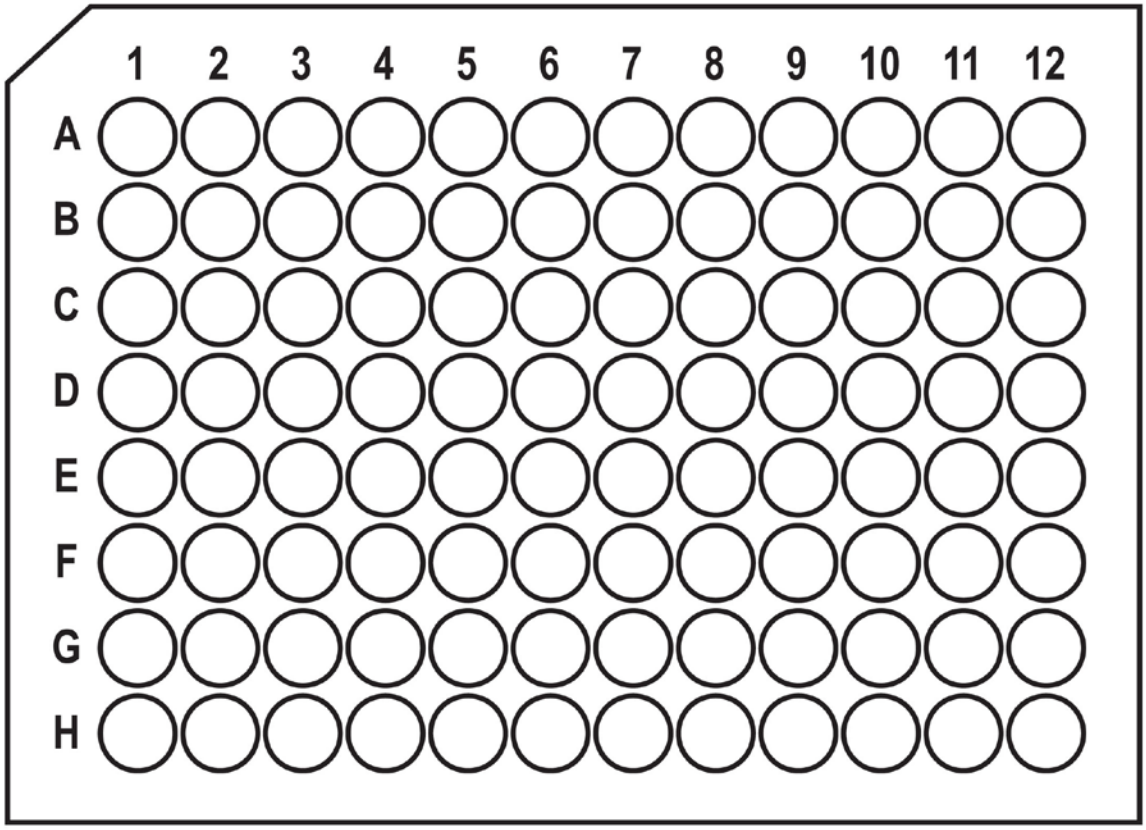
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