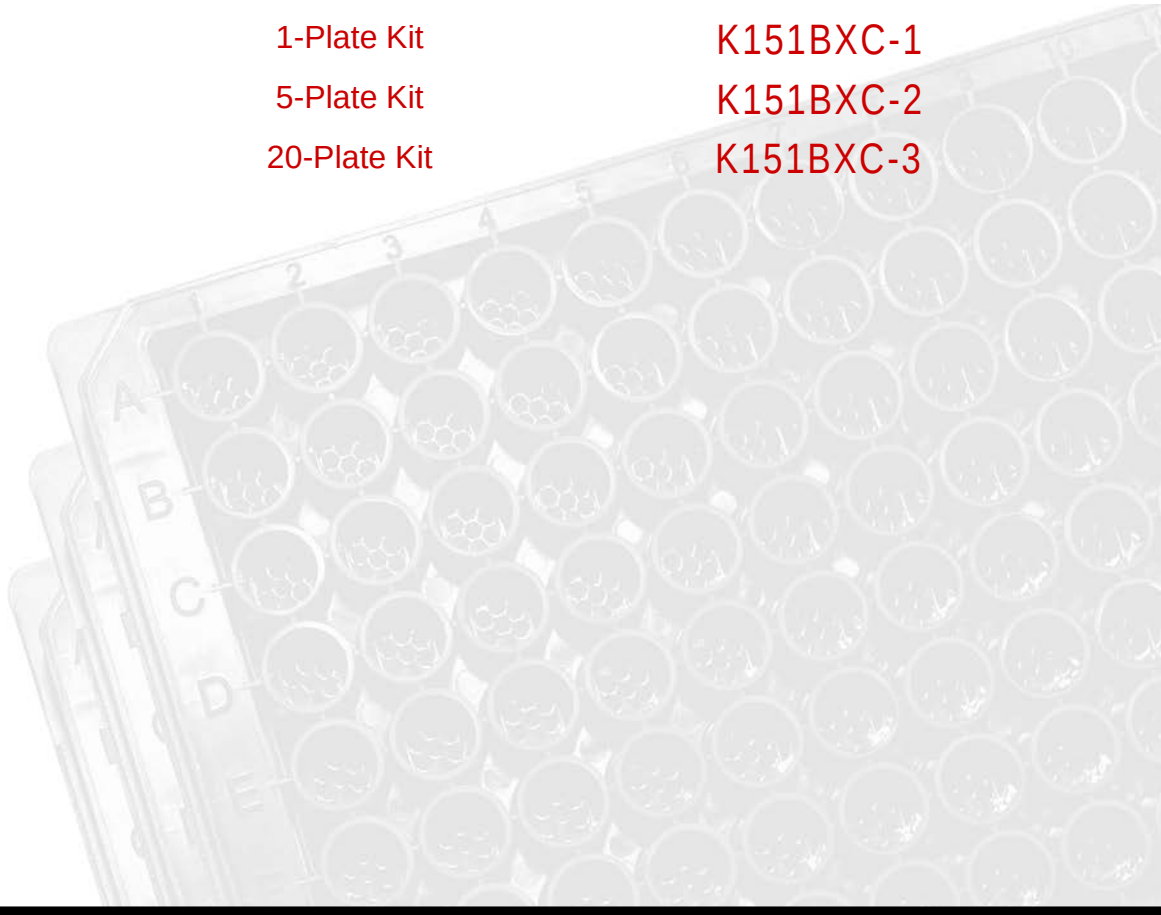
Meso Scale Discovery®

MULTI-ARRAY® Assay System

Human Adiponectin Kit

1-Plate Kit	K151BXC-1
5-Plate Kit	K151BXC-2
20-Plate Kit	K151BXC-3



MSD Metabolic Assays

Human Adiponectin Kit

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

Adiponectin is a 30 kD protein secreted exclusively by adipocytes and is thought to play a role in lipid and glucose metabolism. Adiponectin enhances insulin action by activating glucose uptake and fatty acid oxidation. In addition, this adipokine has potent anti-inflammatory and anti-atherosclerotic properties. Adiponectin circulates at high levels (between 2-20 µg/mL) in plasma as trimers, hexamers and high molecular weight multimers. In contrast to most adipokines, plasma adiponectin is often negatively correlated with body mass index (BMI) in humans and rodents. However, expression in various adipose tissues and disease models may vary.

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 Adiponectin Assay detects adiponectin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with adiponectin antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-adiponectin labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Adiponectin 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 adiponectin present in the sample.

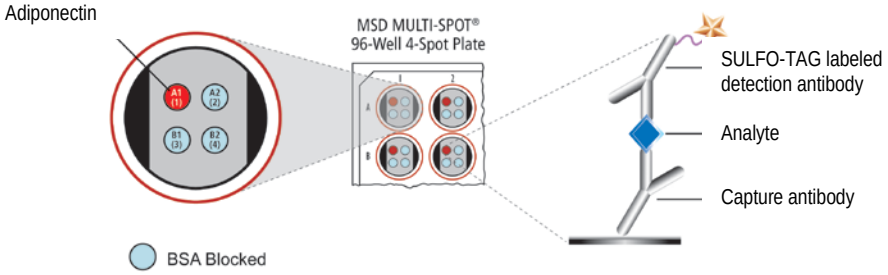


Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding 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 K151BXC-1	K151BXC-2	K151BXC-3
MULTI-SPOT 96-well Human Adiponectin Plate(s) N451BXA-1	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-hAdiponectin Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
Human Adiponectin Calibrator 100 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 100 R50AA-2 (200 mL) R50AA-3 (1 L)	2-8°C	1 bottle (200 mL)	2 bottles (200 mL ea)	2 bottles (200 mL & 1 L)
Diluent 12 R50JA-4 (10 mL) R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	4 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 25 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.

> **Important:** Upon first thaw, separate Diluent 12 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

Calibrator for the Human Adiponectin Assay is supplied at 100 µg/mL. For the assay, an 8-point standard curve is recommended with 5-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Adiponectin conc. (ng/mL)	Dilution Factor
Stock Cal. Vial	100000	
STD-01	1000	100
STD-02	200	5
STD-03	40	5
STD-04	8.0	5
STD-05	1.6	5
STD-06	0.32	5
STD-07	0.064	5
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 Diluent 100.
2) Prepare the next Calibrator by transferring 40 µL of the diluted Calibrator to 160 µL of Diluent 100. Repeat 5-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 pre-diluted serum/plasma samples per well. An adequate volume of each sample should be prepared depending upon desired number of replicates. The serum/plasma samples should be pre-diluted with Diluent 100 one thousand fold (some samples may require larger dilutions). A two-step dilution is recommended; an initial 10-fold dilution followed by a second 100-fold dilution.

- 10 µL of sample + 90 µL of Diluent 100
- 10 µL of the above dilution + 990 µL of Diluent 100

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 antibody for the analyte 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 40 µL of Diluent 12 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 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

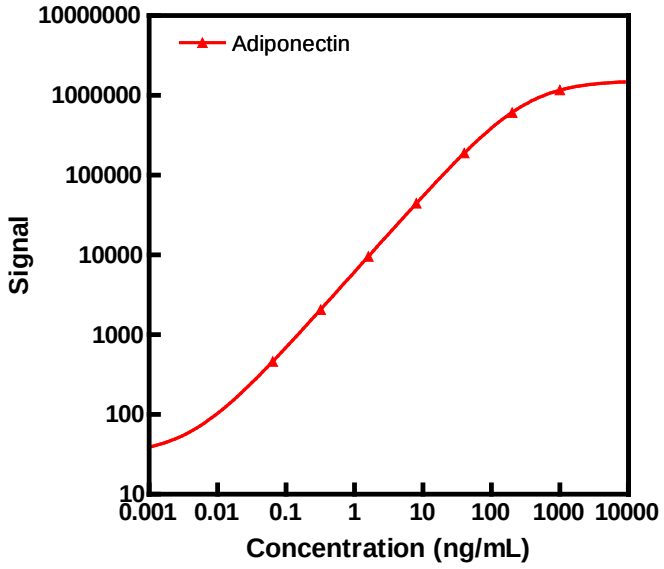
The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have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



	Adiponectin	
Conc. (ng/mL)	Average Signal	%CV
0	62	9.8
0.064	463	6.4
0.32	2063	6.5
1.6	9597	1.3
8.0	44639	5.9
40	189332	1.7
200	607994	2.4
1000	1169321	1.4

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.

	Adiponectin
LLOD (ng/mL)	0.0050

XI Endogenous Levels

endogenous levels

Endogenous levels of human adiponectin in five matched individual serum and plasma samples. Samples were diluted 1000 fold prior to measurement. The data below represents final concentrations which have been corrected for dilutions.

Sample	Serum (µg/mL)	EDTA Plasma (µg/mL)	Heparin Plasma (µg/mL)
1	6.4	9.0	8.8
2	8.6	9.6	9.8
3	23.1	21.8	17.8
4	13.5	12.7	14.8
5	7.1	6.2	7.6

XII Spike Recovery

spike recovery

Diluted 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 pooled human samples.
% Recovery = measured /expected x 100

	Spike Conc. (pg/mL)	% Recovery
Spiked Serum	20	100
	200	93
	500	85
Spiked EDTA Plasma	20	94
	200	98
	500	108
Spiked Heparin Plasma	20	95
	200	91
	500	81

XIII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled human samples 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	107
	4	111
	8	123
EDTA Plasma	2	105
	4	107
	8	119
Heparin Plasma	2	105
	4	107
	8	89

XIV Assay Components

assay components

Calibrator	
Analyte	Human adiponectin
Source	Purified, recombinant human adiponectin fused with a histidine tag and expressed in mouse NSO cells

Capture Antibody	
Analyte	Human adiponectin
Source	Mouse monoclonal
Isoforms Recognized	all monomer and multimeric forms of adiponectin; binds globular domain
Species cross-reactivity	Human; does not bind mouse or rat adiponectin

Detection Antibody	
Analyte	Human adiponectin
Source	Goat polyclonal
Isoforms Recognized	n/a
Species cross-reactivity	Human, 10% mouse

XV References

1. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obesity Reviews. 2005 Feb;6(1):13-21

2. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006 Sep:Oct;6(10):772-83

3. Hada Y, Yamauchi T, Waki H et al. Selective purification and characterization of adiponectin multimer species from human plasma. Biochem Biophys Res Commun. 2007 May 4;356(2):487-93

4. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med. 2001 Aug;7(8):947-53

5. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. Am J Physiol Endocrinol Metab. 2003 Sep;285(3):E527-33

Summary Protocol

MSD 96-well MULTI-ARRAY Human Adiponectin Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Human Adiponectin 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 Calibrator:

- The Calibrator should be diluted in Diluent 100.
- Dilute the stock Calibrator 1:100 in Diluent 100 then perform a series of 5-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-hAdiponectin 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 40 µL/well Diluent 12.

Immediately, dispense 10 µL/well Calibrator or Sample.

- Samples should be diluted 1000-fold as described in the Reagent Preparation section.

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.

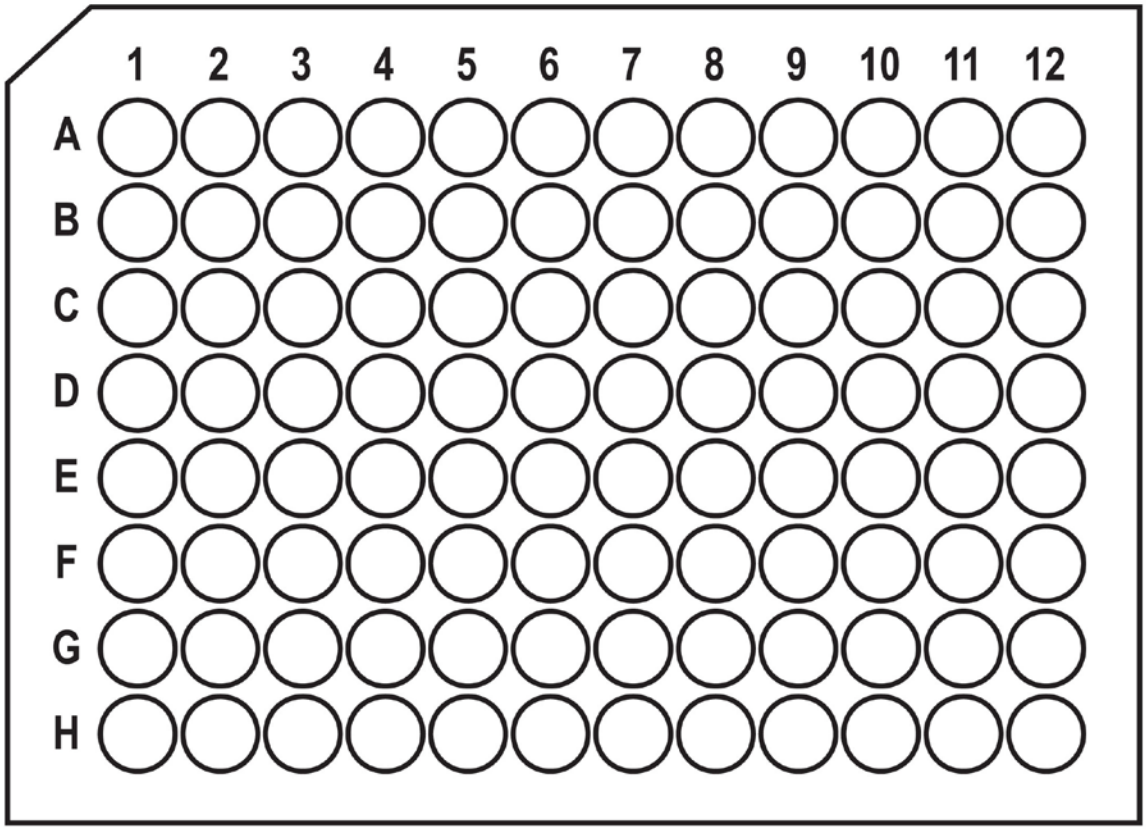
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

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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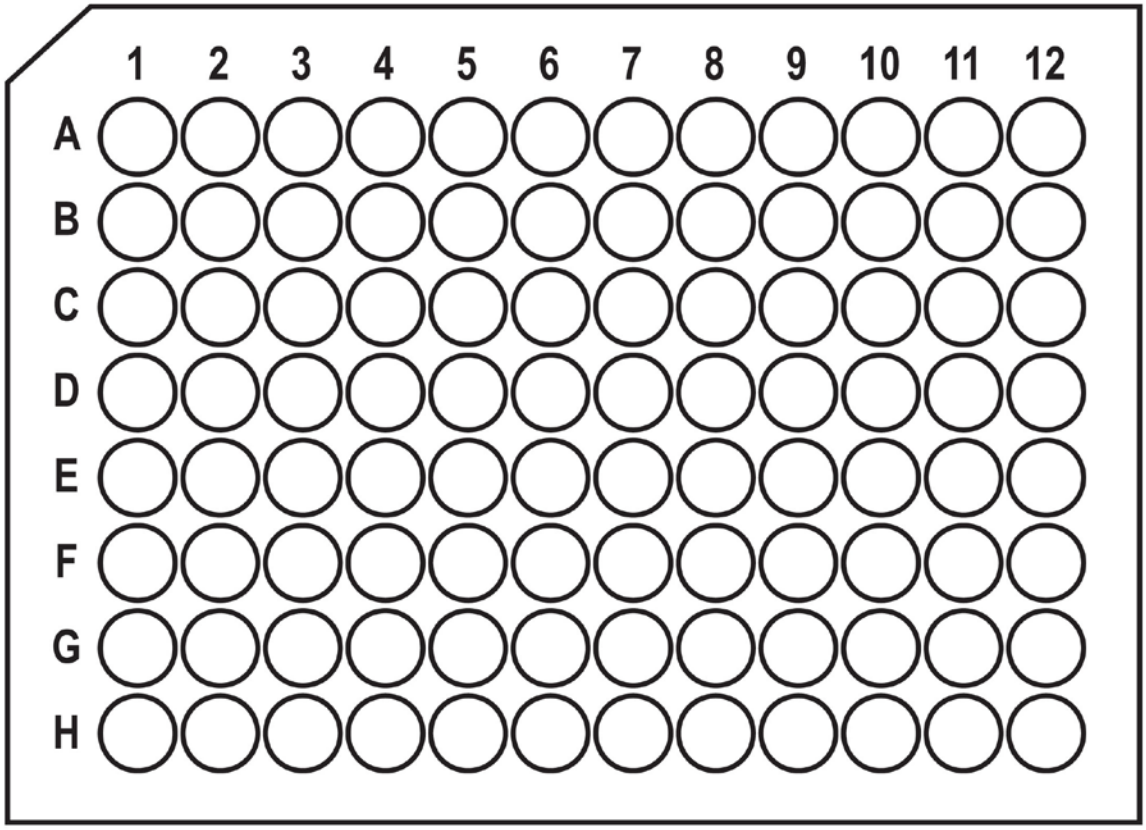
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