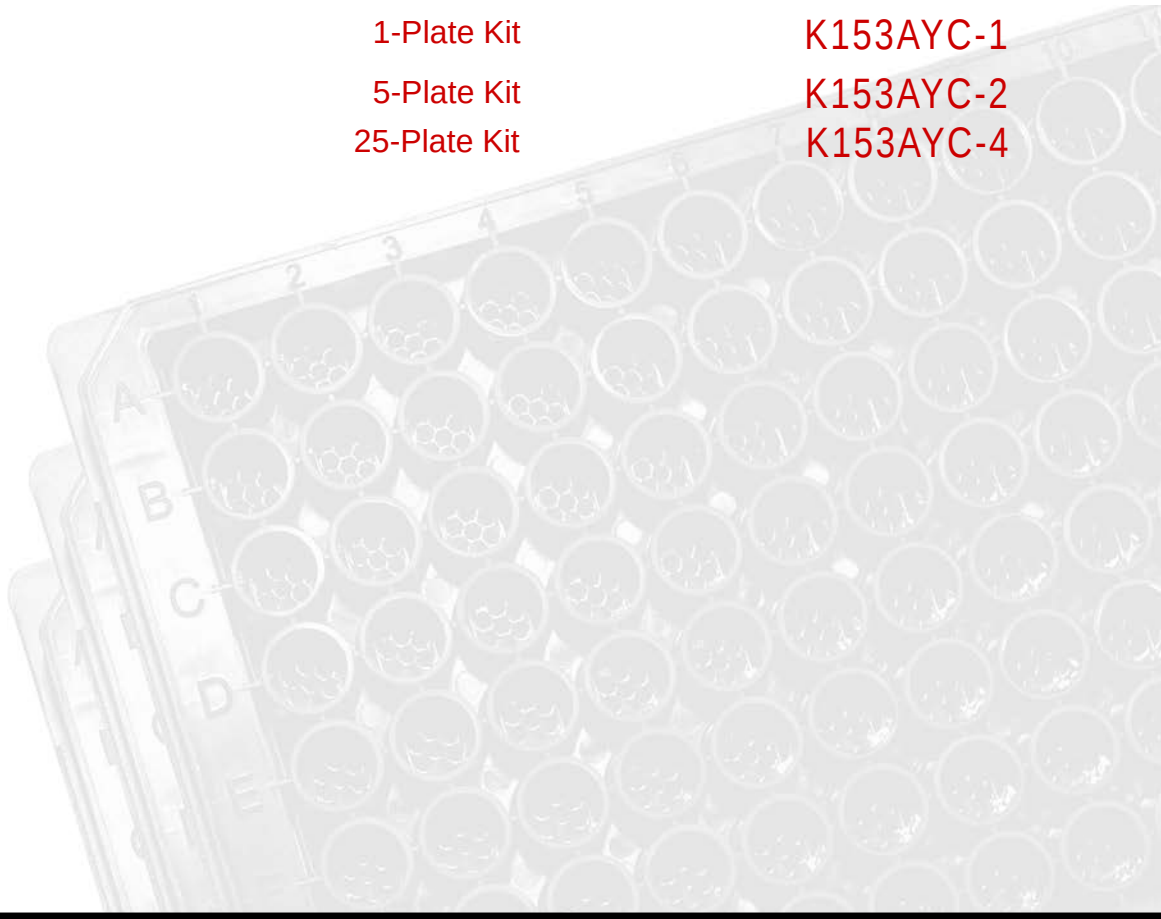
Meso Scale Discovery®

MULTI-ARRAY® Assay System

Rat MCP-1 Assay
Ultra-Sensitive Kit

1-Plate Kit	K153AYC-1
5-Plate Kit	K153AYC-2
25-Plate Kit	K153AYC-4



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MSD MULTI-ARRAY Assay

Ultra-Sensitive Kit

Rat MCP-1 Assay

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

Meso Scale Discovery
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FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Ordering Information

Ordering information

MSD Customer Service

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MSD Scientific Support

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Introduction

introduction

Monocyte chemoattractant protein-1 (MCP-1) is a cytokine that belongs to the family of chemotactic cytokines known as chemokines. It is also called Monocyte Chemotactic and Activating Factor (MCAF) or Chemokine (C-C motif) Ligand 2 (CCL2).[1] Human MCP-1 is encoded by the human JE gene at chromosome 17q11.2-q21.1. The gene encodes a 99 amino acid residue precursor protein with a 23 amino acid hydrophobic signal peptide that is cleaved to generate the 76 amino acid mature protein.[2,3] The rat analogue is 49-amino acids longer than human MCP-1 at the 3'-end and was cloned from a Con A-stimulated rat spleen cDNA library.[4] Mouse MCP-5 shares 65% overall amino acid similarity with human MCP-1.[1]

MCP-1 has been shown to recruit monocytes, memory T cells and dendritic cells to sites of injury and infection.[5,6] Other cell types responding to MCP-1 include NK cells, activated-effector T cells, eosinophils, basophils and hepatic stellate cells.[7-11] MCP-1 typically does not attract neutrophils; however, point mutations have been identified at two amino acid positions, which alter the protein so that it can be chemotactic for neutrophils.[12]

MCP-1 is primarily produced by monocytes, macrophages, fibroblasts and keratinocytes in response to various pathogenic conditions. Low levels of MCP-1 are also secreted by stimulated lymphocytes, endothelial cells and smooth muscle cells in vitro.[13,14] Elevated levels of MCP-1 are observed in atherosclerotic plaques that are rich in macrophages.[15] Furthermore, MCP-1 is produced during inflammatory disease states such as atherosclerosis and rheumatoid arthritis, and it plays an important role in intimal hyperplasia.[16]

Studies in MCP-1 deficient mice have revealed other important functions for this chemokine. MCP-1 is produced by different tumor cell types and has been shown to contribute directly to tumor progression by increasing angiogenesis.[17] Recent studies have also revealed that wound angiogenesis is delayed in MCP-1(-/-) mice suggesting that MCP-1 plays a critical role in the healing response, most likely by influencing the effector state of both lymphoid and non-lymphoid cells.[18] Elevated MCP-1 levels have been observed in Crohn's disease, sepsis, lupus nephritis, multiple sclerosis, rheumatoid arthritis and acute pancreatitis.[19-23] MCP-1 also plays an important role in certain cancers such as gastric carcinoma, malignant glioma, as well as in ovarian, pancreatic and breast cancers.[24-27]

Principle of the Assay

principle of the assay

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

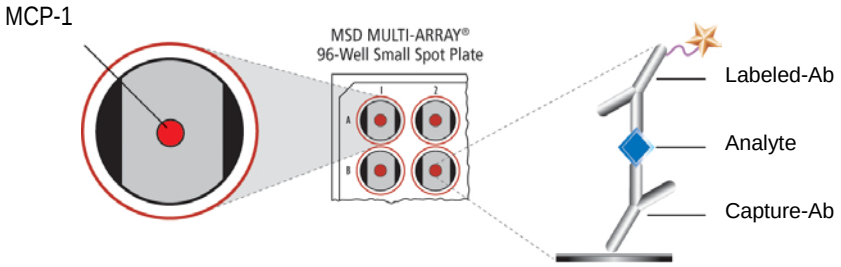


Figure 1. Spot diagram showing placement of analyte capture antibody.
A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K153AYC-1	K153AYC-2	K153AYC-4
MULTI-SPOT® 96-well Small Spot Rat MCP-1 US Plate(s) L453AYA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-m/r MCP-1 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat MCP-1 Calibrator (1 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 5 R52BA-4 (5 mL) R52BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Diluent 6 R53BB-4 (8 mL) R53BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	2 bottles (200 mL ea)

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.

> Important: Upon first thaw, separate Diluent 5 and Diluent 6 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 Calibrator and Control Solutions

MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates of each point. Each well requires 25 µL of Calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 6 alone for the 8th point:

Standard	MCP-1 (pg/mL)	Dilution Factor
25X Stock	1,000,000	
STD-01	40,000	25
STD-02	10,000	4
STD-03	2,500	4
STD-04	625	4
STD-05	156	4
STD-06	39	4
STD-07	9.8	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

1) Prepare STD-01 by transferring 10 µL of the Rat MCP-1 stock Calibrator to 240 µL Diluent 6.
2) Prepare the next Calibrator point (STD-02) by transferring 50 µL of STD-01 to 150 µL Diluent 6. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 6 (i.e. zero Calibrator).

Notes:

a. Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces. If your sample matrix is serum-free tissue culture media, then the addition of 10% FBS or 1% BSA is recommended.
b. The standard curve can be modified as necessary to meet specific assay requirements.

Dilution of Samples

Serum and Plasma

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples. Serum and plasma samples may be diluted 40-fold prior to being used in the MSD Rat MCP-1 Assay. A simple PBS based diluent with 1% BSA may be used for dilution. Alternatively, additional Diluent 100 can be purchased for diluting samples (catalog numbers: R50AA-4 (50 mL), R50AA-2 (200 mL), R50AA-3 (1000 mL)).

Tissue Culture

Tissue culture supernatant samples may be run neat in the MSD Rat MCP-1 Assay. If using serum-free medium, the presence of carrier protein (e.g., 1% BSA) in the solution is helpful to prevent loss of analyte to the labware.

Other Matrices

Information on preparing samples in other matrices, including sputum, CSF, and tissue homogenates can be obtained by contacting MSD Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 50X stock of Anti-m/r MCP-1 Antibody. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 60 µL aliquot of the stock Anti-m/r MCP-1 Antibody into 2.94 mL of Diluent 5.

Prepare Read Buffer

The Read Buffer should be diluted 2-fold in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of 4X Read Buffer T to 10 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

1. Addition of Diluent 6: Dispense 25 µL of Diluent 6 into each well. Seal the plate with an adhesive plate seal and incubate for 30 min with vigorous shaking (300–1000 rpm) at room temperature.
2. Addition of the Sample or Calibrator: Dispense 25 µL of sample or Calibrator into separate 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 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
4. Wash and Read: Wash the plate 3X with PBS-T. Add 150 µL of 2X 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 plate typically accelerates capture at the working electrode.

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

VIII Analysis of Results

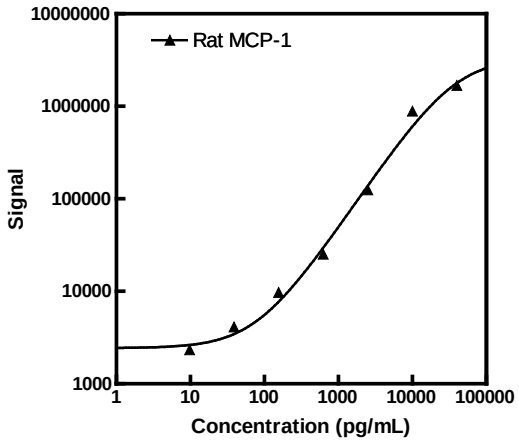
analysis of results

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 function 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 following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



MCP-1		
Conc. (pg/mL)	Average Signal	%CV
0	826	0.9
9.8	2338	3.4
39	4110	2.0
156	9725	3.1
625	25211	4.3
2500	125218	4.0
10000	885788	9.2
40000	1681565	0.6

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.

	MCP-1
LLOD (pg/mL)	2.0

XI Spike Recovery

spike recovery

Rat serum and heparin plasma pooled samples were diluted 40-fold and spiked with Calibrator at multiple values throughout the range of the assay. Each spike was done in ≥ 3 replicates. Representative data from an average of three serum and three heparin plasma are shown here. Results of spike-recovery may vary based on the individual samples.
% Recovery = measured / expected x 100

Sample	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	323	3.7	
	48	337	3.7	91
	167	460	5.0	94
	601	894	3.7	97
Heparin Plasma	0	78	5.2	
	45	107	1.6	87
	174	230	2.1	91
	566	609	3.9	95

XII Linearity

linearity

Three pools each of rat serum and heparin plasma were evaluated; a representative pool of each is shown below. The pooled samples were diluted 40-fold, spiked with Calibrator and then diluted with Diluent 6. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). 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

Sample	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	5	3,965	2.4	
	10	4129	4.7	104
	20	4234	2.3	103
	40	4561	4.2	108
Heparin Plasma	5	4772	4.6	
	10	4780	5.4	100
	20	4812	4.4	101
	40	5184	4.2	108

XIII Samples

samples

Eight normal rat samples were measured for each of the following sample types: serum, EDTA plasma, and heparin plasma. Samples were run with a 40-fold dilution.

		MCP-1 (pg/mL)
Serum	Min	12912
	Max	19156
	Median	13532
EDTA Plasma	Min	14241
	Max	17055
	Median	20261
Heparin Plasma	Min	23302
	Max	20812
	Median	23861

XIV References

references

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Summary Protocol

MSD 96-well MULTI-ARRAY Rat MCP-1 Assay: Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Rat MCP-1 Assay.

Step 1 : Sample and Reagent Preparation

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

Samples may require 40-fold dilution prior to use in this assay.

Prepare calibrator solutions and standard curve.

Use the 1 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 6.

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

Prepare Detection Antibody Solution by diluting Detection Antibody to 1X in a final volume of 3.0 mL Diluent 5 per plate.
Prepare 20 mL of 2X Read Buffer T by diluting 4X Read Buffer T with deionized water.

SERUM OR PLASMA SAMPLES

Step 2: Add Diluent 6

Dispense 25 µL/well Diluent 6.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

Step 3: Add Sample or Calibrator

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.

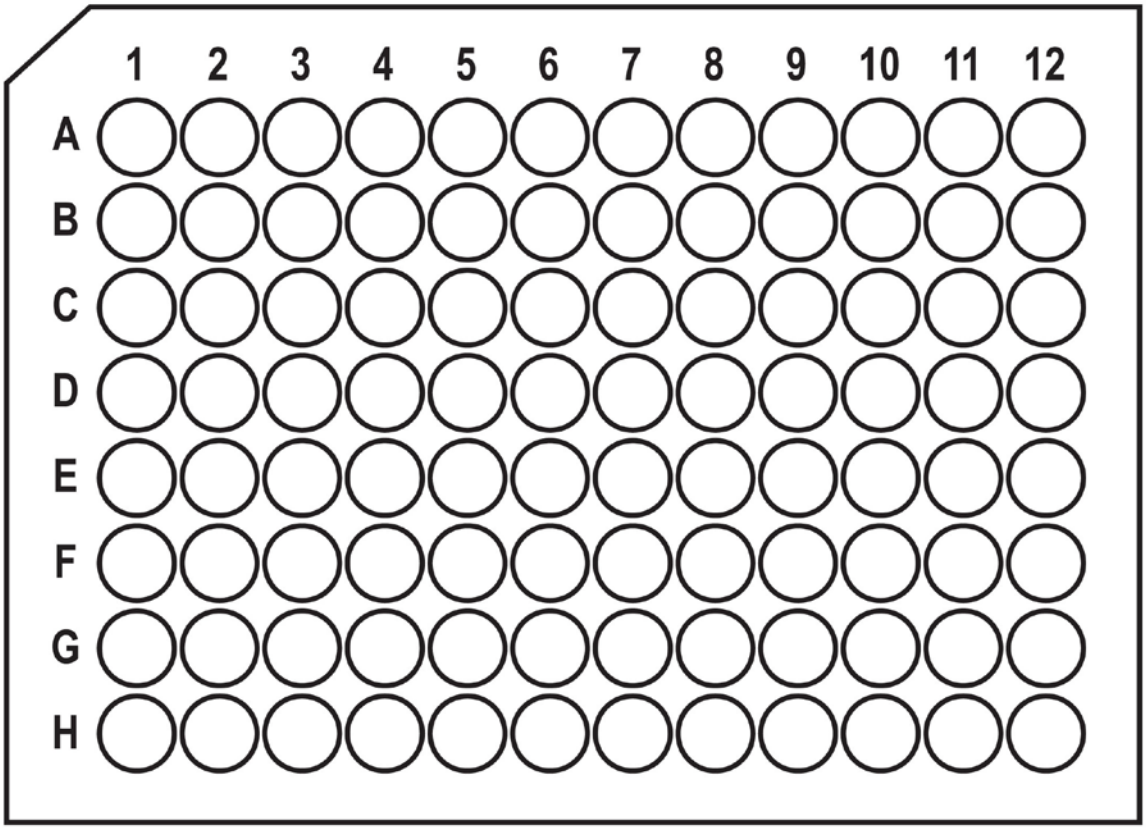
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 5: Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.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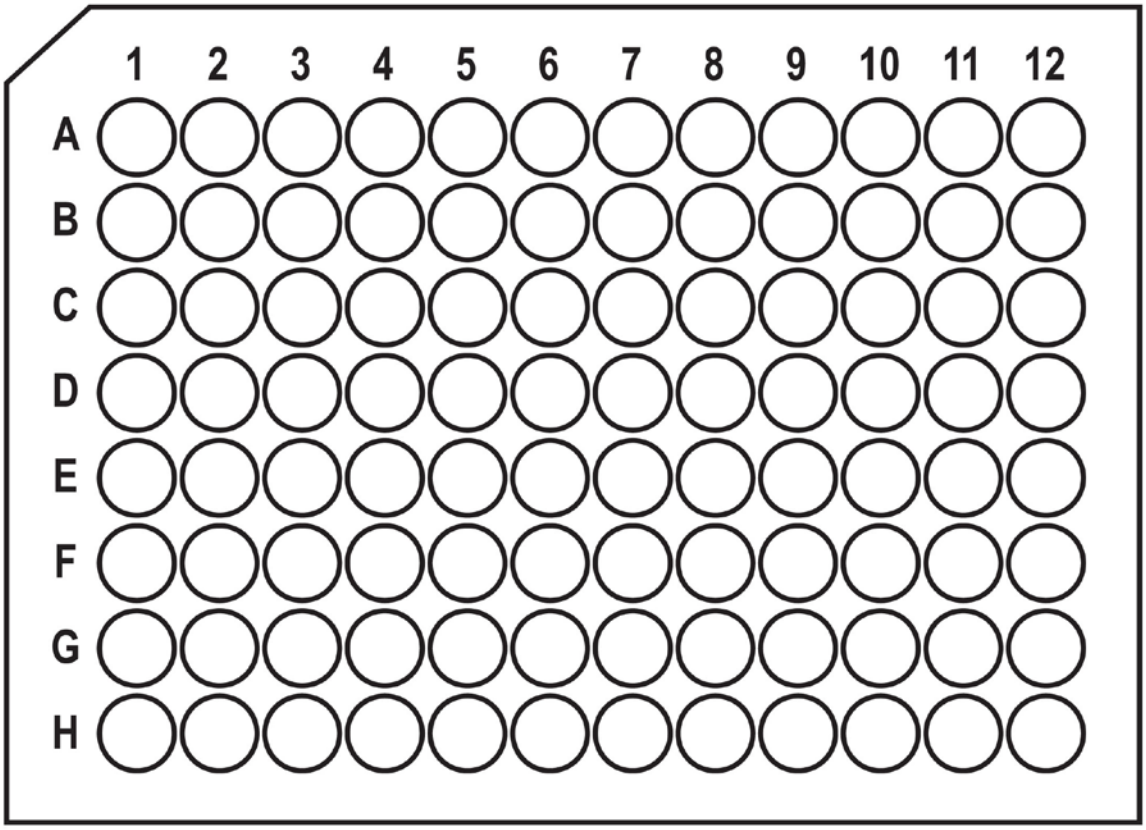
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