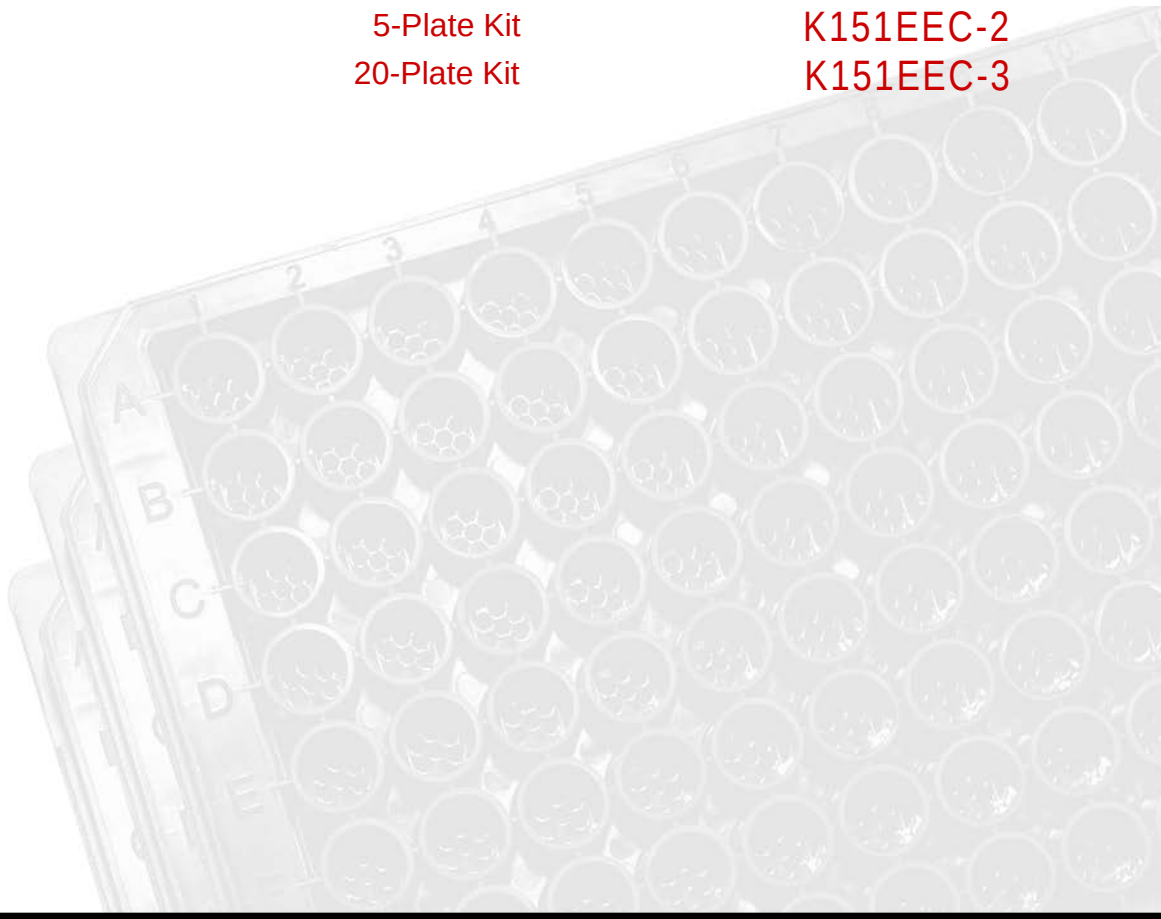
Meso Scale Discovery®

MULTI-ARRAY® Assay System

Human Myeloperoxidase (MPO) Assay Kit

1-Plate Kit	K151EEC-1
5-Plate Kit	K151EEC-2
20-Plate Kit	K151EEC-3



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MSD Cardiac Assays

Human Myeloperoxidase (MPO) Assay

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

MSD Customer Service

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MSD Scientific Support

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Introduction

introduction

Myeloperoxidase (MPO) is a member of the heme protein superfamily that is abundantly present in azurophilic granules of polymorphonuclear neutrophils (1,2). It is a 140 kDa tetramer, consisting of two alpha chains of 60 kDa and two beta chains of 14 kDa. It is produced by neutrophils and monocytes in response to leukocyte activation. These phagocytic cells undergo respiratory burst on stimulation. The extra oxygen that is consumed during this process is converted to hydrogen peroxide (3). MPO released from the azurophilic granules of neutrophils and monocytes react with this hydrogen peroxide to form potent oxidizing complexes. One such molecule is chloride which gets oxidized to hypochlorous acid, and later to chlorine and chloramines. These oxidants of the MPO-H_2O_2-halide system have potent bactericidal and viricidal activities (4). Thus, MPO plays an important role in many inflammatory reactions and contributes to innate host responses (2).

MPO is the product of a single gene which is 11 kb in size and is composed of 11 introns and 12 exons (5-7). This gene is located on chromosome 17 (8). It gets translated to an 80-kD protein (9), which undergoes proteolytic cleavage and N-linked glycosylation to generate an approximately 90 kDa inactive apopro-MPO (10). This apopro-MPO becomes active after the addition of heme.

Myeloperoxidase and its oxidation products have been found in atherosclerotic lesions (11). High levels of MPO have been demonstrated to predict angiographic coronary artery disease (12). MPO has been implicated in the oxidative modification of HDL with consequent functional inactivation (13). Thus MPO is one of the most promising cardiac markers to identify patients with increased risk of cardiovascular events. There is a suggested role of the MPO system in carcinogenesis by the conversion of several procarcinogens to their carcinogenic form (14). MPO has also been implicated in the pathogenesis of renal disease, lung injury, multiple sclerosis, Alzheimer's disease, brain infarction and Parkinson's disease (15-20).

Principle of the Assay

principle of the assay

MSD® cardiac 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 Myeloperoxidase Assay detects MPO in a sandwich immunoassay format (Figure 1). MSD provides a plate that has been pre-coated with MPO antibody. The user adds the sample and a solution containing the labeled detection antibody— Anti-MPO labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. MPO in the sample binds to capture antibodies 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 MPO present in the sample.

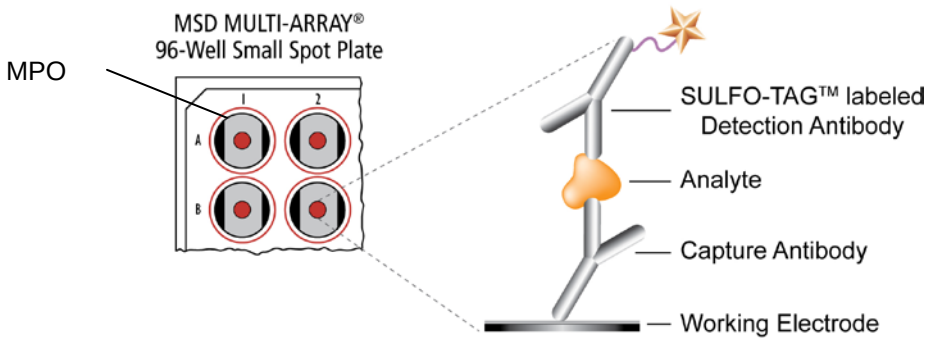


Figure 1. Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K151EEC-1	K151EEC-2	K151EEC-3
MULTI-ARRAY 96-well Human Myeloperoxidase (MPO) Plate L451EEA-1	2–8°C	1 plate	5 plates	20 plates
SULFO-TAG™ Anti-hMPO Detection Antibody[1] (100X)	2–8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
Human Myeloperoxidase (MPO) Calibrator[2] (1 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	20 vials (15 µL ea)
Diluent 27 R50OA-3 (30 mL) R50OA-2 (150 mL)	≤-10°C	1 bottle (30 mL)	1 bottle (150 mL)	4 bottles (150 mL ea)
Blocker A Kit R93AA-2 (250 mL) R93AA-1 (1000 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	1 bottle (1000 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 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.

[2] The Calibrator in this kit is derived from human source material which has been tested and found to be negative for HIV-1 and 2, Hepatitis B, and Hepatitis C. This material should be handled and disposed of in accordance with local, state, and federal guidelines.

VI Reagent Preparation

reagent preparation

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

> Important: Upon first thaw, separate Diluent 27 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 Kit

Follow instructions included with the Blocker A Kit.

Prepare Calibrator and Control Solutions

Calibrator for the Human MPO Assay is supplied at 20-fold higher concentration than the recommended highest Calibrator. MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates of each point. Each well requires 10 µL of Calibrator. For the assay, MSD recommends 3-fold serial dilution steps and Diluent 27 alone for the 8th point. The table below shows the concentrations of the 8-point standard curve:

Standard	Human MPO Calibrator (pg/mL)	Dilution Factor
20X Stock	1000000	
STD-01	50000	20
STD-02	16667	3
STD-03	5556	3
STD-04	1852	3
STD-05	617	3
STD-06	206	3
STD-07	69	3
STD-08	0	n/a

To prepare this 8-point standard curve for up to 3 replicates:

1) Prepare 200 µL of the highest Calibrator by adding 10 µL of the Calibrator stock vial to 190 µL of Diluent 27.
2) Prepare the next Calibrator by transferring 25 µL of the diluted Calibrator to 50 µL of Diluent 27. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 50 µL of Diluent 27 to be used as the 8th (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. *Dilute samples 1:20 in Diluent 27.* For example, add 10 µL of sample to 190 µL of Diluent 27 and mix thoroughly. Each replicate will require 10 µL of diluted sample.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 100X stock of Anti-human MPO Antibody. 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 solution into 2.97 mL of Diluent 27.

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

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 at room temperature.
2. Wash and Addition of Sample or Calibrator: Wash the plate 3 times with PBS-T. Dispense 40 µL/well of Diluent 27. Immediately dispense 10 µL of sample or Calibrator into separate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
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.

Notes

Solutions containing MSD Blocker A should be stored at 4°C and discarded after 14 days.

Plates may also be blocked overnight at 4°C.

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

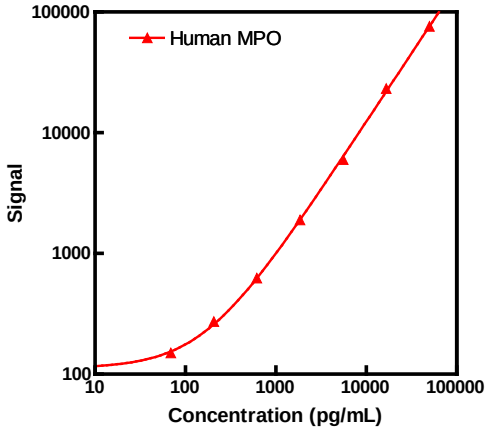
analysis of results

The Calibrator 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.



MPO		
Conc. (pg/mL)	Average Signal	%CV
0	76	6.5
69	150	7.4
206	273	5.1
617	624	4.4
1852	1895	3.6
5556	5989	5.0
16667	23209	3.7
50000	76089	7.7

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 values below represent the average LLOD over multiple kit lots.

	MPO
LLOD (pg/mL)	33

XI Spike Recovery

spike recovery

Four serum samples were spiked with Calibrator at multiple values throughout the range of the assay. Samples were diluted 1:20 in Diluent 27 prior to measurement. Each spike was done in ≥ 3 replicates.
% Recovery = measured / expected x 100

Sample	Spike Conc. (ng/mL)	% Recovery
Serum 1	50	99
	250	99
	500	96
Serum 2	50	96
	250	94
	500	105
Serum 3	50	96
	250	99
	500	106
Serum 4	50	115
	250	96
	500	104

XII Linearity

linearity

Three individual samples of human serum were evaluated and the %recovery is shown below. The samples were diluted 1:20 with Diluent 27 prior to testing for linearity. 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	% Recovery
Serum 1	2	98
	4	102
	8	106
Serum 2	2	100
	4	103
	8	94
Serum 3	2	90
	4	97
	8	89

XIII Samples

samples

Human serum and plasma samples from 20 normal individuals were measured in the Human MPO assay. Median levels and range of concentration are displayed in the table below.

.		MPO (ng/mL)
Serum	Mean	64
	Median	57
	Range	20 – 150
EDTA Plasma	Mean	41
	Median	32
	Range	13 - 127
Heparin Plasma	Mean	104
	Median	99
	Range	25 - 197

XV Assay Components

Assay components

The human MPO capture and detection antibodies used in this assay are listed below.

	Source species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
hMPO	Mouse monoclonal	Rabbit polyclonal

XIV References

references

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Summary Protocol

MSD 96-well MULTI-ARRAY Human MPO Assay

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

Step 1: Sample and Reagent Preparation

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

Serum and plasma samples should be diluted 20-fold in Diluent 27.

Prepare an 8-point standard curve using supplied calibrator and conducting 3-fold dilution in Diluent 27. Use Diluent 27 as zero calibrator blank.

Prepare Detection Antibody Solution by diluting the 100X Anti-hMPO Antibody to 1X in a final volume of 3.0 mL of Diluent 27 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 MSD Blocker A Solution.

Incubate at room temperature for 1 hour.

Step 3: Wash and Add Sample or Calibrator

Wash plate 3 times with PBS-T.

Dispense 40 µL/well Diluent 27.

Immediately dispense 10 µL/well Calibrator or sample.

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

Step 4: Wash and Add Detection Antibody Solution

Wash plate 3 times with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

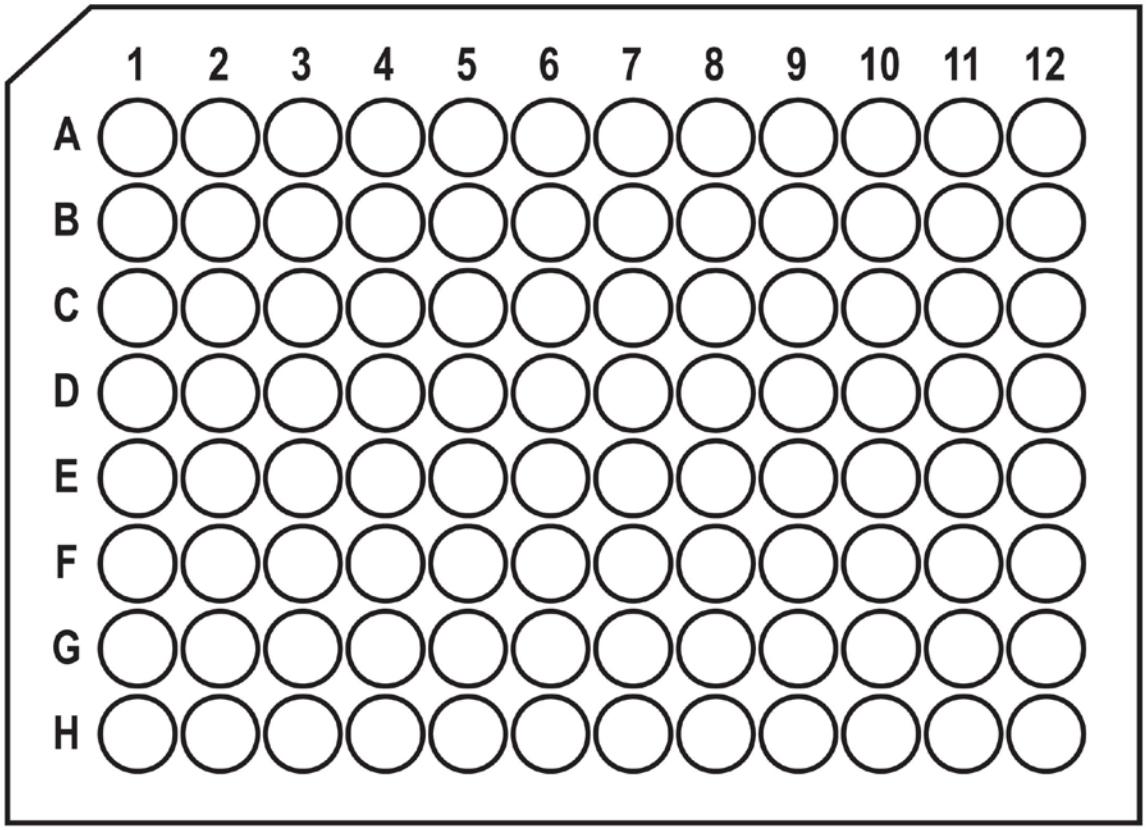
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 1 hour.

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 Imager instrument.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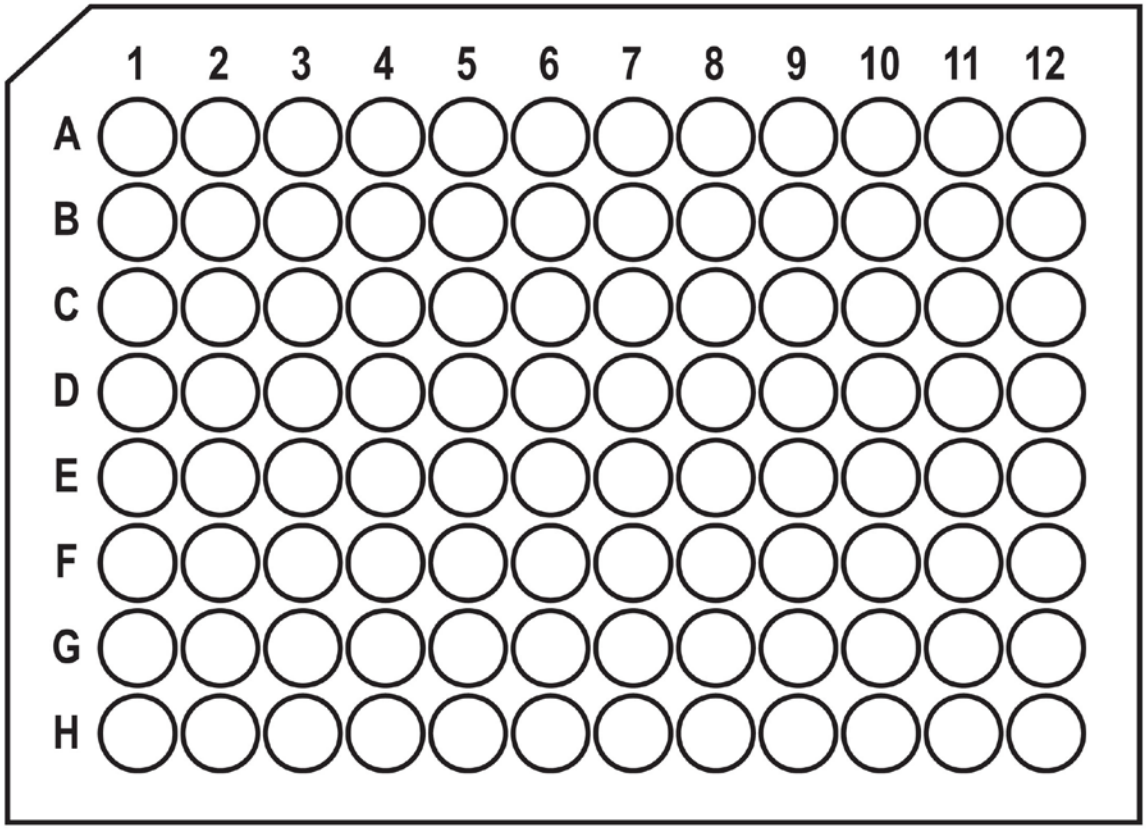
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