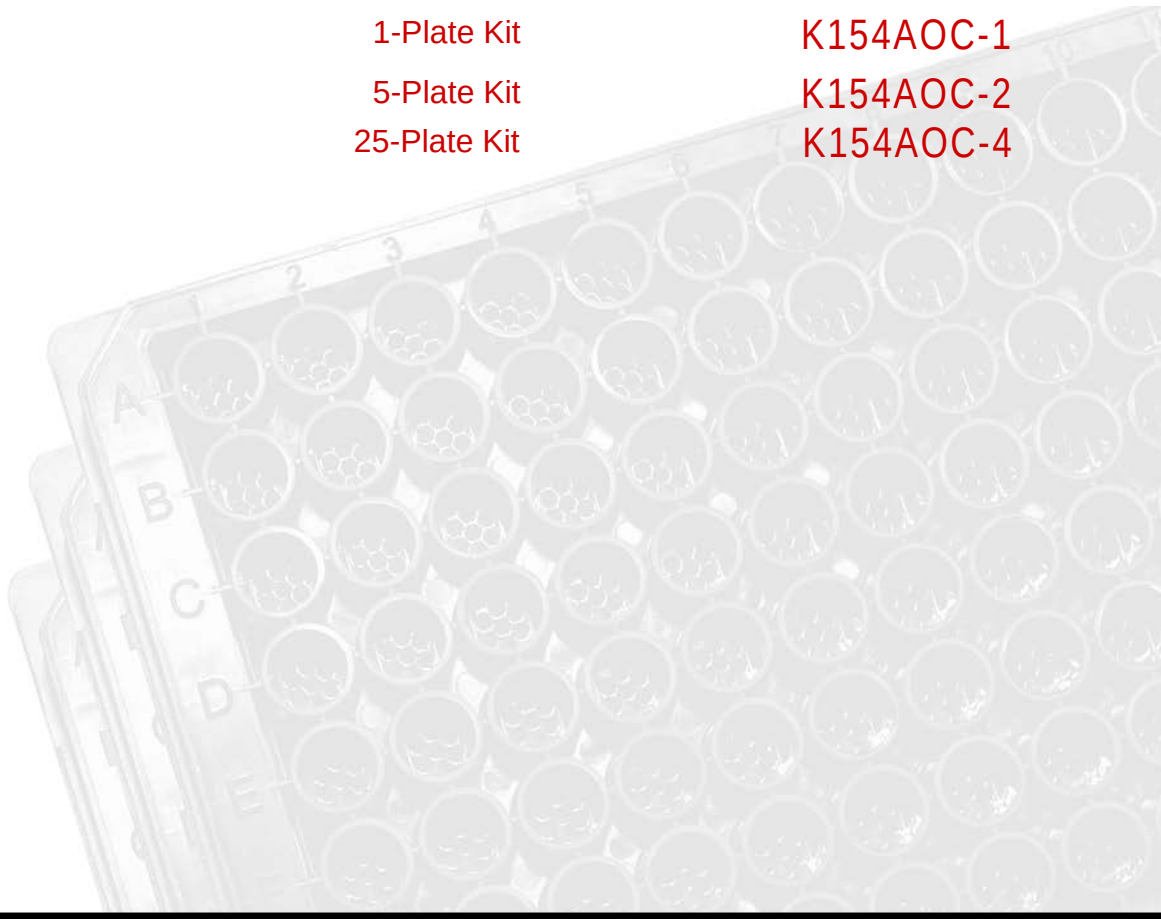
MESO SCALE DISCOVERY®

MULTI-ARRAY Assay System

Canine IL-10 Assay
Ultra-Sensitive Kit

1-Plate Kit	K154AOC-1
5-Plate Kit	K154AOC-2
25-Plate Kit	K154AOC-4



MSD MULTI-ARRAY Assay

Ultra-Sensitive Kit

Canine IL-10 Assay

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
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Ordering Information

Ordering information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-240-314-2798
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Email: ScientificSupport@mesoscale.com

MSD Advantage

MSD advantage

MESO SCALE DISCOVERY'S MULTI-ARRAY® Technology is a multiplex immunoassay system that enables the measurement of biomarkers utilizing the next generation of electrochemiluminescent detection. In an MSD® assay, specific Capture Antibodies for the analytes are coated in arrays in each well of a 96-well carbon electrode plate surface. The detection system uses patented SULFO-TAG™ labels, which emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT® plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable and are non-radioactive. Additionally, only labels near the electrode surface are detected, enabling non-washed assays.

One of the advantages of MSD assays is the minimal sample volume required as compared to a traditional ELISA, which is also limited by its inability to measure more than a single analyte. With an MSD assay, ten different biomarkers can be analyzed simultaneously using as little as 10-25 µL of sample. These assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. Further, the simple and rapid protocols of MSD assays provide a powerful tool to generate reproducible and reliable results. The MSD product line offers a diverse menu of assay kits for profiling biomarkers, cell signaling pathways, and other applications, as well as a variety of plates and reagents for assay development.

Introduction

introduction

Interleukin (IL)-10 is a regulatory cytokine belonging to the IL-10 family of cytokines. Other key members of this family include IL-19, IL-20, IL-22, IL-24 and IL-26.[1] IL-10 was originally described as Cytokine Synthesis Inhibitory Factor (CSIF) due to its ability to inhibit cytokine production by Th1 clones. It is an anti-inflammatory cytokine, capable of inhibiting synthesis of pro-inflammatory cytokines like IFN-γ, IL-2, IL-3, TNF-α and GM-CSF.[2-4] It also down-regulates the expression of MHC class II antigens and can suppress antigen presentation capacity.[5] It enhances B cell survival, proliferation, and antibody production.[6] IL-10 can block NF-κB activity and is involved in the regulation of the JAK-STAT signaling pathway.[7,8] IL-10 is also a co-stimulator for the growth of mature and immature thymocytes.[9]

IL-10 is produced primarily by monocytes and to a lesser extent by lymphocytes. Human IL-10 is an 18.5 kDa protein containing 160 amino acids and appears to function as a homodimer.[10] It shows 73 percent homology with murine IL-10, which is a 35kDa homodimeric protein and differs from the human IL-10 due to glycosylation at the N-terminus. Both mouse and human IL-10 contain intrachain disulfide bonds that are essential to the biological function of IL-10.[11] The canine homologue of IL-10 has also been cloned. It shares 80% amino acid sequence homology with human IL-10 and 72% sequence homology with mouse IL-10.[12] Additionally, there are several viral homologs of IL-10 such as Epstein-Barr virus, herpes virus type 2, cytomegalovirus, etc.[13,14]

IL-10 has been shown to play an important role in many immune-related diseases. Recent research suggests that due to the anti-inflammatory properties of IL-10, it has a favorable impact on inflammatory diseases such as multiple sclerosis.[15,16] IL-10 transgenic mice have been shown to be resistant to the development of multiple sclerosis.[17] IL-10 has been useful in the treatment of Crohn's disease.[18] IL-10 may also be useful for the development of new therapeutic strategies for some cancers since it is produced in tumors such as melanoma, ovarian carcinoma and B-cell lymphoma.[19-21]

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. The antibody for a specific protein target is coated on one electrode (or "spot") per well. The Canine IL-10 Assay detects IL-10 in a sandwich immunoassay format (Figure 1). For this assay, MSD provides a Canine IL-10 Ultra-Sensitive Plate that has been pre-coated with the capture antibody on a spatially distinct spot. The position of IL-10 capture antibody is indicated in Figure 1 and on the plate packaging. The user adds the sample and a solution containing the labeled detection antibody—anti-IL-10 labeled with an electrochemiluminescent compound, MSD SULFO-TAG—over the course of one or more incubation periods. IL-10 in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound IL-10 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 instrument for analysis. Inside the 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 IL-10 present in the sample.

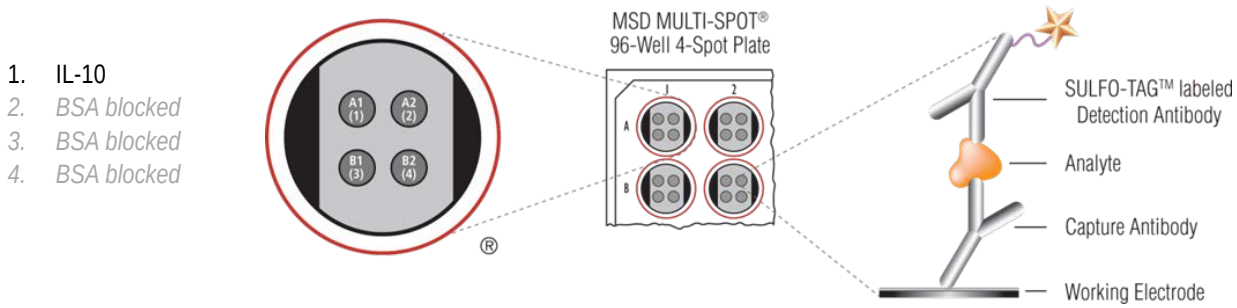


Figure 1. Spot diagram showing placement of analyte capture antibody on Canine IL-10 Ultra-Sensitive Plate. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

IV Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K154AOC-1	K154AOC-2	K154AOC-4
MULTI-SPOT 96-well, 4-Spot Canine IL-10 Ultra-Sensitive Plate(s) N45038A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-canine IL-10 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Canine IL-10 Calibrator (5 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Blocker D-M (2%)	≤-10°C	1 vial (0.2 mL)	1 vial (0.9 mL)	5 vials (0.9 mL ea)
Blocker D-G (10%)	≤-10°C	1 vial (0.05 mL)	1 vial (0.2 mL)	1 vial (1 mL)
Diluent 2 R51BB-4 (8 mL) R51BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 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)	2 bottles (200 mL ea)

V 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

VI 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.

VII Reagent Preparation

reagent preparation

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

> Important: Upon first thaw, separate Diluent 2 and Diluent 3 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 2 alone for the 8th point:

Standard	Canine IL-10 Calibrator (pg/mL)	Dilution Factor
100X Stock	5 000 000	
STD-01	50 000	100
STD-02	12 500	4
STD-03	3125	4
STD-04	781	4
STD-05	195	4
STD-06	49	4
STD-07	12	4
STD-08	0	n/a

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

1) Prepare the highest Calibrator point (STD-01) by transferring 10 µL of the Canine IL-10 stock Calibrator to 990 µL Diluent 2.
2) Prepare the next Calibrator by transferring 50 µL of the diluted Calibrator to 150 µL of Diluent 2. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 2 (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. Normal serum and plasma samples may not require a dilution prior to being used in the MSD Canine IL-10 Assay. Serum or plasma with high levels of these analytes may require 2-fold dilution in Diluent 2.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Canine IL-10 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. Samples from experimental conditions with extremely high levels of cytokines may require a dilution.

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 as a 50X stock solution. The working Detection Antibody Solution should contain 1X as final concentration of the antibody and 0.1% of each blocker.

In a 15 mL tube combine (per plate):

- ❑ 60 µL of 50X SULFO-TAG Anti-canine IL-10 Antibody
- ❑ 30 µL of Blocker D-G (10%)
- ❑ 150 µL of Blocker D-M (2%)
- ❑ 2760 µL of Diluent 100

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.

VIII Assay Protocol

assay protocol

1. Addition of Diluent 2: Dispense 25 µL of Diluent 2 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 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 2 hours 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 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the MSD instrument. 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.

IX Analysis of Results

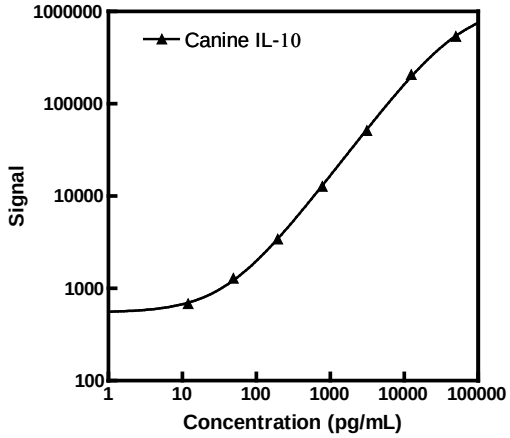
analysis of results

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 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.

X 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 quantification of unknown samples.



IL-10		
Conc. (pg/mL)	Average Signal	%CV
0	591	2.0
12	683	11.6
49	1286	2.3
195	3421	2.8
781	12 724	4.0
3125	51 361	2.4
12 500	207 666	3.8
50 000	537 050	4.9

XI 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.

	IL-10
LLOD (pg/mL)	5.9

XII Spike Recovery

spike recovery

Beagle and Mongrel serum and plasma samples were spiked with Calibrator at multiple values in the linear range of the assay. Each spike was done in ≥ 3 replicates.
% Recovery = measured / expected x 100

Sample	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Beagle Serum	0	3	14	
	308	313	5	101
	1237	1305	3	105
	5249	5564	1	106
Beagle Plasma	0	5	52	
	308	287	5	92
	1237	1156	5	93
	5249	4459	3	85
Mongrel Serum	0	4	20	
	308	244	5	78
	1237	943	4	76
	5249	3366	21	64
Mongrel Plasma	0	13	23	
	308	264	9	82
	1237	1127	0	90
	5249	4351	1	83

XIII Linearity

linearity

Dilution linearity was tested by spiking calibrators in different matrices including beagle serum, beagle plasma, mongrel serum and mongrel plasma and then conducting 2-fold dilutions.
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
Beagle Serum	1	5692	1	
	2	5413	5	95
	4	5431	2	100
	8	5291	3	97
Beagle Plasma	1	5663	2	
	2	5466	5	97
	4	5499	1	101
	8	5066	4	92
Mongrel Serum	1	5619	0	
	2	5468	4	97
	4	5222	1	95
	8	5167	3	99
Mongrel Plasma	1	5827	2	
	2	5222	5	90
	4	5467	1	105
	8	4964	3	91

XIV Samples

samples

Serum and plasma samples from Beagle and serum from Mongrel were measured and the endogenous levels were found to be below LLOD.
The endogenous level in Mongrel plasma was measured to be 10 pg/mL.

XV References

references

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Summary Protocol

MSD 96-well MULTI-ARRAY Canine IL-10 Assay: Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Canine IL-10 Assay.

Step 1 : Sample and Reagent Preparation

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

Samples may not require dilution prior to use in this assay.

Prepare Calibrator solutions and standard curve.

Use the 5 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 2.

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

Prepare Detection Antibody Solution by diluting Detection Antibody to 1X, Blocker D-M to 0.1% and Blocker D-G to 0.1% in a final volume of 3.0 mL of Diluent 100 per plate.

Prepare 20 mL of 2X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 2: Add Diluent 2

Dispense 25 µL/well Diluent 2.

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 3 times with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

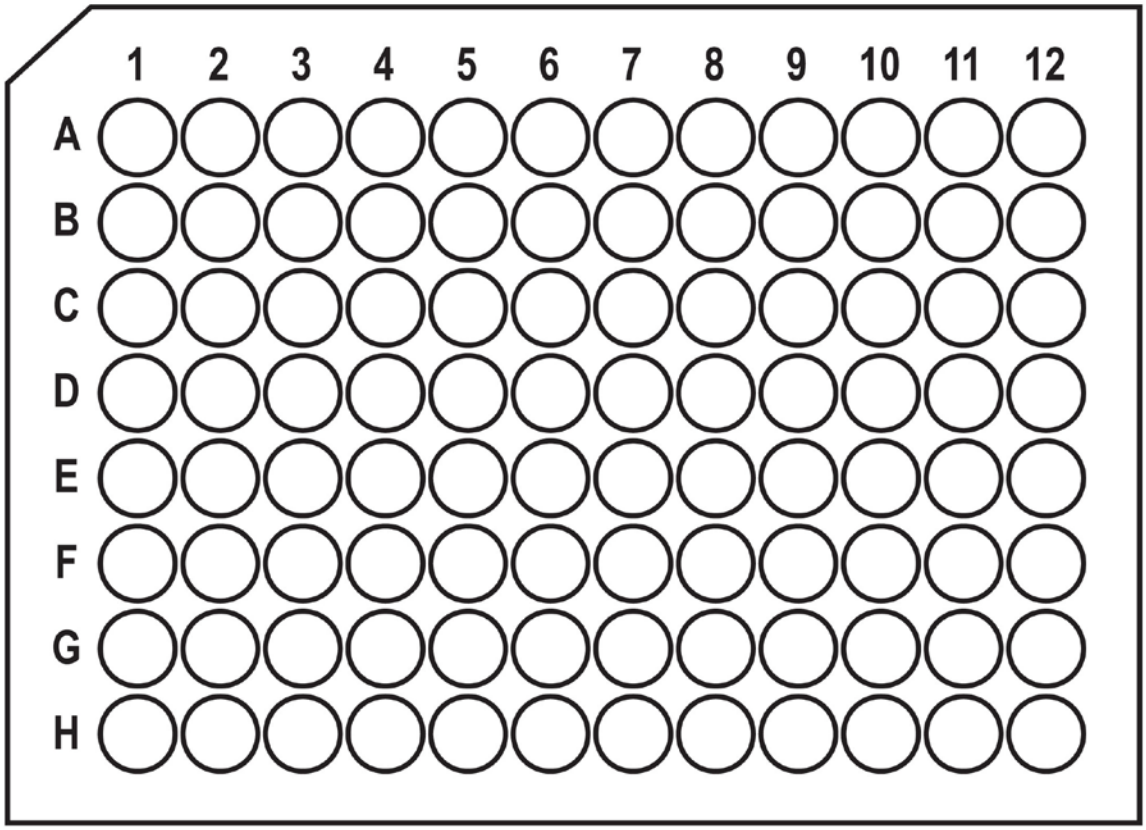
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

Step 5: Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on MSD instrument.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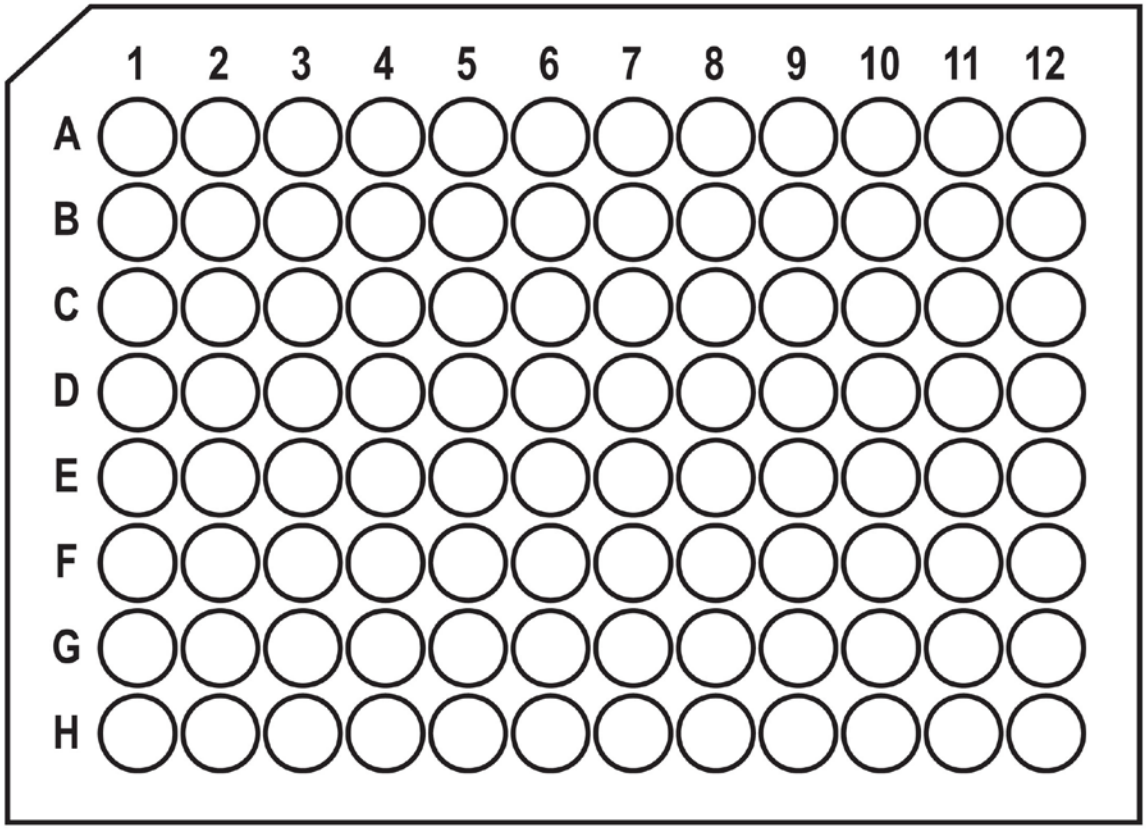
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