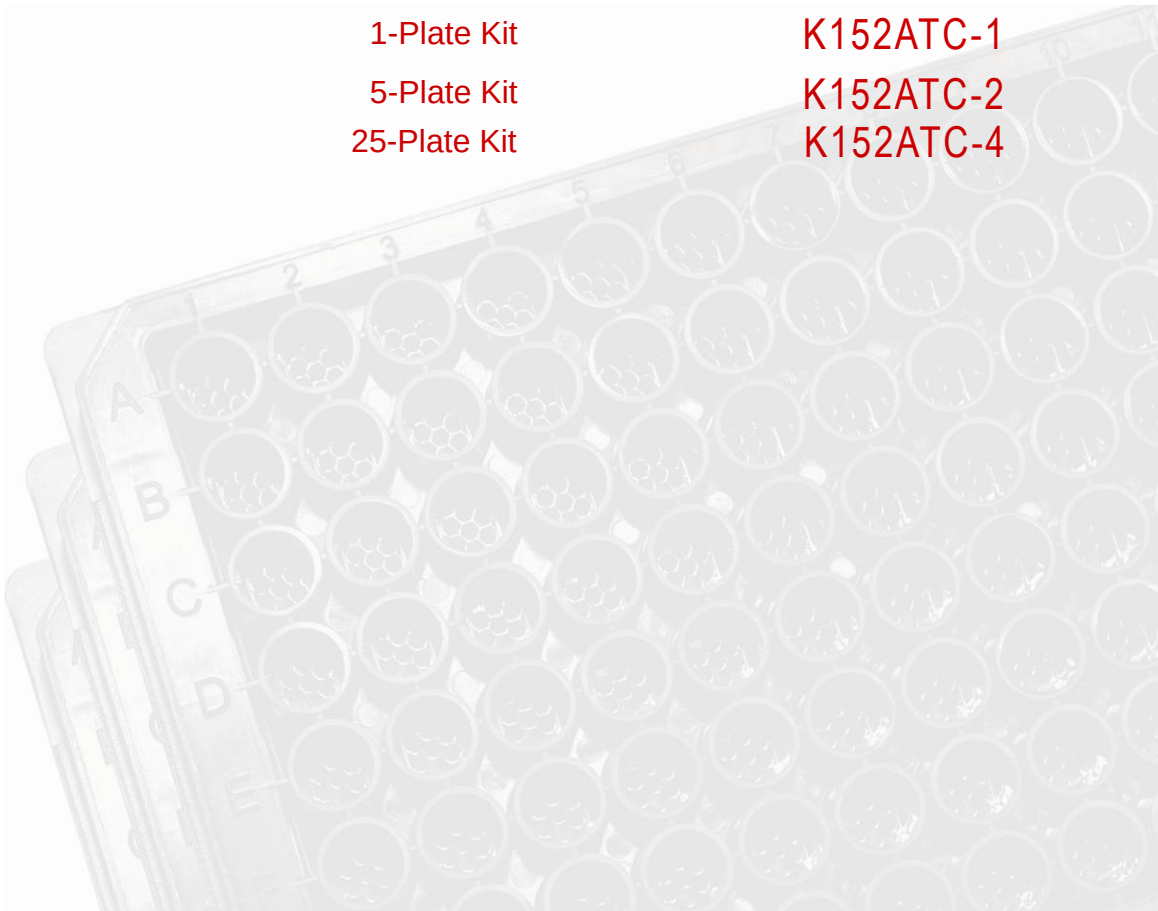
Meso Scale Discovery®

MULTI-ARRAY® Assay System

Mouse IL-17 Assay
Ultra-Sensitive Kit

1-Plate Kit	K152ATC-1
5-Plate Kit	K152ATC-2
25-Plate Kit	K152ATC-4



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MSD MULTI-ARRAY Assay

Ultra-Sensitive Kit

Mouse IL-17 Assay

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Table of Contents

table of contents

Ordering Information

Ordering information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

Introduction

introduction

Interleukin-17 (IL-17) is produced in large amounts exclusively by T cells, [1,2,3] in particular the inflammatory T helper cells. IL-17 cDNA was first isolated from a subtracted cDNA library of a PMA+ionomycin-activated T cell hybridoma produced by fusion of a mouse cytotoxic T cell clone and a rat T cell lymphoma. [3]

A single copy of the IL-17 gene has been found in the human, mouse and rat genomes. Mouse IL-17 has been mapped to chromosome 1 and human IL-17 to chromosome 2.[3] Comparison between the IL-17 nucleotide sequences of different species reveals a conserved region of several AU-rich repeats in the 3' UTRs. [4]

Even though IL-17 secretion is restricted to specific subsets of T cells, the IL-17 receptor is expressed ubiquitously. IL-17 does not seem to have any direct effect on the growth of either tumor cell lines[5] or normal stromal cells.[1]

In vitro, IL-17 activates fibroblasts and macrophages to produce other proinflammatory mediators such as TNF-α, IL-1β, IL-6, IL-8, GM-CSF, G-CSF and SCF. [1,6] A number of these cytokines have a significant impact on hematopoietic response[6,7] including the stimulation of neutrophil proliferation and differentiation [8,9] as well as being involved in inflammatory airway diseases within the lungs of asthmatic individuals. [10,11,12] IL-17 has been shown to be important in many other inflammatory diseases including systemic lupus erythematosus, rheumatoid arthritis,[13,14,15] multiple sclerosis,[16] and inflammatory skin diseases.[17]

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 Mouse IL-17 Assay detects IL-17 in a sandwich immunoassay format (Figure 1). For this assay, MSD provides a Cytokine Panel 15 plate that has been pre-coated with capture antibodies on spatially distinct spots. The position of IL-17 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-17 labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. IL-17 in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound IL-17 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 IL-17 present in the sample.

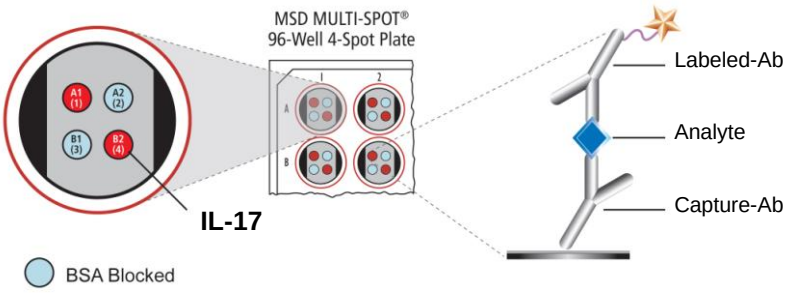


Figure 1. *Spot diagram showing placement of analyte capture antibody on Cytokine Panel 15 Plate. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody 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 K152ATC-1	K152ATC-2	K152ATC-4
MULTI-SPOT® 96-well 4 Spot Cytokine Panel 15 Plate(s) N45037B-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-mIL-17 Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Mouse IL-17 Calibrator (2 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 4 R52BB-4 (8 mL) R52BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL 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)
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 4 and Diluent 5 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 calibration 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 4 alone for the 8th point:

Standard	IL-17 (pg/mL)	Dilution Factor
100X Stock	2000000	
STD-01	20000	100
STD-02	5000	4
STD-03	1250	4
STD-04	313	4
STD-05	78	4
STD-06	20	4
STD-07	4.9	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 Mouse IL-17 Calibrator Blend to 990 µL Diluent 4.
2) Prepare the next Calibrator by transferring 50 µL of the diluted Calibrator to 150 µL of Diluent 4. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th Standard is Diluent 4 (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 run neat in the MSD Mouse IL-17 Assay.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Mouse IL-17 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 at 50X stock of Anti-mIL-17 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-mIL-17 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 4:** Dispense 25 µL of Diluent 4 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

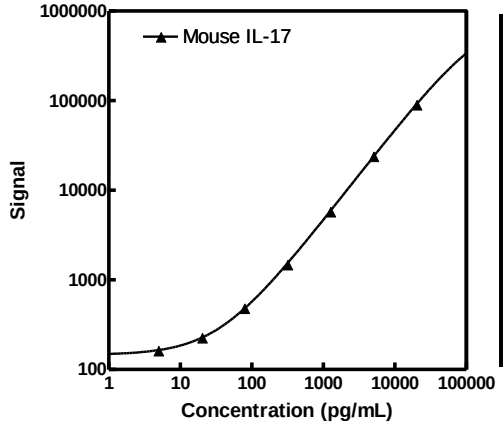
analysis of results

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.



IL-17		
Conc. (pg/mL)	**Average Signal**	**%CV**
0	143	6.4
4.9	163	6.9
20	227	2.9
78	486	4.3
313	1484	1.8
1250	5799	1.2
5000	24042	2.7
20000	90452	7.8

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.

	IL-17
LLOD (pg/mL)	5.5

XI Spike Recovery

spike recovery

Mouse serum, EDTA plasma and heparin plasma pooled samples were spiked with Calibrator at multiple values throughout the range of the assay. Each spike was done in ≥ 3 replicates. An average of two serum and three EDTA 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	0	8.7	
	78	84	3.4	108
	313	336	2.5	107
	1250	1213	1.3	97
EDTA Plasma	0	6	11.5	
	78	95	2.3	113
	313	338	4.1	106
	1250	1286	1.1	102
Heparin Plasma	0	6	9.3	
	78	102	5.6	121
	313	352	2.6	110
	1250	1266	1.8	101

XII Linearity

linearity

Three pools each of mouse serum and heparin plasma were evaluated; a representative pool of each is shown below. The pooled samples were spiked with Calibrator and then diluted with Diluent 4. 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	1	1084	2.1	
	2	1143	3.4	105
	4	1191	2.4	104
	8	1200	0.9	101
Heparin Plasma	1	896	2.0	
	2	1074	2.0	120
	4	1110	8.0	103
	8	1106	3.5	100

XIII Samples

samples

Eight normal mouse serum samples were measured.

		IL-17 (pg/mL)
Serum	Min	<LLOD
	Max	29
	Median	20

XIV References

references

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Summary Protocol

MSD 96-well MULTI-ARRAY Mouse IL-17 Assay: Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Mouse IL-17 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 2 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 4.

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 3.0 mL of Diluent 5 (per plate).

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

SERUM OR PLASMA SAMPLES

Step 2: Add Diluent 4

Dispense 25 µL/well Diluent 4.

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.

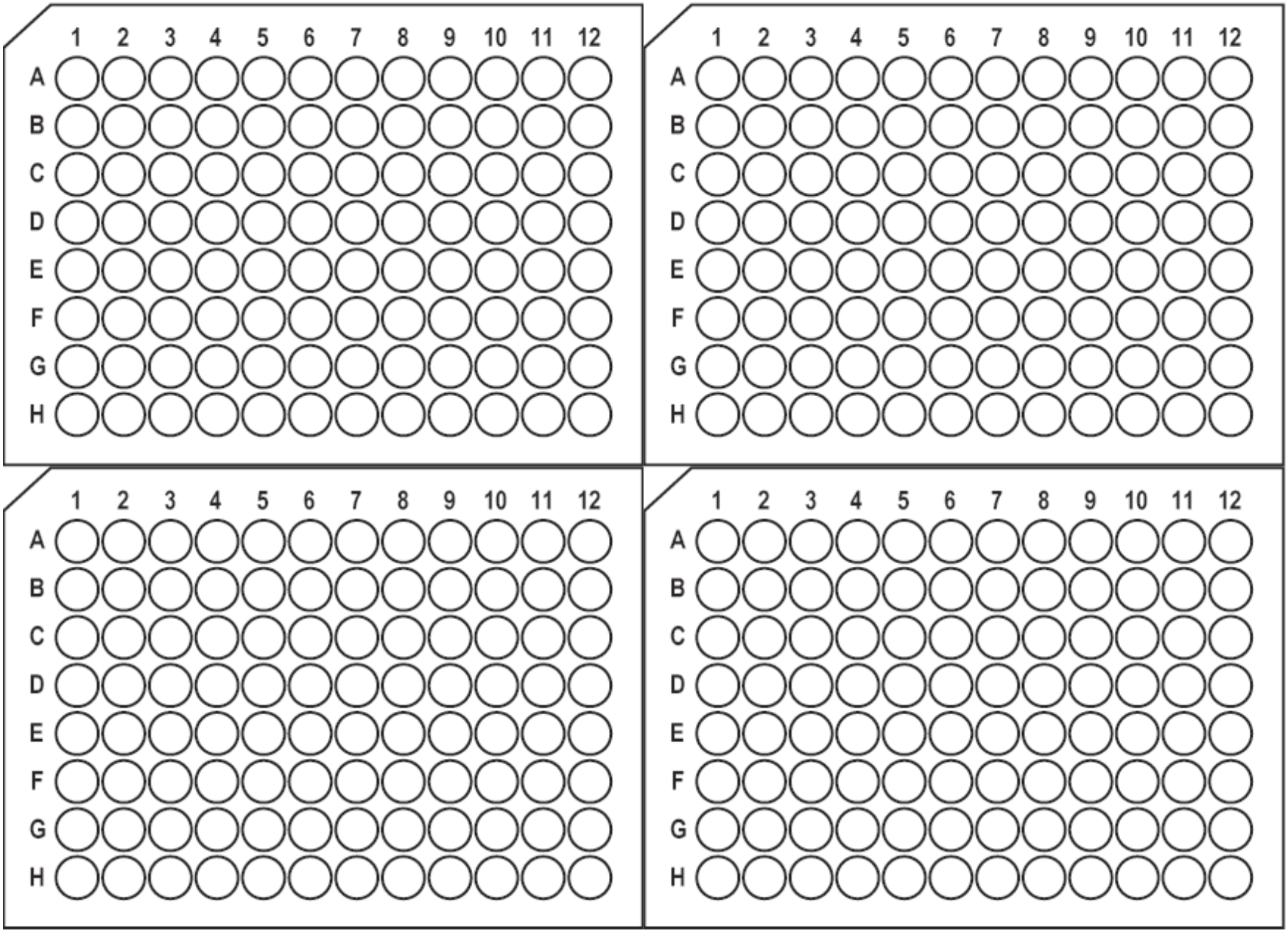
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 2 hours.

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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