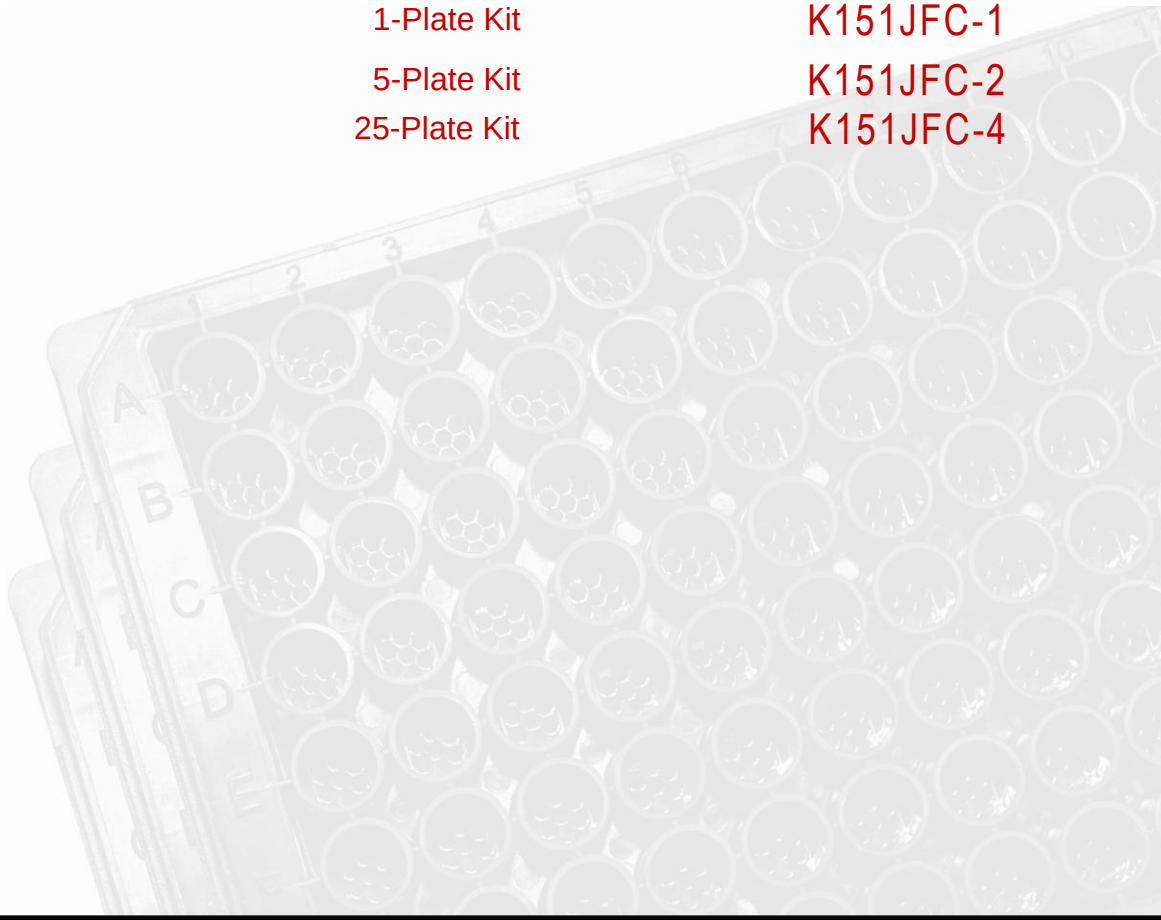
# Meso Scale Discovery®

## MULTI-ARRAY® Assay System

### Human TIMP-1 Assay Kit

| | |
|---|---|
| 1-Plate Kit | K151JFC-1 |
| 5-Plate Kit | K151JFC-2 |
| 25-Plate Kit | K151JFC-4 |



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# MSD Toxicology Assays

## Human TIMP-1 Assay Kit

*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



# Ordering Information

ordering information

## MSD Customer Service

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## Meso Scale Discovery

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# Introduction

introduction

**Tissue inhibitor of metalloproteinase 1 (TIMP-1)** is a glycoprotein that regulates the activation and inhibition of the matrix metalloproteinases (MMPs). The N-terminal site of TIMP-1 forms a non-covalent complex with the active site of the MMPs to inhibit active MMP-mediated proteolysis. TIMP-1 is widely present in many tissues and cell types. Transcription of this gene is inducible by many cytokines and hormones. Overexpression of TIMP-1 correlates with aggressive pathogenesis of a multitude of tumors [1] [2]. It has also been shown that plasma total TIMP-1 can be related to cardiovascular risk factors influencing cardiovascular remodeling via extracellular matrix degradation [3].

# Principle of the Assay

principle of the assay

MSD® toxicology 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 panel formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or "spot") per well. In a panel assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Human TIMP-1 Assay detects TIMP-1 in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with TIMP-1 antibody. The user adds the sample and a solution containing the labeled detection antibody—Anti-hu TIMP-1 labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. TIMP-1 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 TIMP-1 present in the sample.

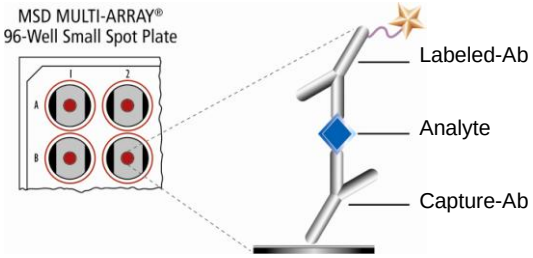



***Figure 1.*** *Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

# III Reagents Supplied

reagents supplied

| Product Description | Storage | Quantity per Kit K151JFC-1 | K151JFC-2 | K151JFC-4 |
|---|---|---|---|---|
| MULTI-ARRAY® 96-well Human TIMP-1 Plate<br>L451JFA-1 | 2–8°C | 1 plate | 5 plates | 25 plates |
| SULFO-TAG™ Anti-hu TIMP-1 Antibody (50X)[1] | 2–8°C | 1 vial<br>(75 µL) | 1 vial<br>(375 µL) | 5 vials<br>(375 µL ea) |
| Human TIMP-1 Calibrator (10X)<br>400 ng/mL | ≤ -70°C | 1 vial<br>(30 µL) | 5 vials<br>(30 µL ea) | 25 vials<br>(30 µL ea) |
| Diluent 2<br>R51BB-3 (40 mL)<br>R51BB-2 (200 mL) | ≤ -10°C | 1 bottle<br>(40 mL) | 1 bottle<br>(200 mL) | 5 bottles<br>(200 mL ea) |
| Diluent 3<br>R51BA-4 (5 mL)<br>R51BA-5 (25 mL) | ≤ -10°C | 1 bottle<br>(5 mL) | 1 bottle<br>(25 mL) | 5 bottles<br>(25 mL ea) |
| Read Buffer T (with surfactant), 4X<br>R92TC-3 (50 mL) | RT | 1 bottle<br>(50 mL) | 1 bottle<br>(50 mL) | 5 bottles<br>(50 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

[1] Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

# 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.

# VI Reagent Preparation

reagent preparation

Bring all reagents to room temperature and thaw Calibrator 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

Calibrator for the Human TIMP-1 Assay is supplied at 10-fold higher concentration than the recommended highest Calibrator. For the assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

| Standard | TIMP-1 (pg/mL) | Dilution Factor |
|---|---|---|
| Stock Cal.vial | 400000 | |
| STD-01 | 40000 | 10 |
| STD-02 | 13333 | 3 |
| STD-03 | 4444 | 3 |
| STD-04 | 1481 | 3 |
| STD-05 | 494 | 3 |
| STD-06 | 165 | 3 |
| STD-07 | 55 | 3 |
| STD-08 | 0 | n/a |

To prepare this 8-point standard curve:

1) Prepare the highest Calibrator by adding 20 µL of the Calibrator stock vial to 180 µL of Diluent 2 (10-fold dilution).
2) Prepare the next Calibrator by transferring 80 µL of the diluted Calibrator to 160 µL of Diluent 2. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 160 µL of Diluent 2 to be used as zero calibrator.

Calibrators should be prepared no more than 20 minutes before use.

## Dilution of Samples

Human serum and plasma samples should be diluted prior to the assay in order to get the analyte levels into the detection range. A 50 to 500-fold dilution into Diluent 2 is recommended for this assay. Depending on the sample set under investigation, higher or lower dilution factors may be necessary.

## Prepare Detection Antibody Solution

The Detection Antibody is provided as a 50X stock of Anti-hu TIMP-1 Antibody. The working Detection Antibody Solution should contain 1X as final concentration. For each plate used, dilute 60 µL of the stock Detection Antibody stock into a final volume of 3 mL of Diluent 3.

## Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of stock Read Buffer T (4X) to 10 mL of deionized water for each plate.

## Prepare MSD Plate

This plate has been pre-coated with antibody as 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 Diluent 2:** Dispense 25 µL of Diluent 2 into each well. Pipette to the bottom of the plate so as to allow the fluid to cover the entire bottom of the well. A slight tap may be necessary to allow the fluid to settle to the bottom. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (300–1000 rpm) at room temperature.
2. **Addition of Sample or Calibrator:** Dispense 25 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-ARRAY plate typically accelerates capture at the working electrode.*

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.

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

# VIII Analysis of Results

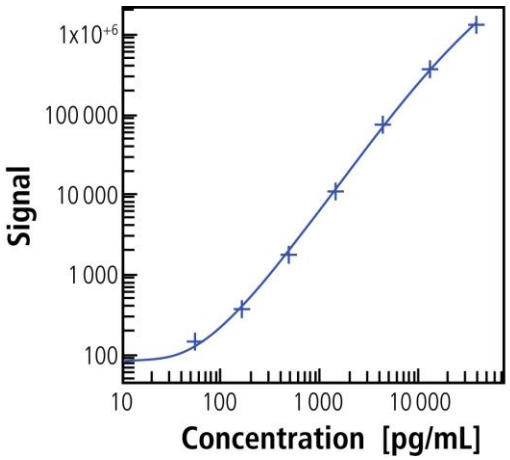
analysis of results

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 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 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.




| Conc. (pg/mL) | Average Signal | %CV |
|---|---|---|
| **0** | 73 | 11 |
| **55** | 149 | 4.1 |
| **165** | 381 | 4.7 |
| **494** | 1806 | 9.7 |
| **1481** | 11178 | 6.3 |
| **4444** | 74415 | 12 |
| **13333** | 365913 | 7.9 |
| **40000** | 1304070 | 8.8 |

# 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.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

| | TIMP-1 (pg/mL) |
|---|---|
| **LLOD** | 17.8 |
| **LLOQ** | 494 |
| **ULOQ** | 32000 |

# XI Precision

precision

A multi-day, multi-plate study over 9 plates was performed to show reproducibility and assay precision. Human serum samples of high, mid, and low levels of Human TIMP-1 were chosen to span the dynamic range of the assay and measured in triplicate on each plate. The average intra-plate %CV and inter-day %CV and the dilution-adjusted concentrations are shown in the table below.
The Controls were run at a 50-fold dilution.

| | Control | Plates | Average Conc. (pg/mL) | Average Intra-plate %CV | Interday %CV |
|---|---|---|---|---|---|
| **TIMP-1** | High | 9 | 808187 | 5.4 | 10 |
| | Mid | 9 | 125419 | 7.1 | 15 |
| | Low | 9 | 83317 | 11 | 14 |

# XII Spike Recovery

spike recovery

Human serum samples were spiked with the TIMP-1 calibrator at multiple values throughout the range of the assay. Spikes were tested at a 50-fold dilution into the assay diluent. A representative spike recovery in human serum and the corrected concentrations and % recovery are presented below.
% Recovery = measured / expected x 100

| | Spike Conc. (pg/mL) | Measured Conc. (pg/mL) | Measured Conc. %CV | % Recovery |
|---|---|---|---|---|
| Human Serum | 13333 | 753850 | 2.6 | 92 |
| | 4444 | 238550 | 5.7 | 97 |
| | 494 | 47900 | 3.5 | 97 |
| | 0 | 24750 | 4.5 | |

# XIII Linearity

linearity

Multiple human serum samples were assayed neat and diluted 100, 200, 400, 800, and 1600-fold in Human Serum Cytokine Assay Diluent to test linearity. A representative serum sample with high endogenous levels of TIMP-1 is shown below. 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 for the previous dilution (expected).
The value in italics is above the assay ULOQ.
% Recovery = (measured x dilution factor) / expected x 100

| | Fold Dilution | Conc. (pg/mL) | Conc. %CV | % Recovery |
|---|---|---|---|---|
| Human Serum | 1 | *971185* | 1.5 | |
| | 100 | 937500 | 1.2 | 97 |
| | 200 | 971000 | 1.3 | 104 |
| | 400 | 828400 | 4.3 | 85 |
| | 800 | 840000 | 7.0 | 101 |
| | 1600 | 822400 | 5.7 | 98 |

# XIV Samples

samples

Human serum samples from 10 normal individuals were measured in the Human TIMP-1 assay. Samples were run on multiple days. Median levels and range of concentration and %CV are displayed in the table below.

| | | Human TIMP-1 |
|---|---|---|
| Serum | Median (pg/ml) | 48792 |
| | Range (pg/ml) | 38302-69792 |
| | # of samples | 10 |

# XV Calibrator

calibrator

Recombinant human TIMP-1 was calibrated against an internal control and diluted to a final concentration of 400 ng/mL to make Human TIMP-1 Calibrator.

# XVI References

references


1. Wang CS, Wu TL, Tsao KC and Sun CF. Serum TIMP-in Gastric Cancer Patients: A Potential Prognostic Biomarker. Annals of Clinical & Laboratory Science 2006; 36:23-30
2. Guedez L, Martinez A, Zhao S, Vivero A, Pittaluga S, Stetler-Stevenson M, Raffeld M, Stetler-Stevenson WG. Tissue inhibitor of metalloproteinase 1 (TIMP-1) promotes plasmablastic differentiation of a Burkitt lymphoma cell line: implications in the pathogenesis of plasmacytic/plasmablastic tumors. Blood. 2005 Feb 15; 105(4): 1660-8
3. Sundström J, Evans JC, Benjamin EJ, Levy D, Larson MG, Sawyer DB, Siwik DA, Colucci WS, Wilson PWF and Vasan RS. Relations of plasma total TIMP-1 levels to caridiovascular risk factors and echocardiographic measures: the Framingham heart study. European Heart Journal 2004 25(17):1509-1516

*Summary Protocol*

# MSD 96-well MULTI-ARRAY Human TIMP-1 Assay Kit

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

**Step 1 : Sample and Reagent Preparation**

Serum and plasma samples should be diluted at least 50-fold into Diluent 2 prior to use in the assay.

Bring appropriate diluents and plates to room temperature and thaw Calibrators on ice.

Prepare an 8-point standard curve using supplied calibrator:

- Dilute the stock Calibrator 1:10 in Diluent 2
- Perform a series of 3-fold dilution steps and a zero calibrator blank.

Prepare Detection Antibody Solution by diluting the 50X Anti-hu TIMP-1 Antibody to 1X in 3.0 mL of Diluent 3 per plate.

Prepare 20 mL of 2X Read Buffer T by diluting 4X MSD Read Buffer T (with surfactant), 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 3X with PBS-T.

Dispense 25 µL/well 1X Detection Antibody Solution.

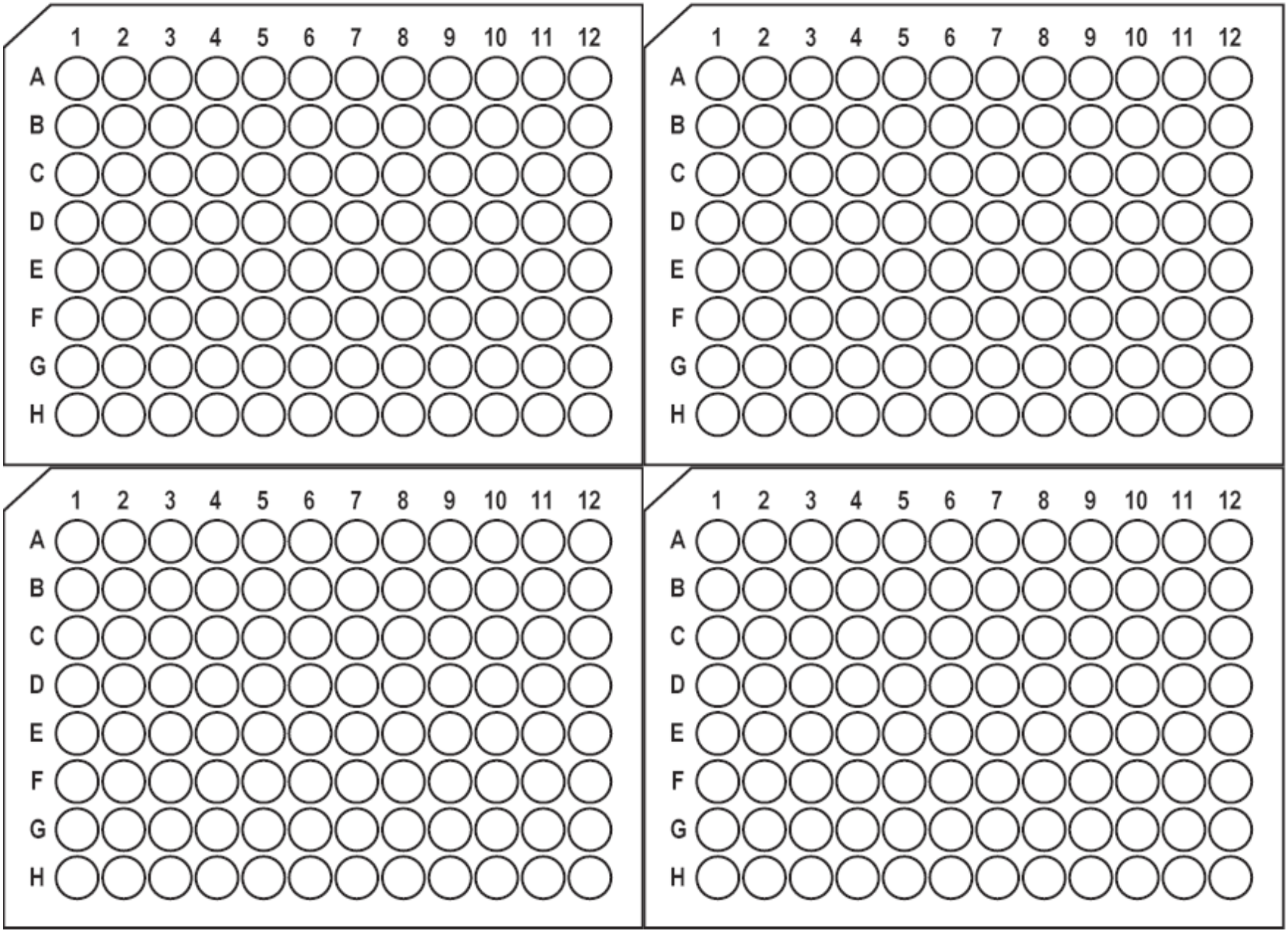
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

**Step 5 : Wash and Read Plate**

Wash plate 3X with PBS-T.

Dispense 150 µL/well 2X Read Buffer T.

Analyze plate on SECTOR instrument.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