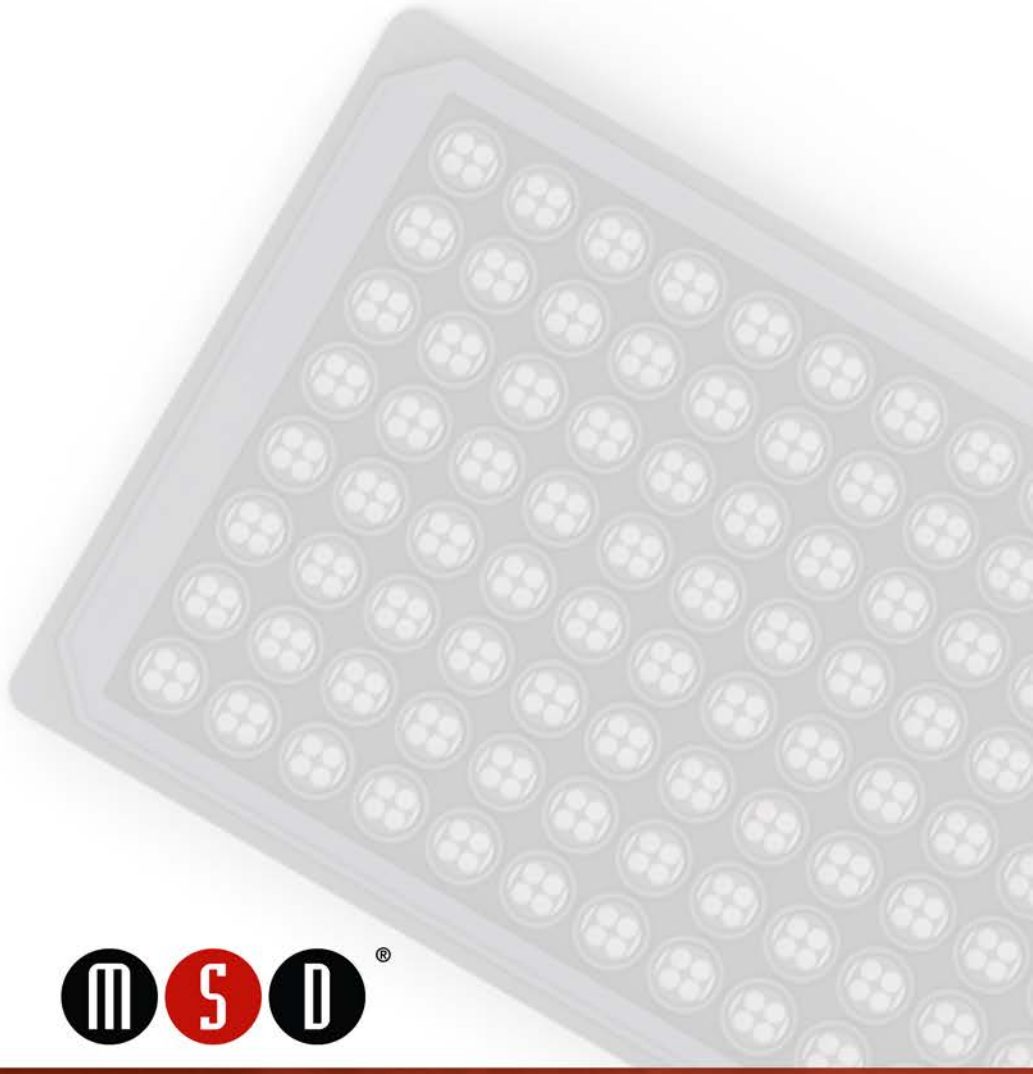
MSD® MULTI-ARRAY Assay System

Rat NT-proBNP Assay Kit

1-Plate Kit	K153JKD-1
5-Plate Kit	K153JKD-2
25-Plate Kit	K153JKD-4



MSD Toxicology Assays

Rat NT-proBNP Assay Kit

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

MSD Customer Service

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MSD Scientific Support

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MSD Advantage

MESO SCALE DISCOVERY'S unique spot patterns are a hallmark of our MULTI-ARRAY® technology, which 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 that 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, up to 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

N-terminal pro-brain (or B-type) natriuretic peptide (NT-proBNP) is produced predominately by the cardiac ventricular myocytes.[1] It is released in response to volume expansion and filling pressure and is involved in maintaining intravascular volume homeostasis.[2] In rats, the generation of NT-proBNP initially starts with the formation of an 121 amino acid (aa) prepro-BNP containing a 26 aa signal sequence. Proteolytic cleavage of the signal peptide releases pro-BNP, which contains 95 aa residues. Further proteolysis of pro-BNP generates a biologically inactive 50 aa NT-proBNP and an active 45 aa BNP molecule.[3]

Elevated plasma levels of BNP and NT-proBNP have been observed at times of cardiac stress and damage. Hence, they are widely used as a diagnostic tool for the occurrence and severity of heart failure and coronary syndrome.[4-6] Measurement of NP levels may help in risk stratification of patients suffering from heart attack in emergency care and in accurate and rapid diagnosis of heart failure in primary care.

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 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. The Rat NT-proBNP Assay is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibody for NT-proBNP. The user adds the sample and a solution containing the detection antibody—anti-NT-proBNP conjugated with an electrochemiluminescent compound, MSD SULFO-TAG label—over the course of one or more incubation periods. Analyte in the sample binds to the capture antibody immobilized on the working electrode surface; recruitment of the conjugated detection antibody by bound analytes 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® Imager for analysis. Inside the SECTOR Imager, 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 provide a quantitative measure of NT-proBNP present in the sample.

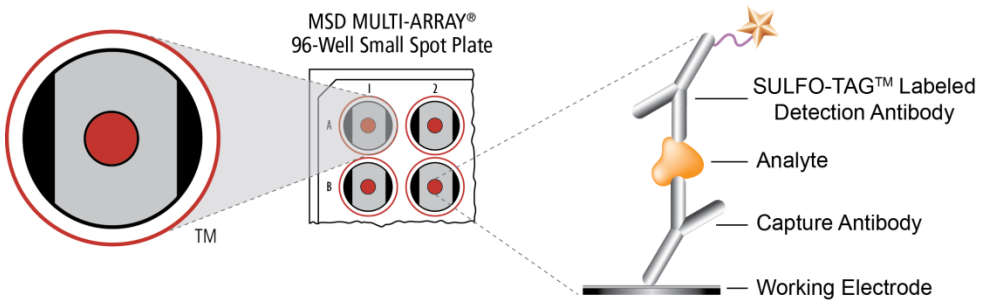


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K153JKD-1	K153JKD-2	K153JKD-4
MULTI-ARRAY 96-Well Small Spot Rat NT-proBNP Plate L453JKA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat NT-proBNP Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat NT-proBNP Calibrator (20X)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	25 vials (20 µL ea)
Diluent 34 R50AC-3 (25 mL), R50AC-6 (125 mL)	≤-10°C	1 bottle (25 mL)	1 bottle (125 mL)	5 bottles (125 mL ea)
Protease Inhibitor Solution (100X)	2–8°C	2 vials (0.5 mL ea)	1 vial (2 mL)	5 vials (2 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

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

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 conjugated detection antibodies may be light-sensitive, so they should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 34 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 Diluent 34 + Protease Inhibitor

For the NT-proBNP Assay, samples and calibrators are diluted in Diluent 34 that contains Protease Inhibitor. This additive must be added into the diluent by the user before each assay is carried out.

For one plate, combine:

- ❑ 200 µL of Protease Inhibitor Solution (100X)
- ❑ 19800 µL of Diluent 34

Prepare Calibrator and Control Solutions

Calibrator for the Rat NT-proBNP Assay is supplied at 20-fold higher concentration than the recommended highest calibrator. An 8-point standard curve is recommended with 4-fold serial dilution steps and a zero calibrator. The stock calibrator should be thawed and kept on ice and then should be added into diluent at room temperature to make the standard curve solutions. For the actual concentration of the calibrator, refer to the certificate of analysis (C of A) supplied with the kit. A copy of the kit-specific C of A can also be found at www.mesoscale.com

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

1) Prepare the highest calibrator by adding 15 µL of the calibrator stock vial to 285 µL of Diluent 34 + Protease Inhibitor. Mix well.
2) Prepare the next calibrator by transferring 60 µL of the diluted calibrator to 180 µL of Diluent 34 + Protease Inhibitor. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3) The recommended 8th standard is Diluent 34 + Protease Inhibitor (i.e. zero calibrator).

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

Dilution of Samples

Serum and plasma samples should be diluted at least 2-fold. Diluent 34 + Protease Inhibitor should be used to dilute the samples.

Prepare Detection Antibody Solution

The detection antibody is provided as a 50X stock solution. 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 detection antibody into 2940 µL of Diluent 34 + Protease Inhibitor.

Prepare Read Buffer

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

Assay Protocol

Notes

(Dilution of samples/calibrators should be completed prior to step 1)

1. Wash and Addition of the Sample or Calibrator: Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 50 µL of calibrator or diluted sample 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.

Shaking a 96-well MSD MULTI-ARRAY plate typically accelerates capture at the working electrode.

2. Wash and Addition of the Detection Antibody Solution: Wash the plate 3 times with 300 µL/well of PBS-T. Dispense 25 µL of 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.

3. Wash and Read: Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

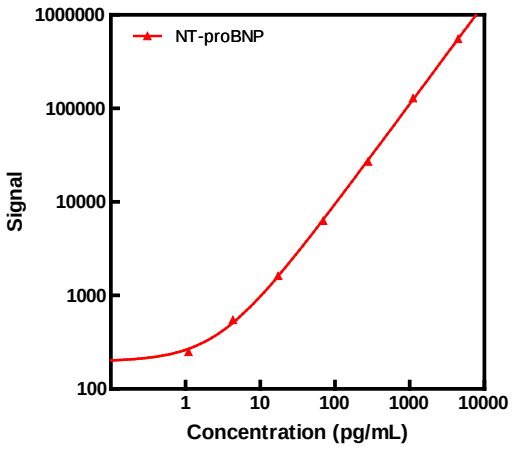
Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to ensure bubbles are not created when dispensing the read buffer.

Analysis of Results

The calibrators should be run minimally 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.

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.



	NT-proBNP	
Conc. (pg/mL)	Average Signal	%CV
0	170	11.9
1.1	249	6.8
4.3	547	5.6
17	1622	3.6
69	6292	7.8
278	27010	6.3
1111	128604	2.5
4445	554796	4.5

Sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the blank (zero calibrator).

	NT-proBNP (pg/mL)
LLOD	0.74

Precision

Control samples of high, mid, and low levels were made by spiking calibrator into rat EDTA plasma and were measured on each plate.

The controls were run in triplicate on multiple days (n>3).

Average intra-plate %CV is the average %CV of the control replicates within an individual plate.

Inter-plate %CV is the variability of controls across 9 plates over 8 days.

	Control	Plates	Average Conc. (pg/mL)	Average Intra-plate %CV	Inter-plate %CV
NT-proBNP	High	9	2268	4.6	7.4
	Mid	9	243	3.7	6.6
	Low	9	18	4.2	14.8

Spike Recovery

Normal rat EDTA plasma and heparin plasma were spiked with the calibrator at multiple levels throughout the range of the assay. The samples were diluted 4-fold, and then spiked with calibrator at the levels indicated in the table below.

% Recovery = measured / expected x 100

	NT-proBNP			
Sample	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. %CV	% Recovery
EDTA Plasma	0	34	1.9	
	8.2	45	2.1	107
	25	65	1.1	111
	74	124	2.3	115
	222	313	2.9	122
	667	855	3.2	122
	2000	2457	2.1	121
Heparin Plasma	0	9.0	4.7	
	1.0	9.3	6.6	94
	3.9	13	2.2	101
	16	25	1.5	101
	63	72	7.9	101
	250	243	1.3	94
	1000	849	1.0	84
	4000	3407	0.7	85

Linearity

To assess linearity, EDTA plasma and heparin plasma samples were diluted 2-fold, 5-fold, 10-fold, 20-fold, and 40-fold prior to testing. 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 measured from the previous dilution (expected).

% Recovery = (measured x dilution factor) / expected x 100

		NT-proBNP		
Sample	Fold Dilution	Conc. (pg/mL)	Conc. %CV	% Recovery
EDTA Plasma	2	97	5.6	
	5	78	3.6	81
	10	74	6.7	95
Heparin Plasma	2	138	5.7	
	5	109	0.9	79
	10	127	4.2	117
	20	148	7.7	116

Specificity

Based on the specificity of the capture and detection antibodies, the MSD Rat NT-proBNP Assay detects the NT-proBNP only and does not cross-react with BNP.

	% Cross-Reactivity	
Assay	BNP Calibrator	NT-proBNP Calibrator
BNP	100	< 0.1
NT-proBNP	< 0.1	100

Samples

Serum, EDTA plasma, and heparin plasma samples collected from normal Sprague-Dawley rats were tested at 2-fold dilution on the Rat NT-proBNP Assay. Shown below are the median and range of concentrations for each sample set. Concentrations have been corrected for sample dilution.

Sample	Statistic	NT-proBNP
Serum	Median (pg/mL)	4.5
	Range (pg/mL)	<LLOD-9.8
	N	8
EDTA Plasma	Median (pg/mL)	52
	Range (pg/mL)	31-130
	N	8
Heparin Plasma	Median (pg/mL)	45
	Range (pg/mL)	19-132
	N	8

Assay Components

Calibrator

Synthesized NT-proBNP peptide is used for this assay.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
NT-proBNP	Mouse Monoclonal	Rabbit Polyclonal

References

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5. Cowie MR, Struthers AD, Wood DA, Coats AS, Thompson SG, Poole-Wilson PA, Sutton GC. Value of natriuretic peptides in assessment of patients with possible new heart failure in primary care. Lancet. 1997 Nov 8;350(9088):1349-53.
6. Hobbs FD, Davis RC, Roalfe AK, Hare R, Davies MK, Kenkre JE. Reliability of N-terminal pro-brain natriuretic peptide assay in diagnosis of heart failure: cohort study in representative and high risk community populations. BMJ. 2002 Jun 22;324(7352):1498.

Summary Protocol

MSD 96-well MULTI-ARRAY Rat NT-proBNP Assay Kit

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

Step 1 : Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.

Prepare Diluent 34 + Protease Inhibitor.

Prepare an 8-point standard curve using the supplied calibrator:

- The calibrator should be diluted in Diluent 34 + Protease Inhibitor.
- Dilute the stock calibrator 20-fold in Diluent 34 + Protease Inhibitor. Then perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- Dilute samples by 2-fold into Diluent 34 + Protease Inhibitor prior to addition to the plate.

Prepare detection antibody solution by diluting the 50X detection antibody to 1X in a final volume of 3.0 mL Diluent 34 + Protease Inhibitor per plate.

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

Step 2 : Wash and Add Sample or Calibrator

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 50 µL/well of calibrator or diluted sample.

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

Step 3 : Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 25 µL/well of 1X detection antibody solution.

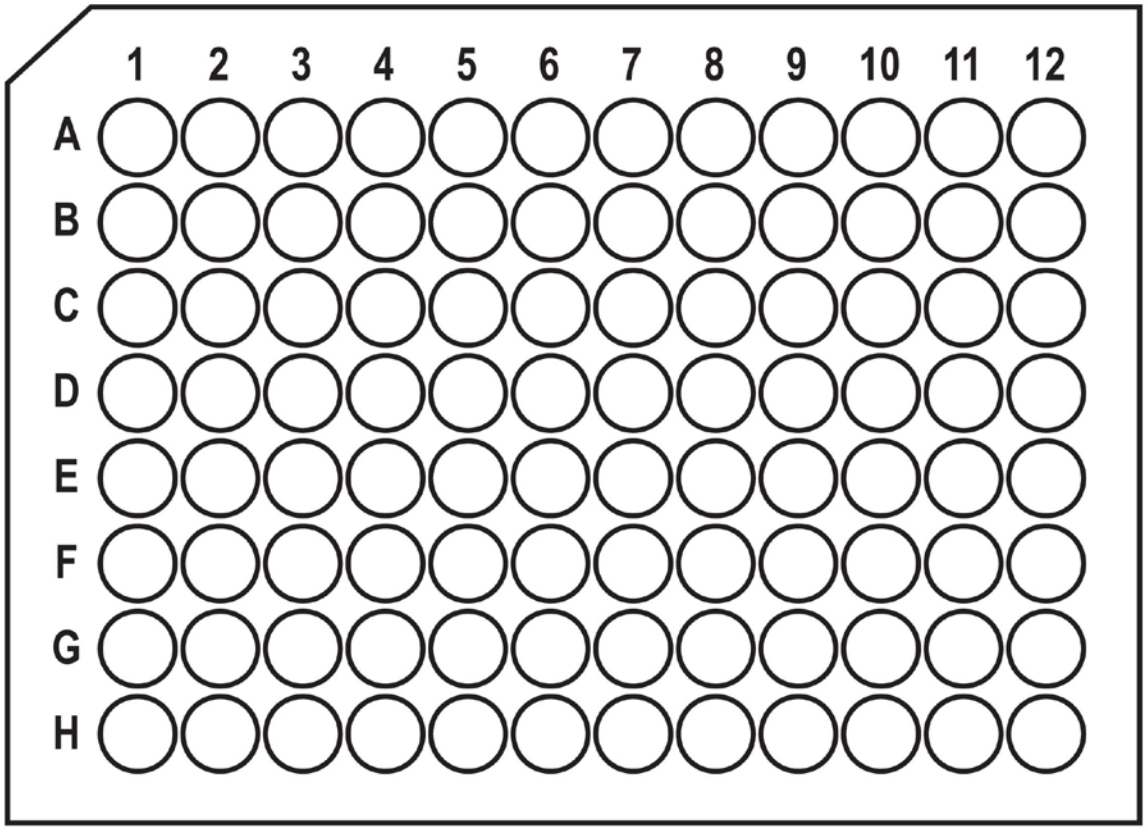
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Dispense 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.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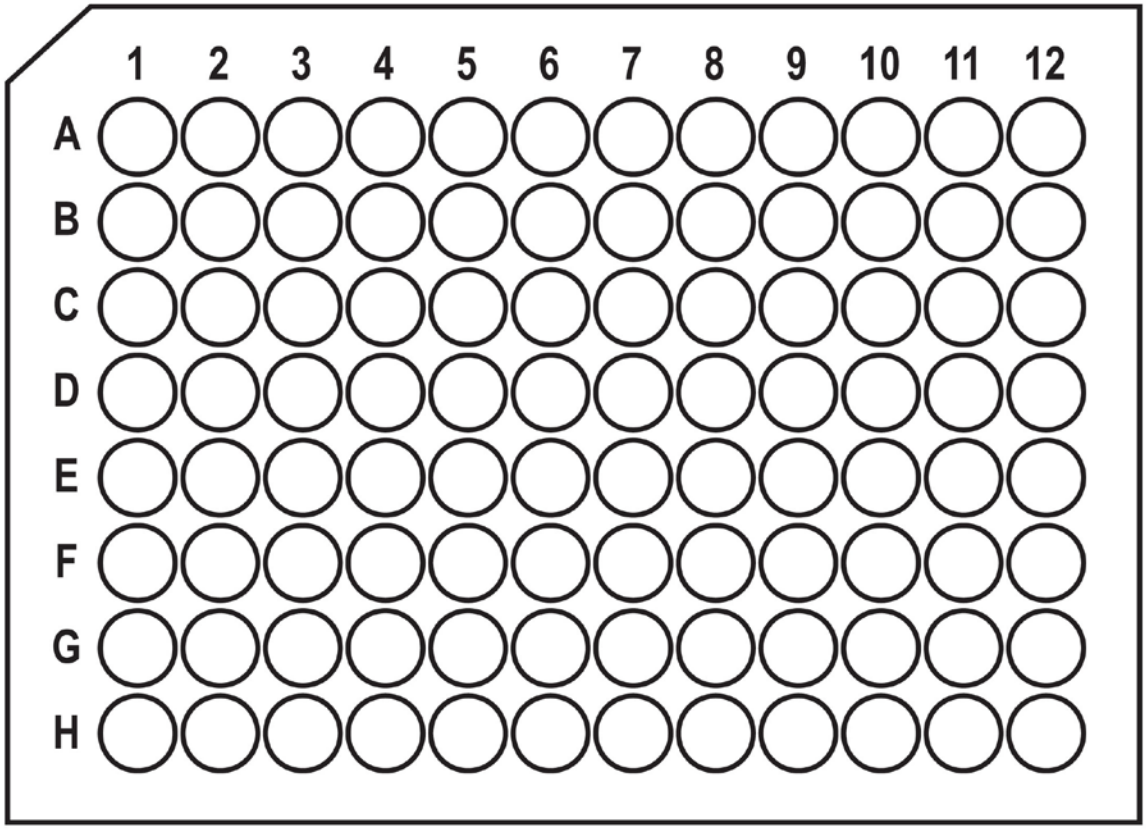
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