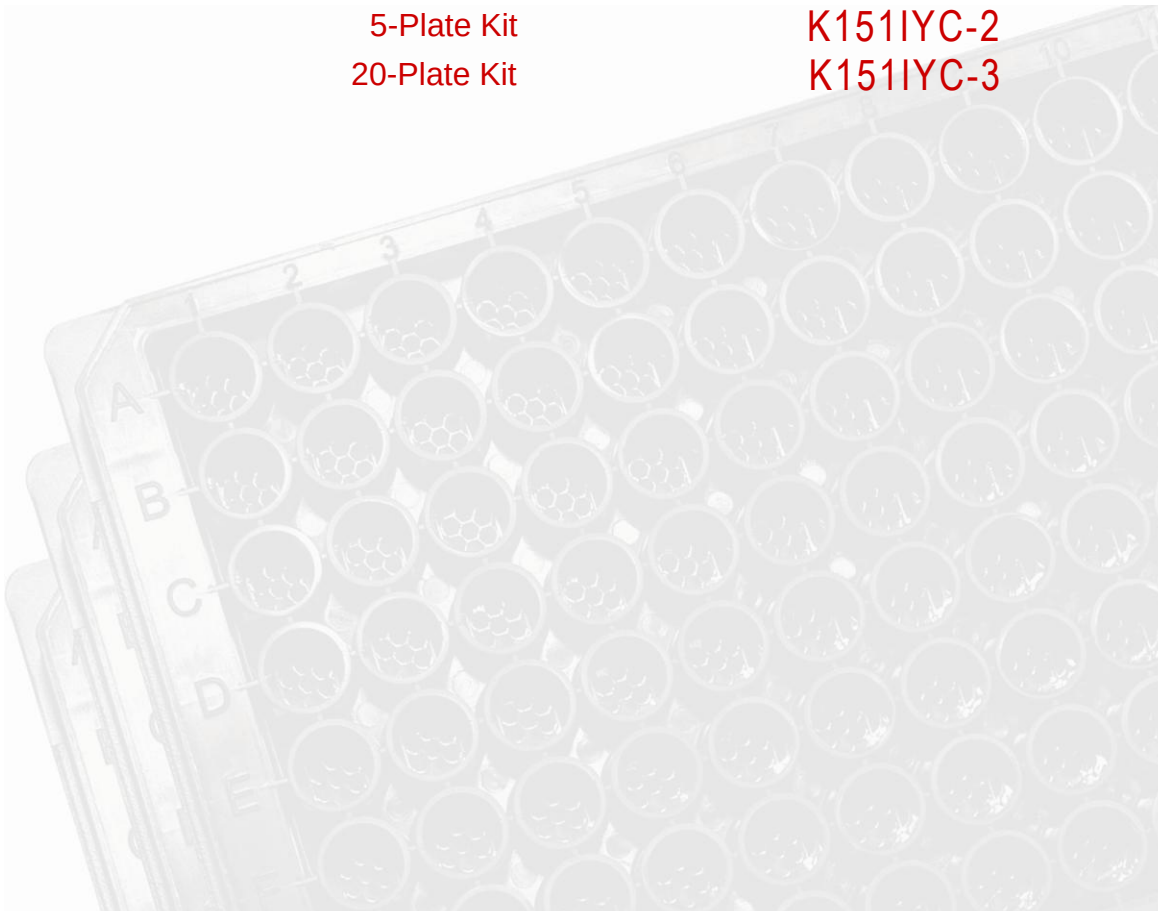
MESO SCALE DISCOVERY

MULTI-ARRAY Assay System

Human LBP Assay Kit

1-Plate Kit	K151IYC-1
5-Plate Kit	K151IYC-2
20-Plate Kit	K151IYC-3



MSD Cardiac Assays

Human LBP 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

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MSD Scientific Support

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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 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, 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

Lipopolysaccharide binding protein (LBP) is an acute phase protein which has been shown to bind to various LPS molecules and to lipid A (1-3). It is an approximately 60 kDa protein that was first isolated from acute phase rabbit serum (2). LBP is constitutively produced by hepatocytes in the liver (4, 5). It binds to LPS and presents it to the CD14 receptors on monocytic cells (6). In the presence of LBP, cytokines are released by monocytes at lower concentrations of LPS as compared to that produced in the absence of LBP (7). Thus, the primary function of LBP is to enhance the ability of the host to detect LPS early in infection. It has been shown that although LBP is required for the induction of inflammatory response, it is not necessary for the clearance of LPS from circulation (8).

In normal serum, LBP is constitutively present at a mean concentration of 5-20µg/mL, and rises up to 200µg/mL in acute phase response (9, 10). One of the functions of LBP is to catalyze the movement of LPS monomers from the aggregates to high density lipoprotein (HDL) particles (11). This leads to the neutralization of LPS. Additionally, LBP associates with lipid A part of gram-negative bacteria resulting in its opsonization (12).

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

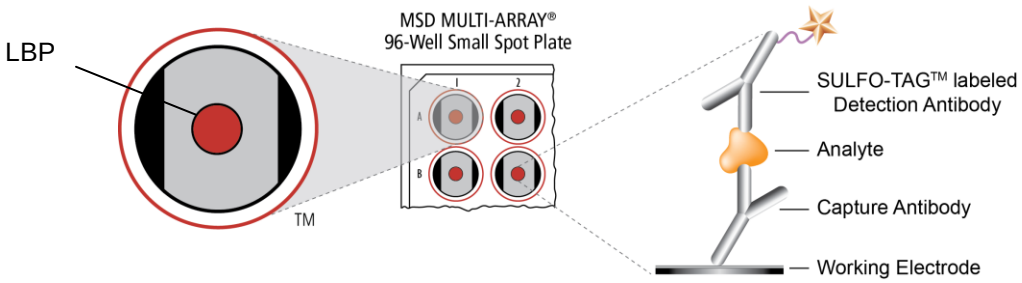


Figure 1. *Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

IV Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K151IYC-1	K151IYC-2	K151IYC-3
MULTI-ARRAY 96-well Human LBP Plate L451IYB-1	2–8°C	1 plate	5 plates	20 plates
SULFO-TAG™ Anti-hLBP Detection Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	4 vials (375 µL ea)
Human LBP Calibrator (1 µg/mL)	≤-70°C	1 vial (100 µL)	5 vials (100 µL ea)	20 vials (100 µL ea)
Diluent 15 R57BB-4 (10 mL) R57BB-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	4 bottles (50 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 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)

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 15 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 LBP Assay is supplied at the concentration of the highest Calibrator. For the assay, an 8-point standard curve is recommended with 7-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Human LBP Calibrator (ng/mL)	Dilution Factor
STD-01	1000	
STD-02	143	7
STD-03	20	7
STD-04	2.9	7
STD-05	0.42	7
STD-06	0.059	7
STD-07	0.0085	7
STD-08	0	n/a

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

1) Calibrator for the Human LBP Assay is supplied at the concentration of the highest Calibrator. Therefore, no dilution is required for top of the curve.
2) Prepare the next Calibrator by transferring 10 µL of the undiluted Calibrator to 60 µL of Diluent 15. Repeat 7-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) Reserve 60 µL of Diluent 15 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.

Prepare 1% Blocker A Solution for Sample Diluent

Determine the amount of 1% Blocker A Solution needed for the experiment. Each sample requires 1990 µL 1% Blocker A Solution for accurate dilution. Dilute 5% Blocker A Solution to 1% with PBS-T.

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 1:200 in 1% Blocker A Solution.* For example, add 10 µL of sample to 1990 µL of Blocker A Solution and mix thoroughly. Each replicate will require 10 µL of diluted sample.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 50X stock of Anti-human LBP 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 Detection Antibody solution into 2.94 mL of Diluent 15.

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.

VIII 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 15. 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.

IX Analysis of Results

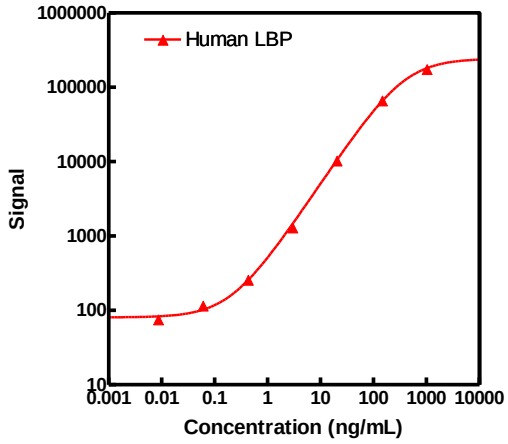
analysis of results

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.

X Typical Standard Curve

typical standard curve

The following standard curves are 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.



LBP		
Conc. (ng/mL)	**Average Signal**	**%CV**
0	67	6.8
0.0085	76	11.9
0.059	117	3.0
0.42	261	1.1
2.9	1314	1.9
20	10454	6.4
143	66914	2.7
1000	177584	2.8

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 values below represent the average LLOD over multiple kit lots.

	LBP
LLOD (ng/mL)	0.038

XII Spike Recovery

spike recovery

Serum and plasma samples were spiked with Calibrator at multiple values throughout the range of the assay. Each spike was done in ≥ 3 replicates. Samples were diluted 1:200 in 1% MSD Blocker A solution prior to measurement.
% Recovery = measured / expected x 100

Sample	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	% Recovery
Serum 1	0	-	-
	2500	4300	100
	5000	6500	95
	10000	11700	99
Serum 2	0	-	-
	2500	5000	91
	5000	7900	99
	10000	12500	96
EDTA Plasma 1	0	-	-
	2500	7200	98
	5000	9500	97
	10000	14200	96
EDTA Plasma 2	0	-	-
	2500	5200	90
	5000	7600	91
	10000	11900	89
Heparin Plasma 1	0	-	-
	2500	6900	94
	5000	9100	93
	10000	13600	91
Heparin Plasma 2	0	-	-
	2500	4800	102
	5000	7300	101
	10000	10200	84

XIII Linearity

linearity

Human serum, EDTA plasma and heparin plasma samples with measurable LBP were evaluated. The samples were diluted 1:200 in 1% MSD Blocker A solution 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	Conc. (ng/mL)	% Recovery
Serum 1	1	-	-
	2	1500	92
	4	700	94
	8	400	90
Serum 2	1	-	-
	2	700	98
	4	300	92
	8	200	92
EDTA Plasma 1	1	-	-
	2	2700	92
	4	1400	92
	8	800	104
EDTA Plasma 2	1	-	-
	2	1400	115
	4	600	92
	8	300	90
Heparin Plasma 1	1	-	-
	2	2500	94
	4	1100	78
	8	600	84
Heparin Plasma 2	1	-	-
	2	1200	113
	4	600	102
	8	300	105

XIV Samples

samples

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

		LBP (ng/mL)
Serum	Mean	3500
	Median	2900
	Range	1400 - 6500
EDTA Plasma	Mean	4300
	Median	4100
	Range	2300 - 9000
Heparin Plasma	Mean	4600
	Median	4400
	Range	2100 - 9300

XV Assay Components

Assay components

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

	Source species	
Analyte	**MSD Capture Antibody**	**MSD Detection Antibody**
hLBP	Mouse monoclonal	Mouse monoclonal

XVI References

references

1. Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. Science. 1990 Sep 21;249(4975):1429–1431
2. Tobias PS, Soldau K, Ulevitch RJ. Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. J Exp Med. 1986 Sep 1;164(3):777–793.
3. Glauser MP, Zanetti G, Baumgartner JD, Cohen J. Septic shock: pathogenesis. Lancet. 1991 Sep 21;338(8769):732–736.
4. Grube BJ, Cochane CG, Ye RD, Green CE, McPhail ME, Ulevitch RJ, Tobias PS. Lipopolysaccharide binding protein expression in primary human hepatocytes and HepG2 hepatoma cells. J Biol Chem. 1994 Mar 18;269(11):8477–8482
5. Ramadori G, Meyer zum Buschenfelde KH, Tobias PS, Mathison JC, Ulevitch RJ. Biosynthesis of lipopolysaccharide-binding protein in rabbit hepatocytes. Pathobiology. 1990;58(2):89–94
6. Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, Busse LA, Zukowski MM, Wright SD. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. J Exp Med. 1994 Jan 1;179(1):269–277
7. Gallay P, Barras C, Tobias PS, Calandra T, Glauser MP, Heumann D. Lipopolysaccharide (LPS)-binding protein in human serum determines the tumor necrosis factor response of monocytes to LPS. J Infect Dis. 1994 Nov;170(5):1319-22.
8. Jack RS, Fan X, Bernheiden M, Rune G, Ehlers M, Weber A, Kirsch G, Mentel R, Fürll B, Freudenberg M, Schmitz G, Stelter F, Schütt C. Lipopolysaccharide-binding protein is required to combat a murine gram-negative bacterial infection. Nature. 1997 Oct 16;389(6652):742-5.
9. Prucha M, Herold I, Zazula R, Dubska L, Dostal M, et al. (2003) Significance of lipopolysaccharide binding protein (an acute phase protein) in monitoring critically ill patients. Crit Care 7: R154–159.
10. Opal SM, Scannon PJ, Vincent JL, White M, Carroll SF, et al. (1999) Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. J Infect Dis 180: 1584–1591.
11. Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. Science. 1990 Sep 21;249(4975):1429–1431
12. Wright SD, Tobias PS, Ulevitch RJ, Ramos R. (1989) Lipopolysaccharide binding protein opsonizes LPS bearing particles for recognition by a novel receptor on macrophages. J Exp. Med. 170, 1231-1241

Summary Protocol

MSD 96-well MULTI-ARRAY Human LBP Assay

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Human LBP 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 200-fold in 1% MSD Blocker A Solution.

Prepare an 8-point standard curve using supplied calibrator and conducting 7-fold dilution in Diluent 15. Use Diluent 15 as zero calibrator blank.

Prepare Detection Antibody Solution by diluting the 50X Anti-hLBP Antibody to 1X in a final volume of 3.0 mL of Diluent 15 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 15.

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.

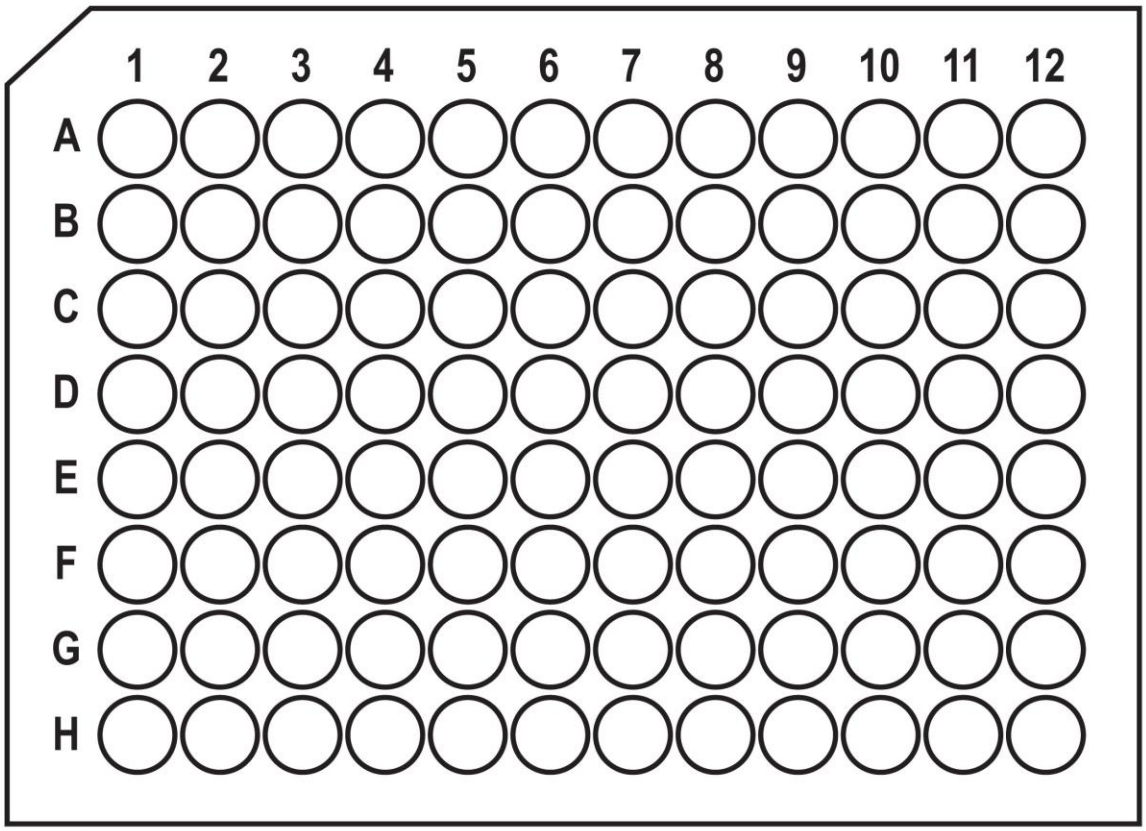
Incubate at room temperature with vigorous shaking (300-1000 rpm) for 1 hour.

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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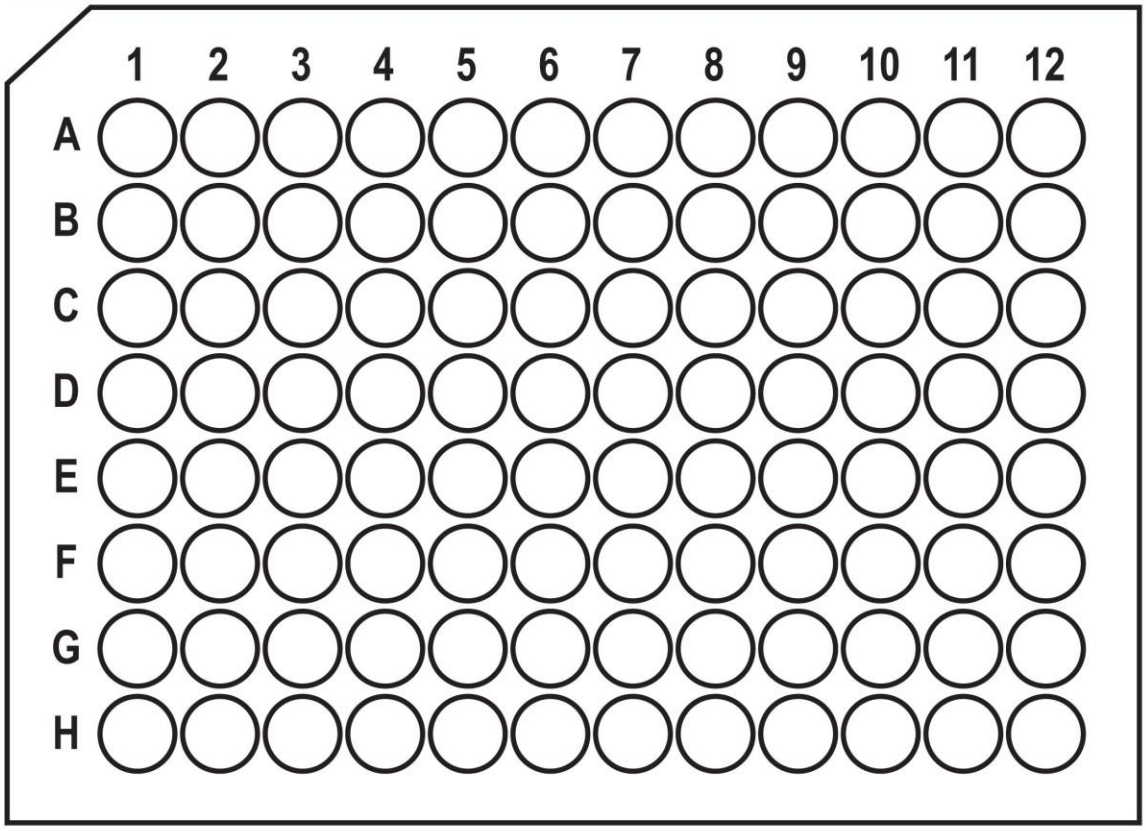
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