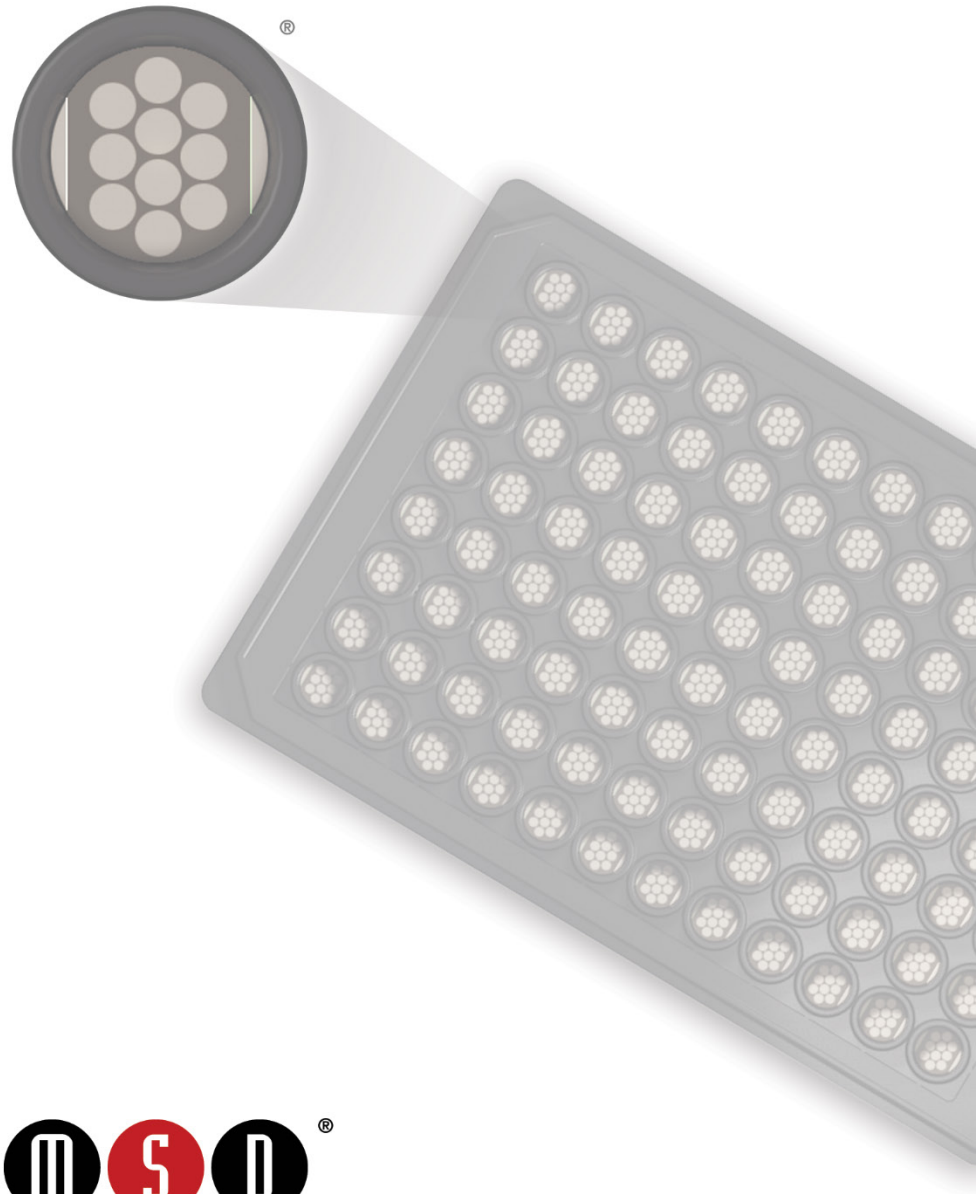
R-PLEX® EV Antibody Sets

& Singleplex Assays



MSD R-PLEX Platform

R-PLEX EV Antibody Sets & Singleplex Assays

For the development of R-PLEX singleplex assays for extracellular vesicles (EV) including exosomes.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

R-PLEX provides an expanded menu of electrochemiluminescence-based assays for biomarker discovery and development.

R-PLEX Antibody Sets include a matched, biotinylated capture and SULFO-TAG™ conjugated detection antibody pair and a calibrator for the quick and easy development of highly sensitive immunoassays on MSD instruments. Diluents, plates, and Read Buffer are available separately.

For greater ordering convenience, R-PLEX assays include an Antibody Set, its primary assay and antibody diluents, MSD GOLD™ Read Buffer, and either SECTOR™ or QuickPlex® plates. Collectively, they provide the complete set of components that is required to develop an MSD immunoassay.

R-PLEX enables the development of singleplex immunoassays on:

- MSD GOLD 96-well Small-Spot Streptavidin SECTOR Plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instruments
- MSD GOLD 96-well Small-Spot Streptavidin QuickPlex plates for use on MESO QuickPlex Q 60MM instrument.

R-PLEX EV Antibody Sets target proteins commonly present on the outer surface of exosomes and other extracellular vesicles (EVs), enabling the development of singleplex and multiplex immunoassays for intact EVs.

This document provides the details for developing a singleplex EV assay on the MSD platform using R-PLEX EV Assays or Antibody Sets. See http://www.mesoscale.com® for more details.

Principle of the Assay

Singleplex assays can be easily developed on MSD GOLD Small-Spot Streptavidin plates. These plates provide high sensitivity, consistent performance, and excellent inter- and intralot uniformity. The typical R-PLEX Antibody Set includes a biotinylated capture antibody that binds to streptavidin on the plate surface. Analyte in the sample binds to the capture reagent; a detection antibody conjugated with an electrochemiluminescent label (MSD GOLD SULFO-TAG label) binds to the analyte to complete the sandwich immunoassay. The R-PLEX EV Antibody Sets are designed to form a sandwich complex only when the capture and detection antibodies bind different copies of the target protein on the surface of an EV (Figure 1). This approach enables the specific quantitation of EV-bound surface proteins in samples that may contain the same proteins in free non-EV-bound form, without requiring the isolation of the EV fraction. Once the immunoassay is complete, the plate is loaded into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of the analyte in the sample.

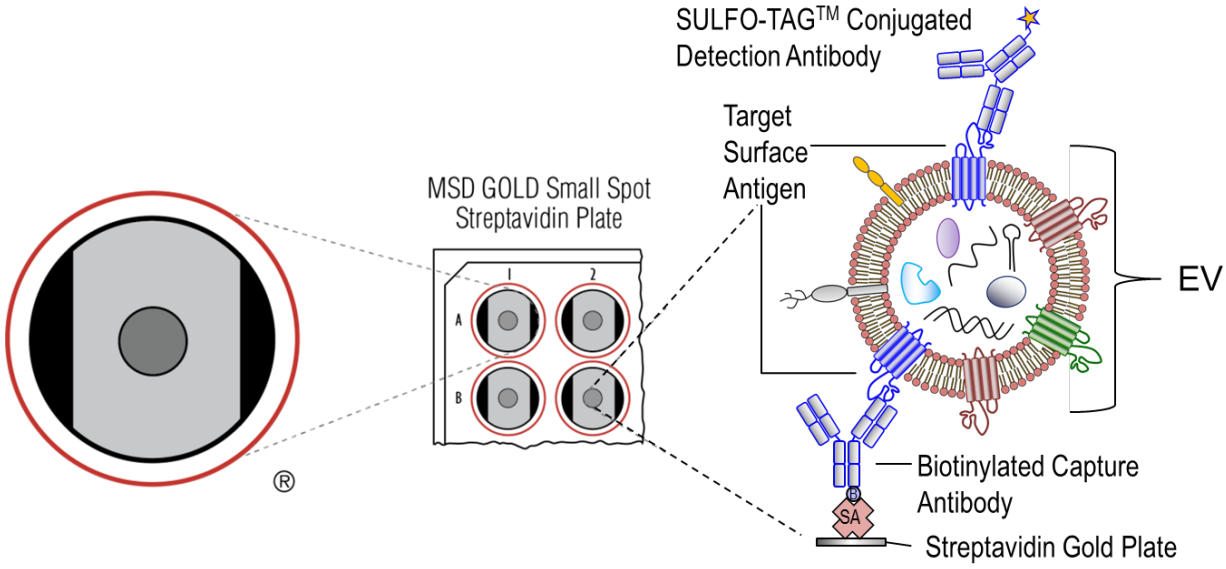

Figure 1. R-PLEX singleplex EV assay on a MSD GOLD Small-Spot Streptavidin Plate.

Components

R-PLEX Assays

For R-PLEX assay instrument compatibility, please refer to the Instrument Compatibility section on page 8.

R-PLEX assays contain the components listed in Table 1 along with the R-PLEX Antibody Set components (Table 2).

Table 1. R-PLEX assay components

Reagent	Storage	Catalog No.	Size	Quantity Supplied	Description
MSD GOLD 96-Well Small-Spot Streptavidin SECTOR Plate	2–8 °C	L45SA-1	1 spot	5 plates	96-well plate—foil sealed, with desiccant
MSD GOLD 96-Well Small-Spot Streptavidin QuickPlex Plate		L4BSA-1			
Assay Diluent	≤–10 °C	The Diluent provided is noted on the assay datasheet.			Diluent for samples and Calibrator; typically contains proteins, blockers, and preservatives
Antibody Diluent	≤–10 °C	The Diluent provided is noted on the assay datasheet.			Diluent for detection antibody; typically contains protein, blockers, and preservatives
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	Plate coating solution; contains protein, blockers, and preservatives
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1 bottle	Buffer to catalyze the electrochemiluminescence reaction

RT = room temperature

R-PLEX assays are provided with sufficient assay diluent to support up to a 10-fold dilution. If additional sample dilution is required for your study, assay diluent is recommended.

See http://www.mesoscale.com/en/products_and_services/assay_kits/r-plex for more information on specific assays. For information on diluent volume requirements, see the R-PLEX Assay Diluent Volume Calculation handout. For information on diluents for R-PLEX, see the R-PLEX Assay/Antibody Diluent Combinations handout. Both are available at www.mesoscale.com/technical_literature/handouts.

R-PLEX Antibody Sets

R-PLEX Antibody Sets contain a biotinylated capture antibody, a SULFO-TAG conjugated detection antibody, and a frozen calibrator. The calibrator is provided at the suggested Calibrator Standard 1 (top of curve) concentration. The representative Calibrator Standard 1 concentration for each assay is shown in the product-specific datasheet.

Table 2. R-PLEX Antibody Set components

Name	Storage	Size	Quantity Supplied	Description
Biotin Capture Antibody (analyte-specific)	2–8 °C	5 Plates	1 vial	Biotinylated capture antibody; provided as one vial per five plates
		50 Plates	10 vials	
SULFO-TAG Detection Antibody (analyte-specific)	2–8 °C	5 Plates	1 vial	SULFO-TAG conjugated detection antibody (100X); provided as one vial per five plates
		50 Plates	10 vials	
EV Calibrator 1	≤–70 °C	5 Plates	5 vials	EVs derived from a human cell line; provided in a buffered diluent
		50 Plates	50 vials	

Plates and Reagents for Separate Purchase

MSD offers a range of plates and reagents to enable assay development using R-PLEX Antibody Sets. Plates and reagents are also available in different pack sizes for individual purchase. MSD GOLD singleplex plates are included in the assays (Table 3). For a complete listing of all available assay development plates and reagents, visit our website at www.mesoscale.com.

Plates

Table 3. MSD GOLD singleplex plates (included in assays)

Name	1 Plate	5 Plates	30 Plates	120 Plates	510 Plates
MSD GOLD 96-well Small-Spot Streptavidin SECTOR Plates	L45SA-1	L45SA-2	L45SA-5	L45SA-6	L45SA-7
MSD GOLD 96-well Small-Spot Streptavidin QuickPlex Plates	L4BSA-1	L4BSA-2	L4BSA-5	L4BSA-6	L4BSA-7

Read Buffer

MSD GOLD Read Buffer B (Table 4) is recommended for use with EV assays. It is included in R-PLEX Assays and is provided at the working concentration. Dilution of MSD GOLD Read Buffer B may compromise the results of the assay.

Table 4. MSD GOLD Read Buffer B

Name	Storage	Catalog No.	Description
MSD GOLD Read Buffer B, 90 mL	15–30 °C	R60AM-2	Buffer to catalyze the electrochemiluminescence reaction; provided at the working concentration of the assay

Diluents

Diluents 52 and 53 are the only recommended diluents for R-PLEX EV assays and Antibody Sets.

Refer to the R-PLEX EV product-specific datasheet supplied with the product for the sample types tested in singleplex EV assays. The datasheets are also available at www.mesoscale.com.

The catalog numbers for required diluents in singleplex EV assays are provided in Table 5.

Table 5. Diluents used in R-PLEX singleplex EV assays

Name	Catalog No.	Size
Diluent 52	R52AA-1	40 mL
Diluent 53	R52AB-1	40 mL

Wash Buffer

See Table 6 for Wash Buffer information. MSD Wash Buffer is recommended for use with R-PLEX EV assays. Other wash buffers may disrupt extracellular vesicles.

Table 6. Catalog number of Wash Buffer

Name	Storage	Catalog No.	Size	Description
MSD Wash Buffer (20X)	RT	R61AA-1	100 mL	Phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T)

Notes: This size of Wash Buffer is sufficient for washing four plates manually or for washing two plates with an automated plate washer. To prepare a 1X working solution of MSD Wash Buffer, combine 15 mL of MSD Wash Buffer (20X) with 285 mL of deionized water for each plate.

Instrument Compatibility

MSD offers R-PLEX singleplex assays designed for use on specific instrument platforms (Table 7). SECTOR plate-based assays are offered for use on SECTOR instruments and the MESO QuickPlex SQ 120/120MM. The MESO QuickPlex Q 60MM only reads R-PLEX assays on QuickPlex plates.

Table 7. Instrument compatibility

Instrument	Assays on SECTOR Plate (Catalog No. -1/-2/-4)	Assays on QuickPlex Plate (Catalog No. -21/-22/-24)
MESO QuickPlex SQ 120	Y	—
MESO QuickPlex SQ 120MM	Y	—
MESO SECTOR S 600	Y	—
MESO SECTOR S 600MM	Y	—
MESO QuickPlex Q 60MM	—	Y

dash (—) = not applicable

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid-handling equipment suitable for dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate-washing equipment, e.g., automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer
- ❑ Minicentrifuge

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the applicable safety data sheets, which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Bring frozen diluents to room temperature in a 22–25 °C water bath.
- Prepare Calibrator Standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by briefly vortexing after each dilution.
- Avoid prolonged exposure of the detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light (except for direct sunlight).
- Avoid bubbles in wells during all pipetting steps because they may lead to variable results. Bubbles introduced when adding MSD Gold Read Buffer B may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500–1,000 rpm, optimally at 700 rpm or above.
- Avoid letting the plate surface dry completely between steps. If a step needs to be delayed, leave the antibody coating solution, sample, or detection antibody solution in the plate until you are ready to perform the next step to keep the plate from drying out.
- Remove the plate seal prior to reading the plate.
- Make sure that the Read Buffer is at room temperature when added to a plate.
- Do not shake the plate after adding Read Buffer.
- To improve interplate precision, keep time intervals consistent between adding Read Buffer and reading the plate. Read the plate as soon as possible after adding Read Buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading and follow guidelines on how to read partial plates provided in the instrument manual. Partially used plates may be stored up to 30 days at 2–8 °C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Refer to the Best Practices section (page 9) before beginning the protocol.

Important: Upon first thaw, aliquot diluents into suitable volumes before refreezing.

To prepare supplemental reagents such as MSD Wash Buffer, please refer to the Components section (page 6).

Coat Plate

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 200 µL of biotinylated capture antibody to 5.8 mL of Diluent 100. Mix by vortexing.
- ❑ Add 50 µL of the above solution to each well of the MSD GOLD Small-Spot Streptavidin plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with vigorous shaking (~700 RPM) at room temperature for 1 hour.

Prepare Calibrator Standards

MSD supplies calibrator for R-PLEX EV Antibody Sets at the working concentration for the highest standard (Calibrator Standard 1). We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to assay diluent (Diluent 52) to make the calibration curve solutions.

To prepare 7 calibrator solutions plus a zero calibrator for up to 3 replicates (see Figure 2), perform the following.

- ❑ Human EV Calibrator 1 is used without dilution for Calibrator Standard 1. Vortex briefly and spin down in minicentrifuge before opening tube.
- ❑ Prepare the next calibrator (Calibrator Standard 2) by adding 50 µL of Calibrator Standard 1 to 150 µL of assay diluent. Vortex briefly to mix.
- ❑ Repeat 4-fold serial dilutions (50 µL previous standard into 150 µL diluent) to generate Calibrator Standards 3 through Calibrator Standard 7.
- ❑ Use assay diluent as the blank (Calibrator Standard 8).

Notes:

- Dilution volumes can be adjusted for fewer replicates.
- For the Calibrator Standard 1 concentration, refer to the product-specific datasheet supplied with the R-PLEX Antibody Set. The datasheet is also available at www.mesoscale.com/R-PLEX-documents.

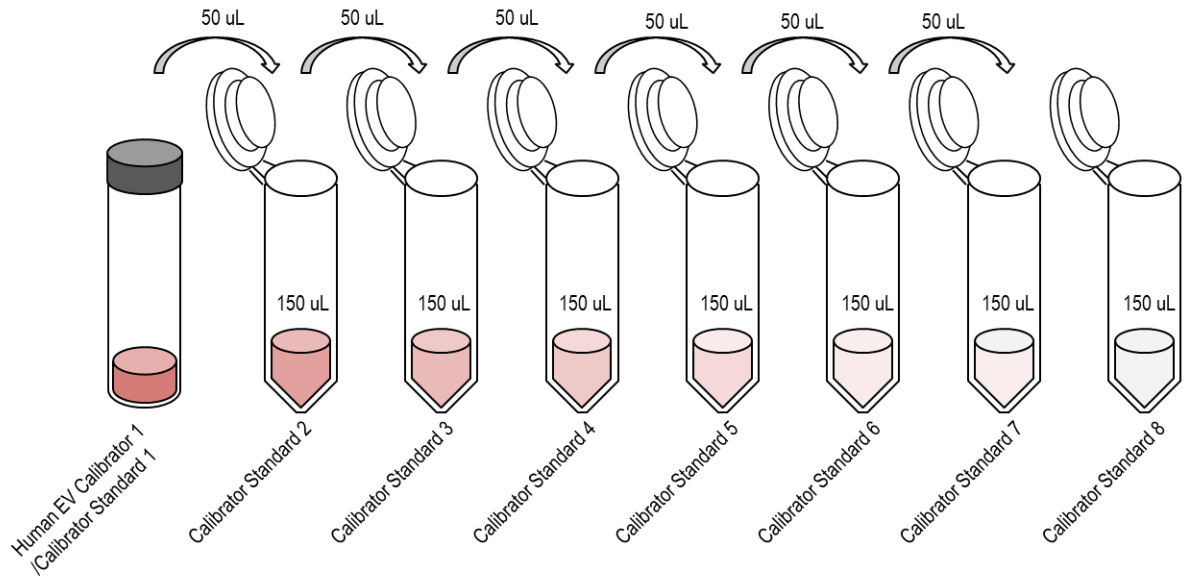

Figure 2. Dilution schema for Calibrator Standards for R-PLEX EV singleplex assays.

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X. Prepare the detection antibody solution in Diluent 53 immediately prior to use.

For one plate, combine:

- ❑ 30 µL of the supplied 100X detection antibody
- ❑ 2.97 mL of antibody diluent (Diluent 53)

Dilute Samples

Depending on the sample set under investigation, a dilution may be necessary. Suggested dilution factors are provided in the R-PLEX product-specific datasheet. Assay diluent should be used for sample dilution. The dilution factor for a given sample type should be optimized. Additional assay diluent may be necessary for samples that are diluted greater than 10-fold.

Prepare MSD GOLD Read Buffer B

MSD provides MSD GOLD Read Buffer B at the working concentration of the assay; do not dilute. Equilibrate the Read Buffer to room temperature before use. To avoid bubbles, do not vortex.

Assay Protocol

Note: Before beginning STEP 1, prepare the plate as described on page 10.

STEP 1: Wash and add Samples and Calibrators

- ❑ Prior to adding samples, remove coating solution and wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of assay diluent to each well. Tap the plate gently on all sides to ensure even coating of the plate surface.
- ❑ Add 25 µL of the prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with vigorous shaking (~700 rpm) for at least 1 hour. Longer incubation times can improve EV assay sensitivities for dilute samples.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with vigorous shaking (~700 rpm) for 1 hour.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well and promptly analyze the plate on an MSD instrument. Incubation in Read Buffer is not required before reading the plate.

Assay Performance

A representative data set for each assay is presented in the product-specific datasheet shipped with the product; it is also available at http://www.mesoscale.com/R-PLEX-documents.The data represent performance of the assay tested in singleplex format on U-PLEX® plates. The data were generated during the development of the assay and do not represent the product specifications. Under your experimental conditions, the assay may perform differently than the representative data shown.

Plate Diagram

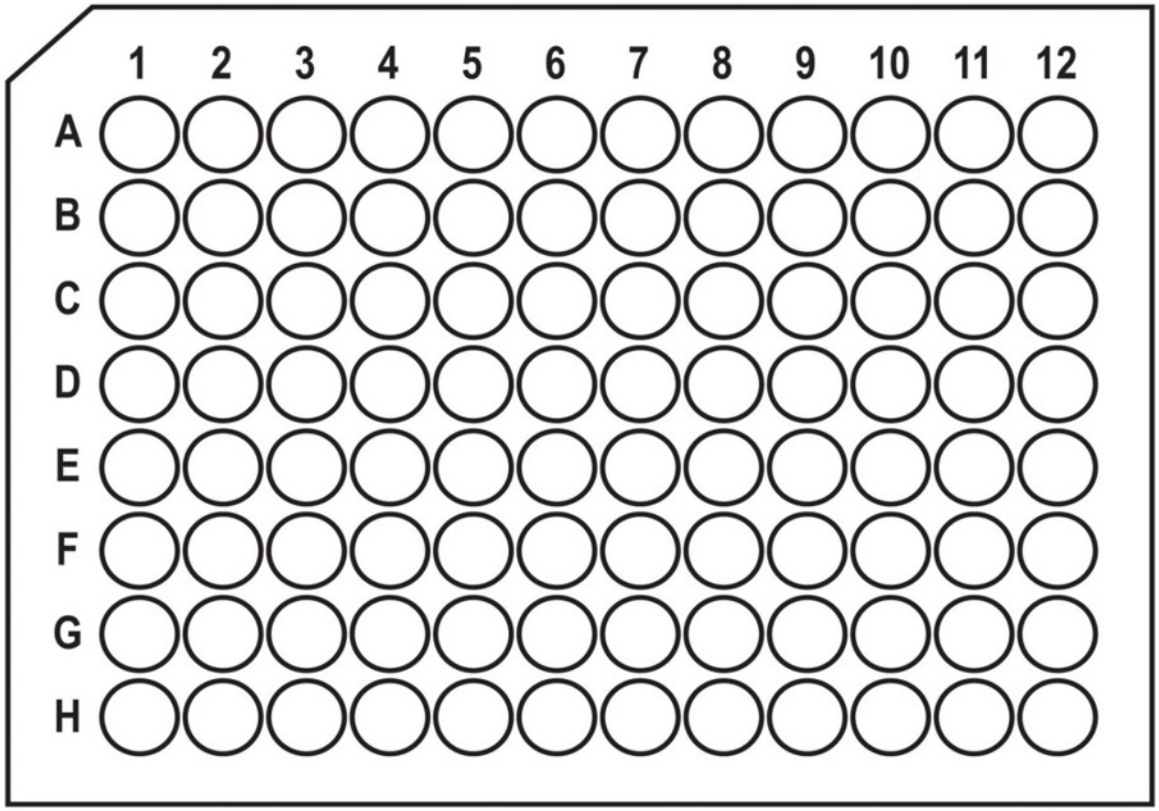

Figure 3. Plate Diagram.