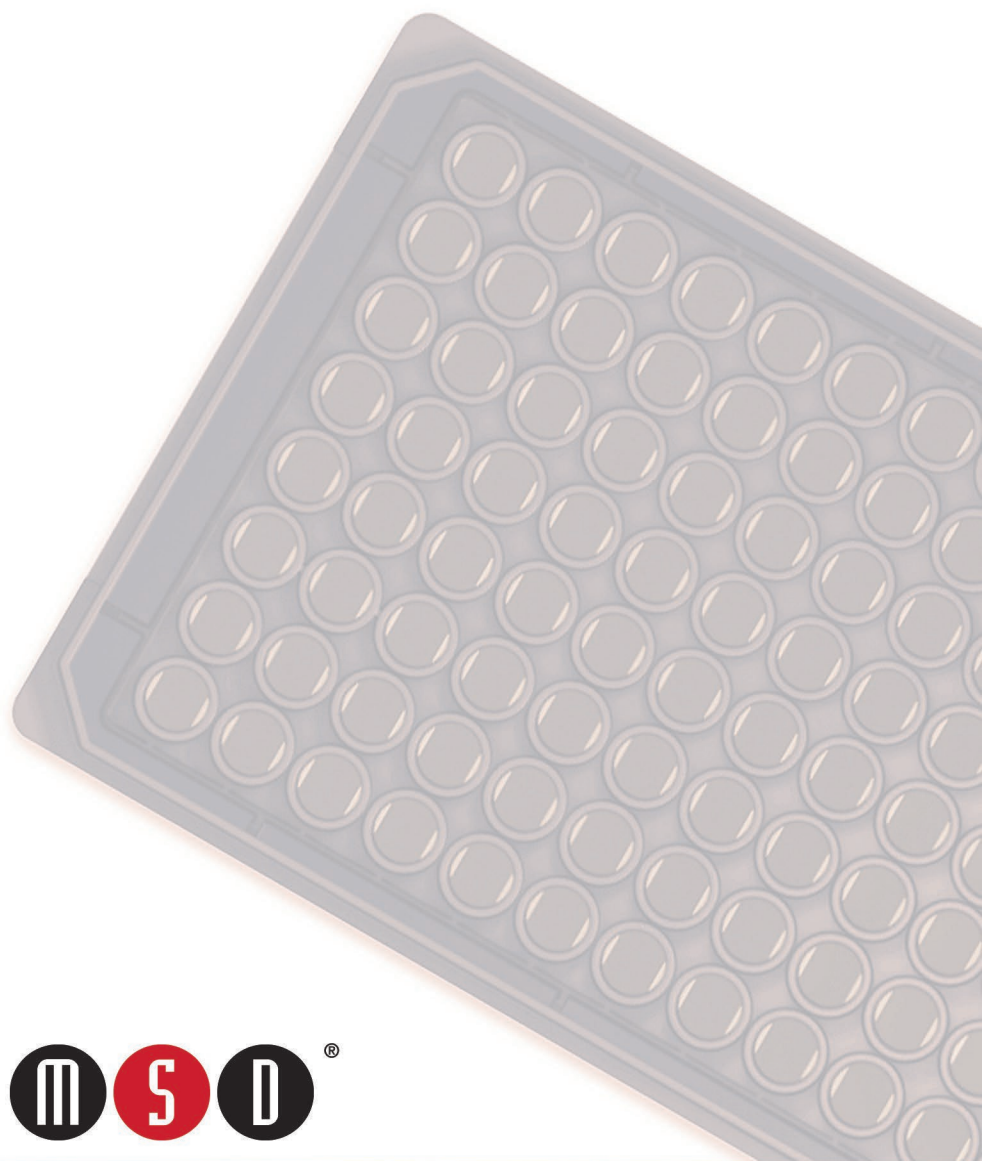


# MSD<sup>®</sup> SECTOR and QuickPlex Plates

## Standard and High Bind Plates



# MSD Plates

## Standard and High Bind Plates

	SECTOR™ Plates	QuickPlex™ Plates	QuickPlex Ultra™ Plates
96-well 1-Spot	L15XA	L55XA	L5BXA
96-well 1-Spot High Bind	L15XB	L55XB	
384-well 1-Spot	L21XA		
384-well 1-Spot High Bind	L21XB		

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

### Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.

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# Introduction

MESO SCALE DISCOVERY® SECTOR and QuickPlex plates offer an excellent platform for the development of immunoassays for measuring biomarkers in life science research. MSD assays have ultralow detection limits, provide up to five logs of linear dynamic range, use minimal sample, and handle difficult sample matrices easily. At the core of this technology are microplates that have been specially developed to take full advantage of the unique benefits of this platform. This document describes how these plates can be used to develop novel, single analyte assays that exploit the advantages of MULTI-ARRAY® technology. After development, assays can be multiplexed using MSD MULTI-SPOT® plates for even more efficiency.

MSD assays follow a workflow similar to that of an ELISA; the main steps are coating the plates with capture reagent, blocking, adding samples/calibrators, adding detection reagent, reading the plate, and analyzing the data. The MSD platform offers several advantages over ELISAs as shown in the table below. Note that typical assay development shows significant conservation of critical reagents (capture antibodies and proteins) over traditional ELISA formats.

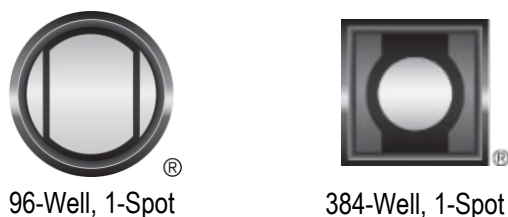
**Table 1.** Comparison of advantages

Comparison of Traditional Immunoassays and MSD assays		
Feature	ELISA	MSD
Conserves samples	50–100 µL	25 µL
Compatible with crude samples (no preps)	No	Yes
Multiplex enabled	No	Yes
Dynamic range (minimal or no sample dilution)	1–2 logs	3–5 logs
Reduced matrix effects	No	Yes
Read time	Slower	Fast (1 min)
Flexible assembly of panels	No	Yes
Simple protocols	No	Yes

This technical note provides instructions for coating and using MSD plates to develop assays. The objectives of successful assay development are high signal-to-background ratio, sensitivity, reproducibility, and a wide linear dynamic range. The signals obtained in the assay should be proportional to the amount of analyte in the samples. Example protocols for typical assays and guidelines on assay optimization are provided in this document.

# Principle

MSD plates provide a rapid and convenient method for the development of new assays and the transfer of existing ELISAs to the MSD platform. The different types of uncoated plates offered by MSD for assay development are graphically represented below.



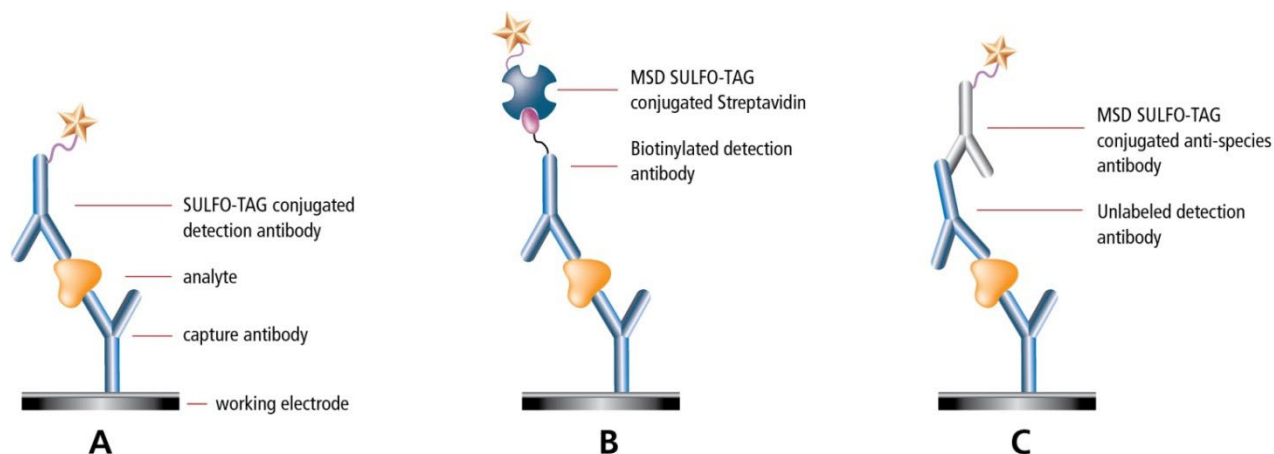
*Figure 1. Spot images of MSD uncoated assay development plates*

Each spot within the wells shown above is a working electrode surface that adsorbs capture reagent. The end user can coat the plates with a variety of capture reagents, including antibodies, carbohydrates, virus-like particles, cells, peptides, lysates, or kinase substrates. After coating, the user adds the sample and a solution containing detection reagent conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) throughout one or more incubation periods. Analytes in the sample bind to the capture reagent immobilized on the electrode surface; recruitment of the detection antibodies by the bound analytes forms the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument. The reader applies a voltage to the plate electrodes, causing the SULFO-TAG in close proximity to the bottom of the plate to emit light through a series of reduction and oxidation reactions (redox). The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

# Assay Formats

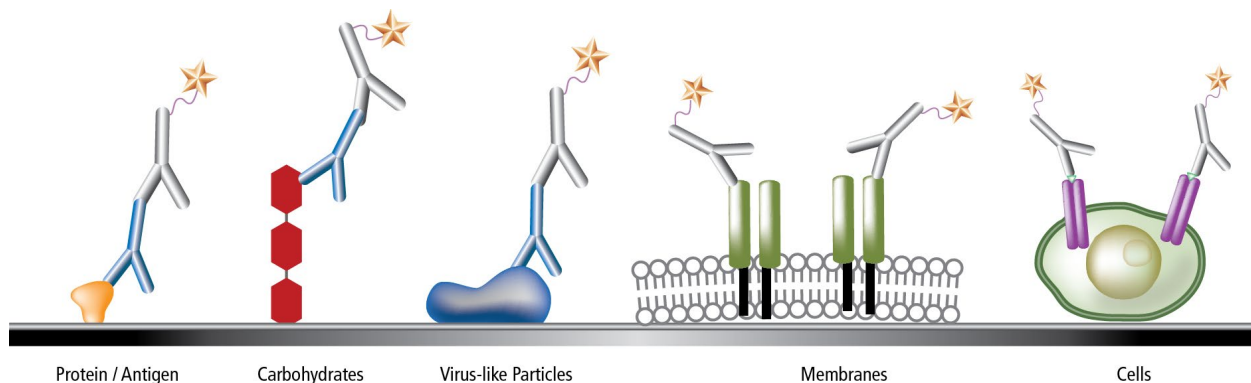
Some of the different sandwich immunoassay formats that may be developed using MSD plates are illustrated below.

**Typical immunoassays:** The graphic below provides examples of different assay formats that are possible using antibodies as capture reagents on MSD plates. (A) MSD SULFO-TAG is directly conjugated to the detection antibody. (B) Biotinylated detection antibody binds to SULFO-TAG streptavidin. (C) Detection antibody binds to SULFO-TAG conjugated anti-species antibody.



*Figure 2. Immunoassay formats using capture antibodies on MSD MULTI-ARRAY plates.*

**Immunoassays using non-antibody capture reagents:** Capture materials, such as antibodies, carbohydrates, virus-like particles, cells, peptides, lysates, or kinase substrates can be directly immobilized onto MSD plates.



*Figure 3. Examples of immunoassay formats using non-antibody molecules as capture reagents on MSD MULTI-ARRAY plates.*

# Plate Types

## Plate Surface

MSD provides plates with two different surface types: High Bind plates have a hydrophilic surface, and Standard plates have a hydrophobic surface. A combination of working electrode size and surface type determines the amount of the capture reagent that can be coated on the plate.

The table below indicates the binding capacity of the different plate types. Binding capacity was measured using IgG as the capture reagent and SULFO-TAG conjugated protein AG as the reporter. These values may vary for non-IgG proteins.

For reproducible performance across different plate lots, we recommend coating the plates at a specific concentration within the binding capacity suggested below. The recommended range of antibody concentrations for **Solution Coating** are included in the **Plate Coating** section of this insert.

*Table 2. Binding Capacities*

Plate Type	Surface Type	Binding Capacity (IgG)	
		96-well, 1 spot	384-well, 1 spot
High Bind Plate	Hydrophilic	5.0 pmol/well	1.0 pmol/well
Standard Plate	Hydrophobic	1.0 pmol/well	0.2 pmol/well

Standard plates tend to offer higher sensitivity while high-bind plates can facilitate the measurement of analytes at higher concentrations. Standard plates frequently exhibit lower nonspecific binding, especially with complex sample matrices. Both plate types can be tested during assay development to determine the optimal plate surface for an assay.

## Instrument Compatibility

Standard and High Bind Plates are compatible with MSD instruments according to the table below. SECTOR plates are read multiple wells at a time, and QuickPlex plates are read one well at a time.

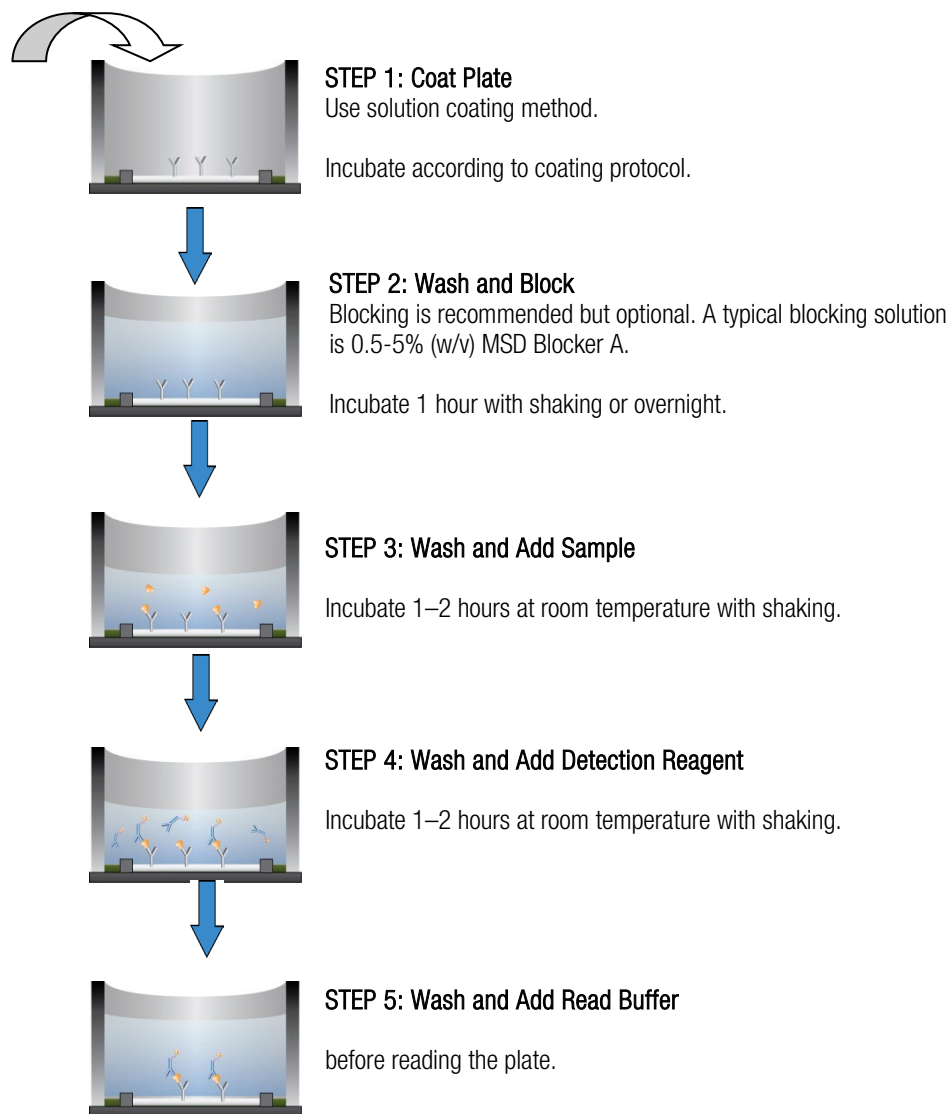
*Table 3. Instrument compatibility*

MSD Instrument	Time and Cycle	Plate Type			
		96-well SECTOR Plates	384-well SECTOR Plates	96-well QuickPlex Plates	96-well QuickPlex Ultra Plates
MESO SECTOR® S 600MM	Read Cycle	6 sectors of 4×4 well arrays	6 sectors of 8×8 well arrays	N/A	N/A
	Read Time	1 min, 10 s	1 min, 10 s		
MESO® QuickPlex SQ 120MM	Read Cycle	24 sectors of 2×2 well arrays	N/A	One well at a time	N/A
	Read Time	1 min, 30 s		2 min, 45 s	
MESO QuickPlex Q 60MM	Read Cycle	N/A	N/A	One well at a time	One well at a time
	Read Time			2 min, 45 s	1 min, 15 s

NA = not applicable

# General Workflow

The general steps in an MSD assay and the recommended volumes are depicted below. Certain steps may be combined or omitted based on assay performance and requirements.



*Figure 4. Schematic diagram of the typical steps in an MSD assay.*

*Table 4. Recommended volumes*

	Calibrator/Sample/Detection Reagent Volume	Blocking Solution/Read Buffer Volume
96-well Assay	25 $\mu$ L/well	150 $\mu$ L/well
384-well Assay	10 $\mu$ L/well	40 $\mu$ L/well

# Plate Coating

MSD plates may be solution-coated. Solution coating MSD plates is similar to coating ELISA plates.

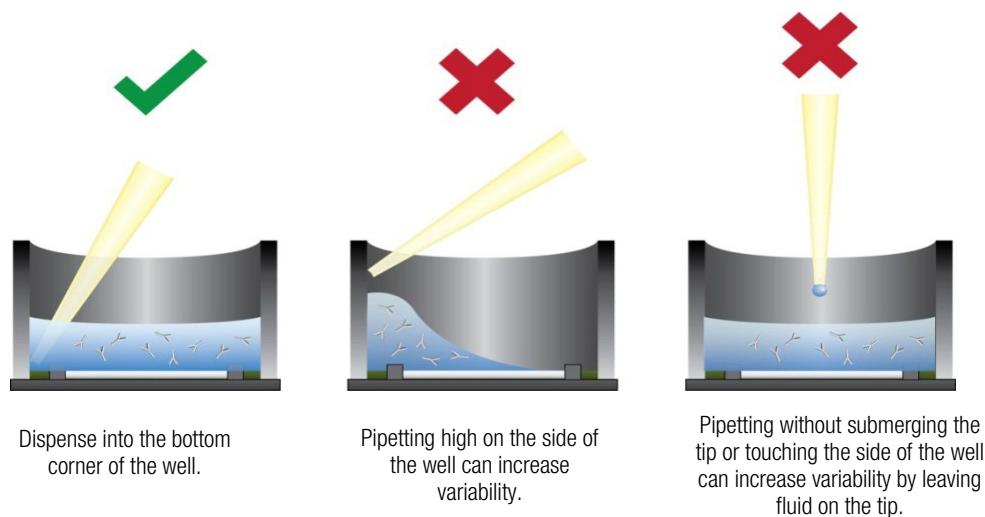
## Solution Coating Protocol

1. Prepare antibody solution in PBS. The recommended coating concentration range for solution coating antibodies is 1–20  $\mu\text{g}/\text{mL}$ .
2. Add an appropriate volume of diluted antibody directly to the bottom corner of each well (Figure 5). Tap the plate firmly to ensure that the solution covers the bottom of each well evenly.
3. Seal the plate with an adhesive plate seal and incubate overnight at 2–8 °C. Do not shake the plate during incubation.

*Table 5. Coating volumes*

	Coating Buffer	Coating Volume
96-well	PBS	25–40 $\mu\text{L}/\text{well}$
384-well	PBS	15–25 $\mu\text{L}/\text{well}$

## Solution Coating of Antibodies



*Figure 5. Appropriate solution-coating technique.*

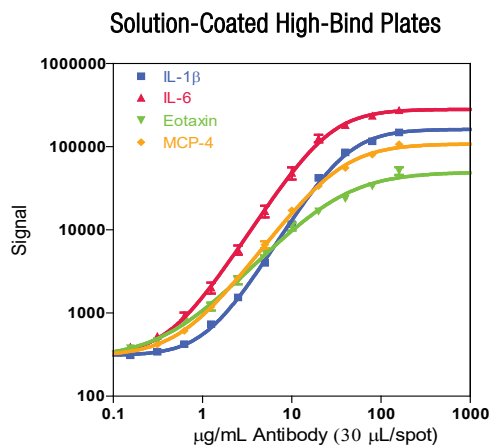
Helpful tips for solution coating MSD MULTI-ARRAY plates is illustrated above. The protocol is the same for High Bind and Standard plates.

### Notes:

- Prepare at least 20% excess volume of coating solution to account for dead volume requirements.
- Do not add detergent to coating buffer for solution coating.
- Other coating buffers, including HEPES, can be used. Coating buffer without capture reagent can be used as an assay background control provided the plate is blocked after coating.

## Examples of Antibodies Coated on High Bind Plates

Four different mouse antibodies (IgG) raised against human cytokines were immobilized on High Bind plates by solution coating (30  $\mu\text{L}$ /well). After coating, the plates were washed and the amount of antibody immobilized on the surface was measured using protein A/G labeled with SULFO-TAG (Figure 6). The maximum signal is dependent on the binding of protein A/G to the antibody and the total amount of antibody immobilized.

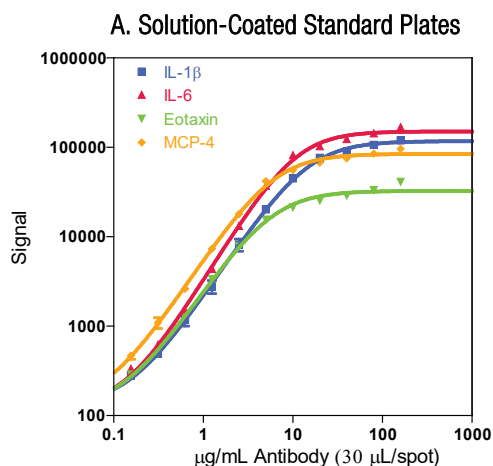


*Figure 6.* Signals from solution-coated antibodies on High Bind plates are compared for four different mouse antibodies raised against human cytokines.

	50% of Max Signal with Solution Coating			
	IL-1 $\beta$	IL-6	Eotaxin	MCP-4
$\mu\text{g/mL}$	41	26	35	35
pmol/well	8.2	5.2	6.9	6.9

## Examples of Antibodies Coated on Standard Plates

Four different mouse antibodies (IgG) raised against human cytokines were immobilized on Standard plates by solution coating. Solution coating results for individual antibodies are compared in Figure 7. The maximum signal is dependent on the binding of protein A/G to the antibody and the total amount of antibody immobilized.



*Figure 7. Signals from solution-coated antibodies on Standard plates are compared for four different mouse antibodies raised against human cytokines.*

50% of Max Signal with Solution Coating				
	IL-1β	IL-6	Eotaxin	MCP-4
μg/mL	86	132	41	65
pmol/well	2.9	4.4	1.4	2.2

## Coating Non-Antibody Molecules

Antigens, peptides, carbohydrates, polysaccharides, and membranes have been successfully immobilized on MSD plates. A range of coating concentrations for non-antibody molecules should be tested during assay optimization. The electrical charge on small proteins and peptides may play a role in determining which plate surface (Standard or High Bind) provides optimal coating.

### Cells and Membranes

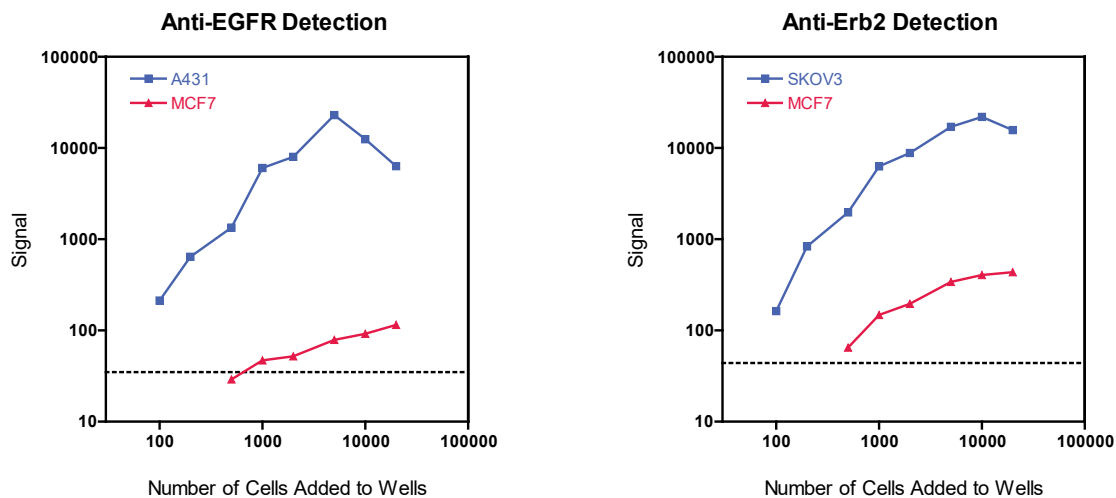
Cells and membranes can be immobilized on MSD High Bind plates by passive adsorption, i.e., cells immobilize directly on the carbon surface without the use of binding agents. Both adherent and suspension cells will bind to the electrode, but they exhibit different binding efficiencies. We recommend sealing the plates with gas-permeable seals during cell coating.

Whole-cell binding assays have been used to determine protein-ligand binding or antibody binding to cell surface protein/receptors, test the efficacy of an inhibitor of ligand binding, detect changes in the expression of a cell surface protein or receptor following treatment of cells, and measure neutralizing antibody (NAb) binding in immunogenicity studies. A cell-surface protein or receptor may be detected directly by using a SULFO-TAG conjugated protein or a SULFO-TAG conjugated antibody.

## Example Cell binding Assay Protocol

1. Solution coat target cells on an MSD High Bind plate. Seal the plate with gas-permeable seals and incubate for 2 hours at 37 °C in a humidified CO<sub>2</sub> incubator.
2. Add 50 µL/well of blocking solution. Incubate for 30 min with gentle shaking.
3. Remove the cells and blocking solution using a multichannel pipet. Add 25 µL/well detection antibody solution. Incubate 1 hour with gentle shaking.
4. Wash twice with 300 µL/well PBS using a multichannel pipet. Add 150 µL/well of Read Buffer T Surfactant Free (R92TD) and analyze.

Illustrated below are results obtained by directly coating A431 or SKOV3 cells on MSD High Bind plates and detecting with SULFO-TAG Anti-EGFR Antibody or SULFO-TAG Anti-ErbB2 Antibody, respectively. MCF7 cells were used as negative controls. With both targets, the signals increased gradually as the number of cells/well increased. At very high cell concentrations, the signals leveled off.

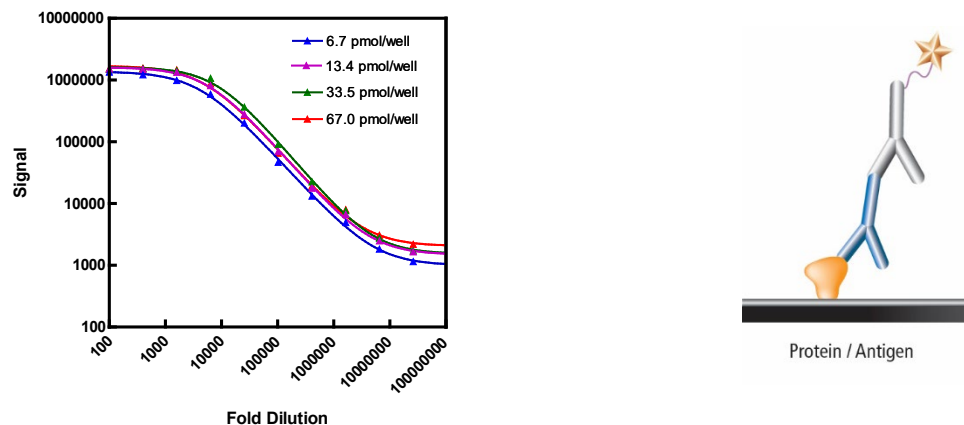


*Figure 8. Cell binding assay on MSD High Bind plates.*

## Peptides

Peptides may be coated directly or first conjugated to carrier proteins such as BSA or ovalbumin and then captured on MSD plates. The volumes specified for antibody coating may be used for non-antibody molecules when solution coating, but the concentrations should be adjusted according to the **binding capacity** of the plates. The response curve below illustrates how coating concentrations affect signals. Different concentrations of a peptide (6.7, 13.4, 33.5, and 67.0 picomoles/well) were coated on MSD plates (Figure 9). Mouse serum containing antibodies raised against the peptide was titrated against all peptide concentrations.

Please see the Appendix for the formula for calculating the picomoles of protein/peptide coated on the spot.



**Figure 9.** Titration of mouse antiserum on MSD plates coated with different concentrations of a peptide used as the capture reagent. Assay diagram shows the use of a SULFO-TAG labeled secondary anti-mouse antibody used to detect mouse serum containing antibodies.

See references below for details on studies that have used MSD plates coated with non-antibody molecules.

**Table 6.** References of coating MSD plates with non-antibody molecules

Coating Molecule	References
Synthetic peptides	Wu, et al. Characterization of the epitope for anti-human respiratory syncytial virus F protein monoclonal antibody 101F using synthetic peptides and genetic approaches. <i>J Gen Virol.</i> 2007;88:2719-23.
Polysaccharides	Goldblatt, et al. Comparison of a new multiplex binding assay versus the enzyme-linked immunosorbent assay for measurement of serotype-specific pneumococcal capsular polysaccharide IgG. <i>Clin Vaccine Immunol.</i> 2011;18:1744-51.
Polysaccharides	Marchese, et al. Optimization and validation of a multiplex, electrochemiluminescence-based detection assay for the quantitation of immunoglobulin G serotype-specific antipneumococcal antibodies in human serum. <i>Clin Vaccine Immunol.</i> 2009;16:387-96.
Cells	Lu, et al. A high throughput electrochemiluminescent cell-binding assay for therapeutic anti-CD20 antibody selection. <i>J Immunol Methods.</i> 2006;31;314:74-9.
Cells	Pang, et al. Improved detection of cell surface proteins using an electrochemiluminescent cell-binding assay. <i>J Immunol Methods.</i> 2010;31;362:176-9.
Antigens	Mao, et al. Spatially addressed combinatorial protein libraries for recombinant antibody discovery and optimization. <i>Nat Biotechnol.</i> 2010;28:1195-202.
Lysates	Gillardon F, et al. ATP-competitive LRRK2 inhibitors interfere with monoclonal antibody binding to the kinase domain of LRRK2 under native conditions. A method to directly monitor the active conformation of LRRK2? <i>J Neurosci Methods.</i> 2013 Mar 30;214(1):62-8.
Lysates	Chung, et al. A well-based reverse-phase protein array applicable to extracts from formalin-fixed paraffin-embedded tissue. <i>Proteomics Clin Appl.</i> 2008;2:1539-47.

# Detailed Sandwich Immunoassay Protocol

Begin this protocol with an MSD plate coated according to the instructions in the **Plate Coating** section. Suggestions for plate layouts for assay optimization and development are provided in the Appendix. This section contains a generalized assay protocol as a starting point for immunoassay development on the MSD platform.

Before beginning the assay, the detection antibody must be conjugated with SULFO-TAG, a necessary component of all MSD assays. The primary detection antibody may be directly conjugated with MSD SULFO-TAG NHS-ester. Alternatively, a secondary SULFO-TAG labeled anti-species antibody can be used in conjunction with an unlabeled primary detection antibody. Available SULFO-TAG labeled anti-species antibodies and the protocol for SULFO-TAG conjugation can be found at [www.mesoscale.com](http://www.mesoscale.com)<sup>®</sup>.

The following is an example of a typical assay format for a 96-well plate. Refer to the Appendix for **Alternative Protocols**.

## STEP 1: Coat Plate

1. Coat the plate following the instructions provided in the **Plate Coating** section.

## STEP 2: Block (optional). Prepare Calibrators/Controls.

1. Add 150  $\mu\text{L}$ /well of blocking solution. A typical blocking solution is 0.5-5.0% (w/v) MSD Blocker A.
2. Seal the plate with an adhesive plate seal and incubate for 30 minutes to 1 hour with shaking at room temperature (or at 2–8 °C overnight).
3. Prepare calibrators and controls during this time. Refer to the Appendix for guidance on calibrators.

**Note:** MSD Blocker A Kit (catalog #R93AA-2) contains the materials to prepare a 5% (w/v) blocking solution in PBS. For phosphoprotein assays, Tris Wash Buffer should be used instead of PBS.

## STEP 3: Wash, Add Sample, and Prepare Detection Antibody Solution.

1. Wash the plate three times with MSD Wash Buffer.
2. Dispense 25–50  $\mu\text{L}$ /well of diluted calibrators, controls, or samples to the bottoms of the wells.
3. Seal the plate and incubate at room temperature with shaking for 1–2 hours. The exact time necessary will vary by application and should be determined experimentally.
4. Prepare the detection antibody during this time. You will need at least 3 mL of detection antibody for each 96-well plate. In a 15 mL tube, combine antibody dilution buffer + SULFO-TAG conjugated detection antibody. You may use 1% (w/v) MSD Blocker A in PBS, PBS + 0.05% Tween 20 (PBS-T), or TBS-T as an antibody dilution buffer. MSD offers Diluent 100 (catalog #R50AA-4), which contains a blend of stabilizers and protein in PBS and is a suitable antibody diluent for this purpose.

## STEP 4: Wash, Add Detection Antibody, and Prepare Read Buffer.

1. Wash the plate three times with MSD Wash Buffer.
2. Add 25  $\mu\text{L}$ /well of detection antibody solution to the bottom corner of wells.
3. Seal the plate and incubate at room temperature with shaking until the binding equilibrium is achieved. This usually takes 1 hour, but the exact time needed will vary by application and should be determined experimentally.
4. Prepare read buffer during this time.

**Note:** MSD offers a variety of read buffers to support different assay and plate types. Contact Scientific Support or your FAS for guidance on selecting the read buffer.

#### STEP 5: Wash and Add Read Buffer.

1. Wash the plate three times with MSD Wash Buffer.
2. Carefully add 150  $\mu\text{L}$ /well of the selected read buffer, being careful to avoid making any bubbles. We recommend using the reverse pipetting technique.

#### STEP 6: Read the plate on an MSD instrument.

## Storage and Expiration

Uncoated MSD plates can be stored at room temperature (18–25 °C). After opening the plate package, we recommend storing unused plates sealed in the original packaging to prevent exposure to dust and contaminants. The plates have a shelf-life of 30 months from the date of manufacture. The expiration date is indicated on the product label.

## Safety

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

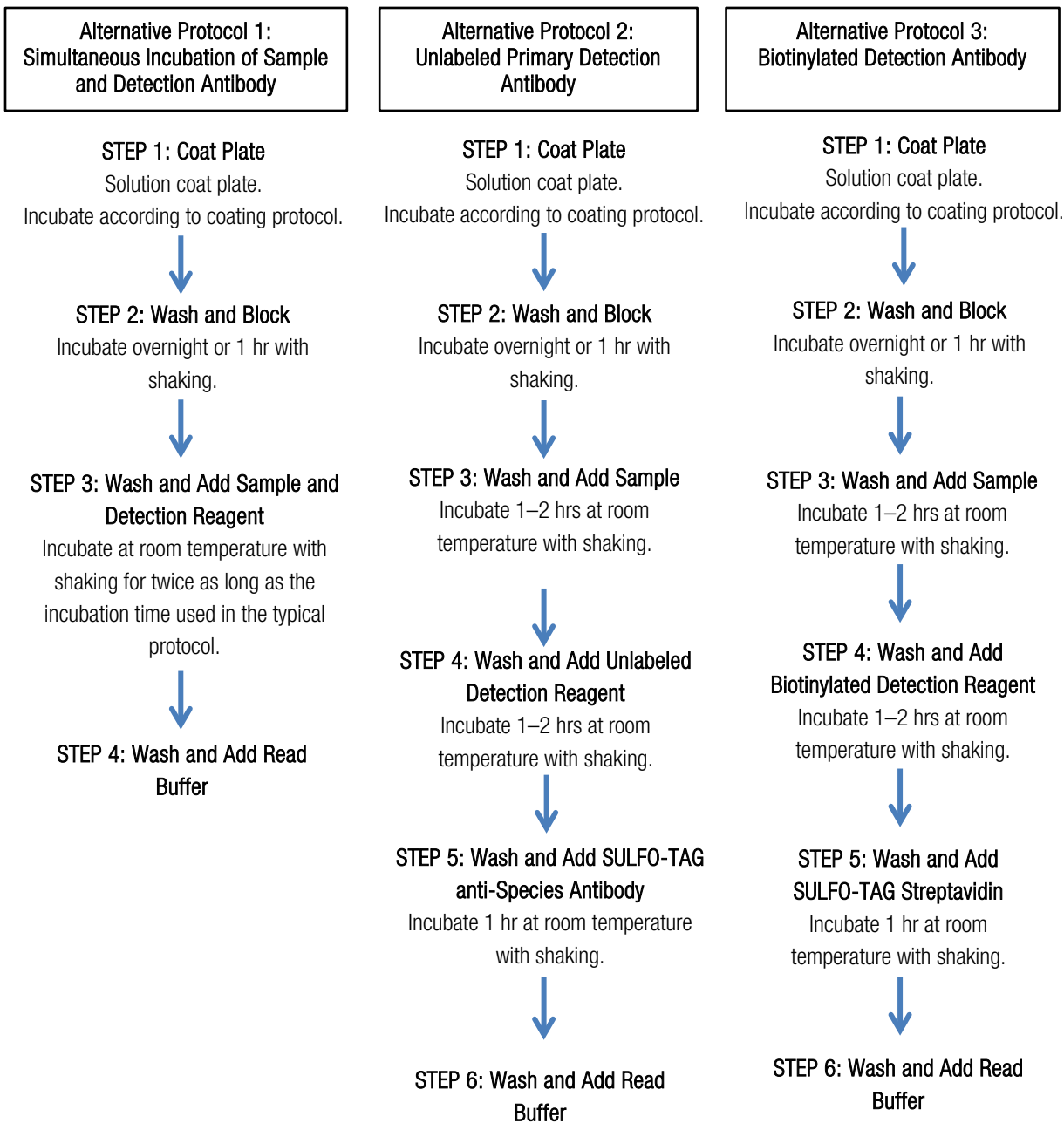
Product-specific safety information is available in the safety data sheet (SDS), which can be found on [www.mesoscale.com](http://www.mesoscale.com).

## Best Practices

- Avoid prolonged exposure of 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.
- Plate shaking should be vigorous with a rotary motion between 500 and 1,000 rpm for 96-well plates and 1,000–1,500 rpm for 384-well plates. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above. For long-term studies using multiple plates, it is recommended that the shaking speed and shaker model be kept consistent.
- Avoid bubbles in wells at all pipetting steps because they may lead to variable results.
- Read buffer should be at room temperature (20–26 °C) when added to the plate.
- Keep time intervals consistent between adding read buffer and reading the plate to improve interplate precision. It is recommended that an MSD instrument be prepared to read a plate before adding read buffer. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
- Do not shake the plate after adding read buffer.
- Remove plate seals before reading the plate.
- Use a new adhesive plate seal for all incubation steps. Avoid reusing plate seals.
- Avoid excessive drying of the plate during the washing step, especially if working inside a laminar flow hood such as a biosafety cabinet or other high air-flow environment. Cover the plate with a new plate seal immediately after washing to protect from airflow and add solutions to the plate as soon as possible.
- Bring frozen diluents to room temperature in a 22–25 °C water bath before use. If a controlled water bath is not available, thaw at room temperature. Diluents may also be thawed overnight at 2–8 °C.

# Appendix

## Alternative Protocols



### Notes:

Alternative protocol 2 requires capture antibody (Step 1) and detection antibody (Step 4) from different species.

The unlabeled detection antibody and SULFO-TAG anti-species antibody (for alternative protocol 2) or the biotinylated detection antibody and SULFO-TAG Streptavidin (for alternative protocol 3) can be blended ≤15 minutes before adding them to the assay plate.

After the addition of the read buffer, the plates should be analyzed on an MSD imager.

## Peptide Coating Calculation

The following formula can be used to calculate the picomoles of protein or peptide coated on each spot of the MSD plate.

$$1000 \times \frac{\text{protein or peptide concentration } (\mu\text{g/mL}) \times \text{coating volume } (\mu\text{L})}{\text{molecular weight of protein or peptide (Da)}} = \text{picomoles of protein or peptide per spot}$$

## Calibrators and Controls

### Intracellular signaling markers

- If recombinant calibrators are not available, an appropriate cell model may be developed to be used for positive and negative controls in the assay. MSD offers cell lysate controls for a range of phosphoprotein and signaling pathway kits. Further details on cell lysate controls are available at [www.mesoscale.com](http://www.mesoscale.com).
- Positive and negative cell lysates may be used neat or diluted. A good starting concentration is 20  $\mu\text{g/well}$  lysate with 2-fold dilutions.
- MSD plates are compatible with most sample matrices. Avoid reagents that could denature the capture antibody (general guidelines are: ionic detergents such as SDS should be  $<0.1\%$ ; reducing agents such as DTT should be  $<1$  mM in the sample when added to the well). If high concentrations of potentially denaturing agents are required for extraction, the sample should be diluted in a suitable buffer lacking denaturing agent before adding to the antibody-coated plate.
- For cell lysates, protease and phosphatase inhibitors may be required in the extraction buffer to preserve the integrity of the analyte.
- Keep diluted samples on ice until use.

### Secreted biomarkers

- For secreted biomarker assays, a calibrator or a recombinant protein that is representative of the native protein can be used for the calibration curve. A good starting concentration is 10 ng/mL for the high calibrator and 0.1 ng/mL for the low calibrator concentration. For initial studies, calibrators can be prepared in 1% Blocker A in PBS. Later, we recommend testing an 8-point titration curve for biomarker assays (for examples, see the **Plate Layouts** section in the Appendix) and to optimize the calibrator diluent if required.
- Denaturing agents should be avoided or kept to a minimum.
- Keep diluted samples on ice until use.

## Assay Optimization

The following steps may be taken to optimize an assay:

Test a full range of titrations for the capture and detection antibodies. This may be useful in increasing the specific signals of the assay as well as for reducing background. See the Assay Variables section below for guidance.

The maximum signal for the assay should be less than 1 million counts because the top of the dynamic range of MSD plate readers is reached at 1 to 1.5 million counts for single spot plates.

If there is high background ( $\geq 1,000$  counts) in the absence of sample, try different blocking solutions or test lower antibody concentrations to reduce the background and maintain the desired assay performance. Alternatively, different blockers (such as MSD Blockers D-M, D-R, D-G, or D-B) can be added to the detection antibody solution at a final concentration of 0.1%. MSD offers a Complete Blocker Kit (catalog #R93AB-1) for testing different blocking agents when background signals need to be reduced. Expected read buffer background signal levels for MSD imagers are around 25–100 counts when using 1X read buffer (in the absence of other assay components).

Vary the incubation times used to optimize signals. The sample/calibrator may be mixed with the detection antibody for simultaneous incubation, which will simplify the protocol but might not result in optimal signals. Coincubation of samples and detection antibodies can result in a hook effect at high analyte concentrations.

We recommend shaking the plates during incubation at  $\geq 500$  rpm. Shaking increases diffusion kinetics and allows the binding equilibrium to be reached in a shorter period. Shaking speed should be kept consistent to minimize variability.

For better recovery and dilution linearity in specific matrices such as serum, plasma, urine, or CSF, it may be useful to test different diluents that are more representative of the sample. MSD offers a range of diluents for serum, plasma, urine, and CSF applications.

## Assay Variables

Suggestions for optimizing three assay variables have been provided below.

Variable	Solution Coating
Capture antibody concentration	4, 2, 1, 0 $\mu\text{g/mL}$
Detection antibody concentration	2, 1, 0.5, 0.25 $\mu\text{g/mL}$
Incubation format	Sequential or simultaneous

## Typical Plate Layouts for Assay Optimization

**Plate layout 1:** For titration of capture and detection antibodies using a known or matched antibody pair.

### Step 1: Capture antibody titration (30 $\mu$ L/well)

*Figure 10a.* For titration of capture and detection antibodies

	1	2	3	4	5	6	7	8	9	10	11	12
A	30 $\mu$ L at 4 $\mu$ g/mL for solution coating						30 $\mu$ L at 2 $\mu$ g/mL for solution coating					
B												
C												
D												
E	30 $\mu$ L at 1 $\mu$ g/mL for solution coating						0 $\mu$ g/mL capture Ab					
F												
G												
H												

### Step 2: Sample addition (25 $\mu$ L/well in appropriate diluent)

*Figure 10b.* For intracellular markers

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive control lysate		Negative control lysate		Lysis buffer No lysate		Positive control lysate		Negative control lysate		Lysis buffer No lysate	
B												
C												
D												
E												
F												
G												
H												

*Figure 10c.* For secreted markers

	1	2	3	4	5	6	7	8	9	10	11	12
A	High calibrator concentration		Mid calibrator concentration		Zero calibrator concentration		High calibrator concentration		Mid calibrator concentration		Zero calibrator concentration	
B												
C												
D												
E												
F												
G												
H												

**Step 3: Detection antibody titration (25  $\mu$ L/well of antibody solution diluted in antibody diluent)**

*Figure 10d. Antibody titration*

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.0 $\mu$ g/mL detection Ab											
B	1.0 $\mu$ g/mL detection Ab											
C	0.5 $\mu$ g/mL detection Ab											
D	0.25 $\mu$ g/mL detection Ab											
E	2.0 $\mu$ g/mL detection Ab											
F	1.0 $\mu$ g/mL detection Ab											
G	0.5 $\mu$ g/mL detection Ab											
H	0.25 $\mu$ g/mL detection Ab											

**Plate Layout 2:** For titration of lysates/calibrator to determine the approximate sensitivity of the assay.

**Step 1: Capture antibody titration (30  $\mu$ L/well)**

*Figure 10e. Titration of lysates/calibrator*

	1	2	3	4	5	6	7	8	9	10	11	12
A	30 $\mu$ L at 4 $\mu$ g/mL for solution coating			30 $\mu$ L at 2 $\mu$ g/mL for solution coating			30 $\mu$ L at 1 $\mu$ g/mL for solution coating			30 $\mu$ L at 0.5 $\mu$ g/mL for solution coating or Uncoated control		
B												
C												
D												
E												
F												
G												
H												

**Step 2: Sample addition (25  $\mu$ L/well in appropriate diluent)**

*Figure 10f. For intracellular markers*

	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive lysate, e.g., 1 $\mu$ g/ $\mu$ L											
B	Positive lysate, e.g., 0.5 $\mu$ g/ $\mu$ L											
C	Positive lysate, e.g., 0.25 $\mu$ g/ $\mu$ L											
D	Positive lysate, e.g., 0.125 $\mu$ g/ $\mu$ L											
E	Negative lysate, e.g., 1 $\mu$ g/ $\mu$ L											
F	Negative lysate, e.g., 0.5 $\mu$ g/ $\mu$ L											
G	Negative lysate, e.g., 0.25 $\mu$ g/ $\mu$ L											
H	Lysis buffer											

**Figure 10g.** For secreted markers

	1	2	3	4	5	6	7	8	9	10	11	12
A	Calibrator 1, e.g.,50 000 pg/mL											
B	Calibrator 2, e.g.,10 000 pg/mL											
C	Calibrator 3, e.g.,2000 pg/mL											
D	Calibrator 4, e.g.,400 pg/mL											
E	Calibrator 5, e.g.,80 pg/mL											
F	Calibrator 6, e.g.,16 pg/mL											
G	Calibrator 7, e.g.,3.2 pg/mL											
H	Calibrator 8, e.g.,0 pg/mL											

**Step 3: Detection antibody titration (25 µL/well of antibody solution diluted in antibody diluent)**

**Figure 10h.** Titration of detection antibody

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.0 µg/mL det Ab	1.0 µg/mL det Ab	0.5 µg/mL det Ab	2.0 µg/mL det Ab	1.0 µg/mL det Ab	0.5 µg/mL det Ab	2.0 µg/mL det Ab	1.0 µg/mL det Ab	0.5 µg/mL det Ab	2.0 µg/mL det Ab	1.0 µg/mL det Ab	0.5 µg/mL det Ab
B												
C												
D												
E												
F												
G												
H												

## Troubleshooting

### High Backgrounds

1. Nonspecific binding of SULFO-TAG conjugated detection antibody to the plate in the absence of capture antibody and sample may cause elevated background signals. Alternative assay diluents and/or blocking solutions should be tested to reduce nonspecific binding.
2. Nonspecific interactions between capture and detection antibodies may sometimes be reduced by lowering the antibody concentrations or by supplementing the detection antibody solution with different blocking agents. MSD offers a Complete Blocker Kit (catalog #R93AB-1) that can be used to test various blockers if reduced background signals are needed.
3. Background signals may be lowered by decreasing the challenge ratio of the detection antibody during SULFO-TAG conjugation.

### Low Assay Signals

1. Low assay signals may result from inefficient conjugation of the detection antibody. Poor conjugation efficiency with SULFO-TAG is often linked to the presence of substances interfering with the labeling reaction (e.g., Tris, glycine, histidine, or azide). Ensure that the antibody is in an amine-free and carrier-free buffer before conjugation. Increasing the conjugation ratio may generate higher signals. Alternate or higher affinity antibodies can also improve assay signals.

## Assay Variability and Signal Reproducibility

Several factors can affect both intra-plate and inter-plate signal reproducibility. These include:

1. **Pipetting variability:** Assay variability is often linked to pipetting differences due to equipment or differences between operators. Ensure that pipettes are calibrated and that the correct pipette tips are used. Repeater pipettes should be checked for accuracy before each dispense step.
2. **Shaking speed:** Differences in plate shaking speed can affect absolute signals since shaking increases diffusion rates and hence binding kinetics of the assay components. Shaking conditions should be kept consistent to ensure optimal signal reproducibility.
3. **Plate washing equipment:** Automated plate washers can lead to signal inconsistencies if some pins are blocked or contaminated. Ensure that plate washers are kept clean and well maintained. Rotating the plate orientation during plate washing can be useful when troubleshooting plate washer problems.
4. **Reagent storage:** Improper storage may cause reagent deterioration leading to variable assay results. The working detection antibody solution should be prepared immediately before use. All solutions must be capped when not in use. Diluted read buffer, for example, can evaporate if not capped. Diluents should be completely thawed and well mixed before use. The recommended number of freeze-thaw cycles must not be exceeded. Ensure that Blocker B powder is kept dry and at room temperature, preferably in a desiccator. Blocker B should be completely dissolved before use and there should be no visible particulate matter.
5. **Dissociation rates:** In MSD assays, the signal is generated from electrochemically stimulated SULFO-TAG conjugated molecules that are near (1 to 10  $\mu\text{m}$ ) the bottom of the well. Before the final wash step, the assay components are at or close to equilibrium. However, if the plate is left too long in wash buffer or read buffer, the assay components may start to dissociate. Since the MSD assay is a proximity assay, the signal will decrease if SULFO-TAG conjugated antibody dissociates from the other assay components on the surface. The signal decrease will not be significant for high-affinity interactions with slow off-rates ( $k_{\text{off}}$ ); however, interactions with fast off rates can result in a time-dependent signal decrease. MSD plates should therefore not be left in wash buffer, and the interval between adding read buffer and reading the plate should be kept consistent until assay stability in read buffer has been established.

## Assay Development Service Options

MSD offers Prototype Printing Services to facilitate assay development by customers. Prototype Printing Services provide the customer with a rapid and convenient way to get MSD MULTI-ARRAY and MULTI-SPOT plates coated with materials of their choice. The end-user defines coating concentration, plate type, and coating buffer.

MSD provides on-site support for assay development through its scientific support team and field application scientists. Contact Scientific Support at 1-240-314-2798 or [ScientificSupport@mesoscale.com](mailto:ScientificSupport@mesoscale.com).

A full suite of in-house assay development and sample testing services are available at MSD. Contact your Account Manager for more information.

# FAQs

## 1. Which type of plate (Standard or High Bind) should I choose for my assay?

Generally, Standard plates provide better sensitivity, and High Bind plates allow you to measure higher analyte concentrations, but there are exceptions. Both plate types can be tested during assay optimization. Use High Bind plates when coating cells.

## 2. What excipients in the capture and detection antibodies can affect my assay performance?

**For capture antibodies:** To obtain the best results with direct coating, the capture antibodies should be free of certain excipients, including gelatin, glycerol, and other chemicals and proteins that significantly alter drying conditions. Large amounts of nonspecific carrier proteins in the coating buffer will compete with the immobilization of the capture antibodies.

The presence of BSA at greater than 5-fold molar excess compromises most assays. To bind antibodies from solutions containing antibodies in serum concentrations, high levels of BSA, or other carrier proteins such as gelatin, use MSD plates coated with anti-species antibodies (e.g., goat anti-rabbit or goat anti-mouse).

**For detection antibodies:** For SULFO-TAG conjugation, the detection antibody must be free of glycerol, carrier protein, and amine-containing molecules such as azide, Tris, histidine, and glycine. Note that the product sheets from most vendors do not reveal if an affinity-purified antibody was eluted from the affinity column with glycine. In these cases, there is usually sufficient residual glycine to inhibit the labeling reaction. To eliminate Tris, glycine, histidine, or azide from the antibody storage solution, the protein should be buffer exchanged into PBS pH 7.4 to 7.9 using a spin column or other suitable technique.

If a purified, carrier-free antibody cannot be obtained for labeling purposes, an MSD SULFO-TAG conjugated anti-species secondary antibody may be used. This can be done only when the capture and detection antibodies are from different species.

MSD offers SULFO-TAG conjugation services. Contact your Account Manager for pricing.

## 3. What concentration of antibody/protein should I use for coating?

When coating antibodies, refer to the **Binding Capacity** table and suggested coating concentrations in the **Solution Coating** protocol. For other proteins, adjust for the protein molecular weight relative to IgG (150,000 Da), e.g., for a 75,000 Da protein, the range to test would be half of that used for the equivalent molar concentration. A titration of coating concentrations should be tested during assay optimization.

## 4. Does pH affect coating efficiency?

Coating is usually carried out at neutral pH unless the molecule to be coated requires a different pH to maintain stability and/or solubility. MSD's process for prototype printing is not compatible with extreme pH coating solutions.

## 5. What substances are unsuitable in coating buffer?

The presence of glycerol, gelatin, and >5-fold molar excess of BSA in the protein solution is unfavorable for coating directly on MSD plates.

## 6. Is it possible to coat if EDTA is present in the capture solution?

Yes.

## 7. Why is higher coating volume not recommended for solution coating?

Higher coating volume ( $\geq 40$   $\mu\text{L}/\text{well}$ ) means more of the well is coated with the capture molecule, which enables analyte to be captured in areas away from the active electrode. Since these areas will not generate a signal at read time, using larger volumes of coating solution has the potential to reduce assay sensitivity.

## 8. Why is detergent not added for solution coating on Standard plates?

Detergent is only required to disrupt surface tension on the hydrophobic surface of Standard plates. With solution coating, the volume of the coating solution (30  $\mu\text{L}/\text{well}$ ) is sufficiently large that surface tension is not a problem.

**9. What are the recommended concentrations when a primary detection antibody and a secondary reporter are used for the assay?**

If the primary and secondary detection antibodies are combined (see simultaneous incubation), we recommend using both at the same concentration. If there is a wash step between the primary and the secondary detection antibody, then the secondary antibody may be used at 1 µg/mL concentration.

**10. What is the recommended concentration for secondary reporters?**

SULFO-TAG Streptavidin may be used at 0.5 to 1 µg/mL. SULFO-TAG anti-species antibody may be used at 0.5 to 1 µg/mL or at a concentration similar to that of the primary detection Ab.

## Catalog Numbers

MSD Standard and High Bind plates are offered in 96-well and 384-well formats. Tables 7 and 8 list the catalog numbers for the different plates as well as the most common assay development reagents. For a complete list of assay development products, please visit our website at [www.mesoscale.com](http://www.mesoscale.com).

### Plates

*Table 7. Catalog numbers for 96-well uncoated SECTOR, QuickPlex, and QuickPlex Ultra plates.*

	10 Plates	100 Plates	500 Plates
96-well 1-Spot SECTOR Plates <sup>1</sup>	L15XA-3	L15XA-6	L15XA-7
96-well 1-Spot High Bind SECTOR Plates <sup>1</sup>	L15XB-3	L15XB-6	L15XB-7
96-well 1-Spot QuickPlex Plates <sup>2</sup>	L55XA-3	L55XA-6	L55XA-7
96-well 1-Spot High Bind QuickPlex Plates <sup>2</sup>	L55XB-3	L55XB-6	L55XB-7
96-well 1-Spot QuickPlex Ultra Plates <sup>3</sup>	L1BXA-3	-	

1. For use on SECTOR S 600MM, SECTOR S 600, QuickPlex SQ 120MM, QuickPlex SQ 120, SECTOR Imager 6000, and SECTOR Imager 2400 instruments.
2. For use on QuickPlex SQ 120MM, and QuickPlex SQ 120 instruments.
3. For use on QuickPlex Q 60MM instruments.

*Table 8. Catalog numbers for 384-well uncoated SECTOR plates.*

	15 Plates	120 Plates	510 Plates
384-well 1-Spot SECTOR Plates <sup>3</sup>	L21XA-4	L21XA-6	L21XA-7
384-well 1-Spot High Bind SECTOR Plates <sup>3</sup>	L21XB-4	L21XB-6	L21XB-7

For use on SECTOR S 600MM, SECTOR S 600, and SECTOR Imager 6000 instruments.

**Table 10. Reagents**

Reagents	Catalog #	Description
SULFO-TAG Streptavidin	R32AD-1/-5	Labeling reagent for use as a secondary reporter with biotinylated detection reagents
SULFO-TAG Anti-Mouse Antibody (Goat)	R32AC-1/-5	SULFO-TAG labeled reagent for use as a secondary reporter
SULFO-TAG Anti-Rabbit Antibody (Goat)	R32AB-1/-5	SULFO-TAG labeled reagent for use as a secondary reporter
Tris Wash Buffer (10X)	R61TX-1/-2	Buffer for washing assay plates for phosphoprotein assays
Tris Lysis Buffer	R60TX-2/-3	For use in the preparation of cell lysates
Inhibitor Pack	R70AA-1	Protease and phosphatase inhibitors for use in intracellular signaling assays
Blocker A Kit	R93AA-1/-2	Blocking buffer to reduce the nonspecific binding of proteins to the plate
Diluent 100	R50AA-2/-3/-4	For use as a detection antibody diluent
MSD GOLD™ Read Buffer B	R60AM	Ready-to-use read buffer
MSD Read Buffer T (4X), Surfactant Free	R92TD	read buffer recommended for cell-based assays
MSD Wash Buffer	R61AA	Phosphate buffered wash solution (20X)

MSD also offers a variety of blockers, secondary reporters, and diluents for use in assay development. Information and technical notes on these products are available at [www.mesoscale.com](http://www.mesoscale.com).

# Plate Diagrams

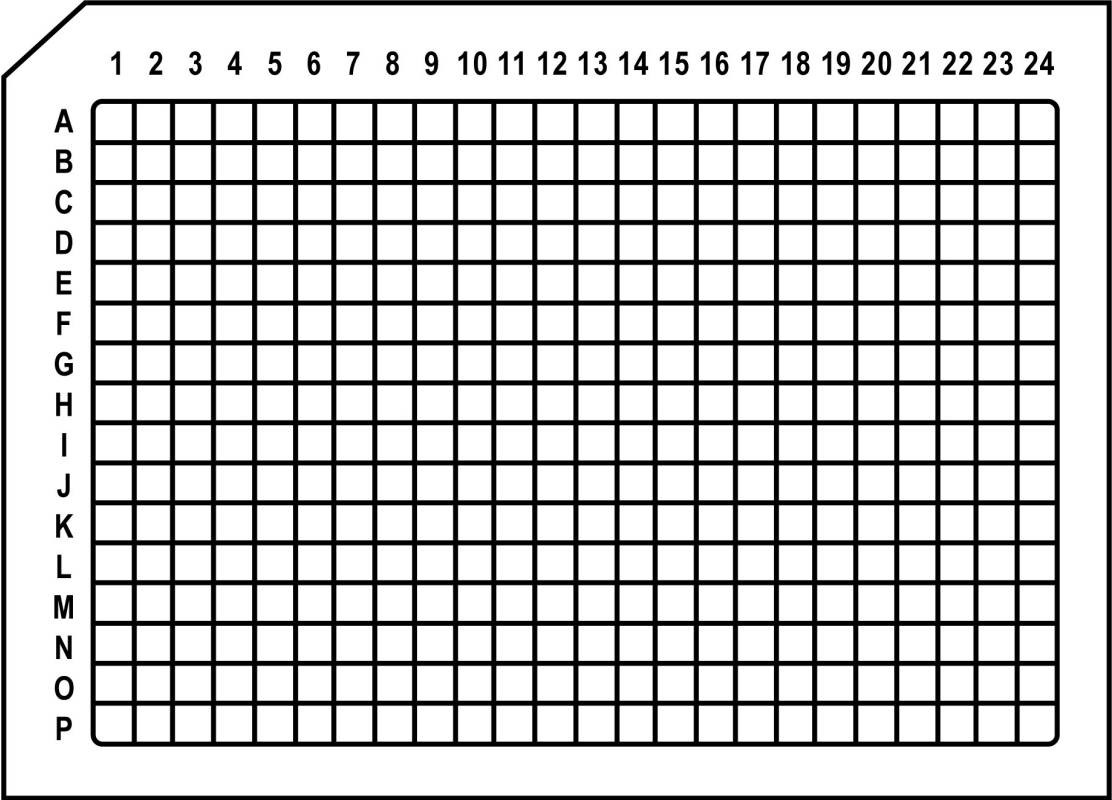
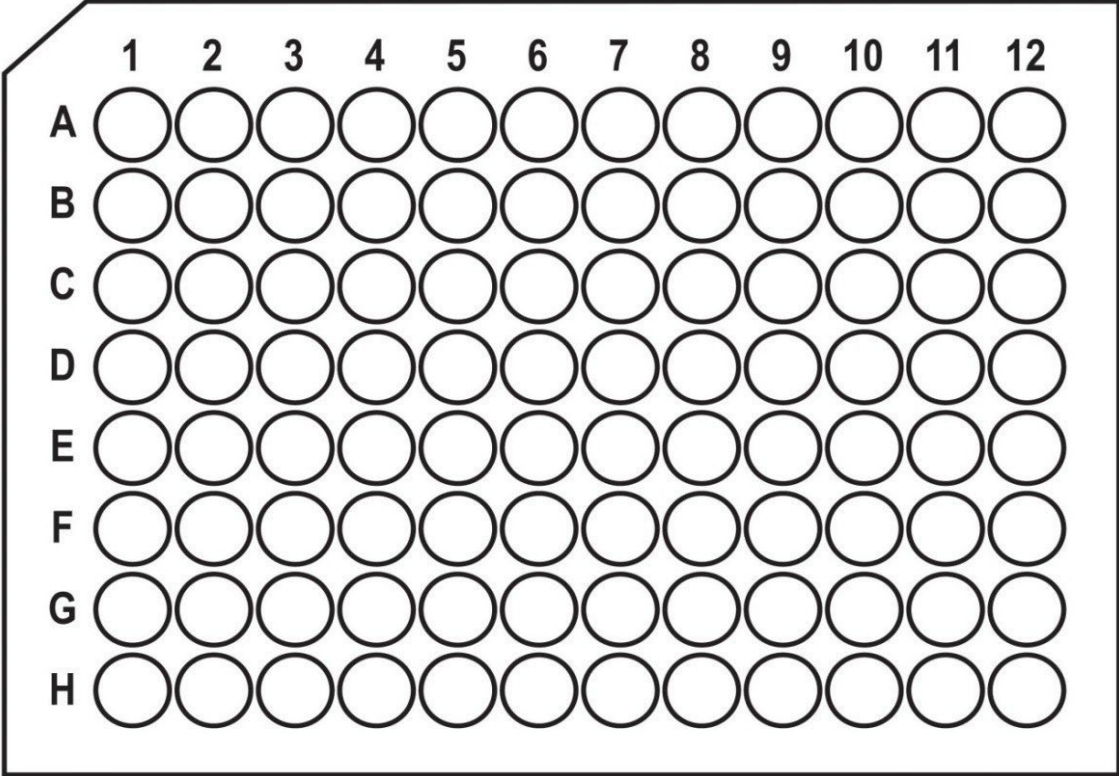


Figure 11. Plate diagrams. Similar plate layouts can be created in Excel and in the DISCOVERY WORKBENCH® software.