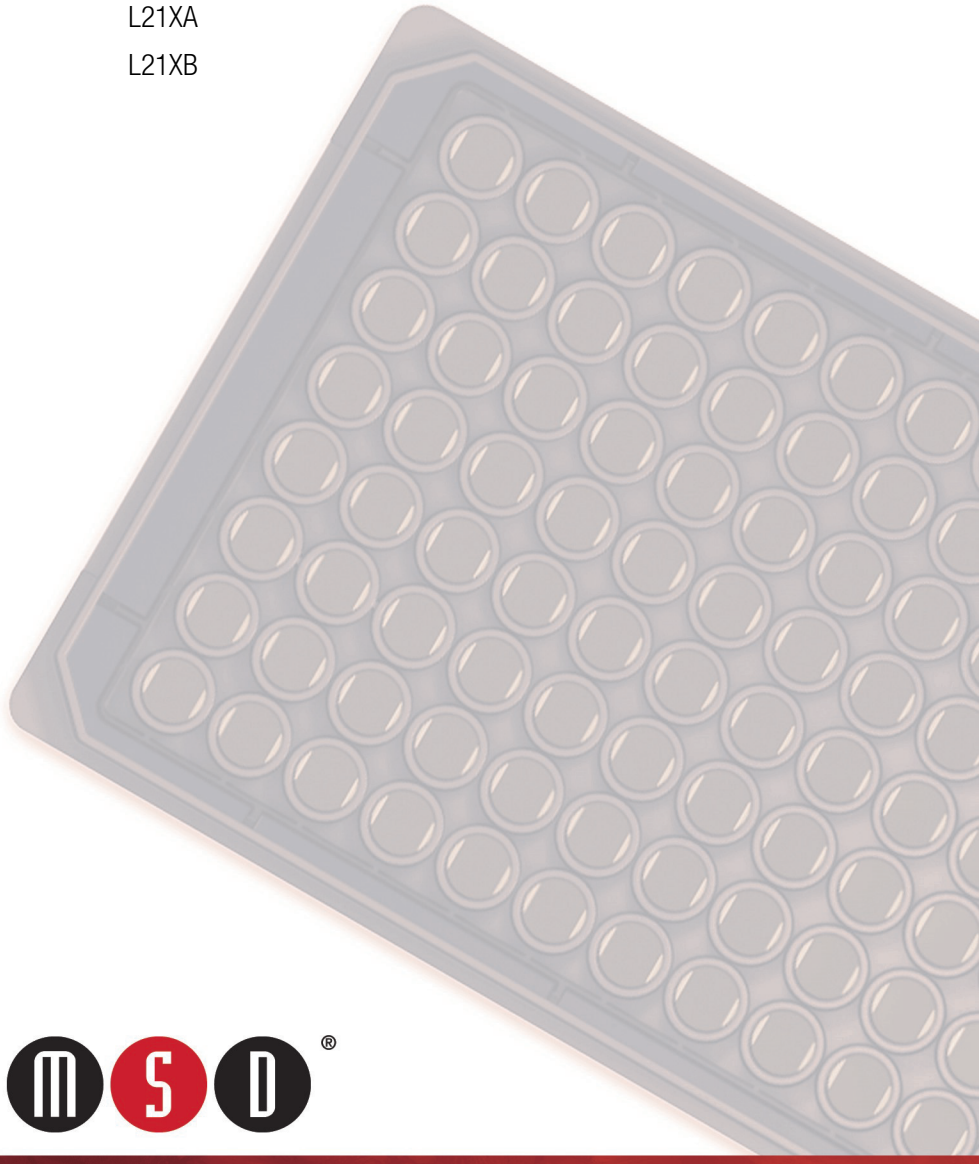
MSD® SECTOR and QuickPlex Plates

Standard and High Bind Plates

	SECTOR™ Plates	QuickPlex® Plates
96-well Standard	L15XA	L55XA
96-well High Bind	L15XB	L55XB
96-well Small Spot Standard	L45XA	
96-well Small Spot High Bind	L45XB	
384-well Standard (SI 2400)	L25XA	
384-well High Bind (SI 2400)	L25XB	
384-well Standard (SI 6000)	L21XA	
384-well High Bind (SI 6000)	L21XB	



MSD Plates

Standard and High Bind Plates

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

MESO SCALE DISCOVERY's MULTI-ARRAY® technology offers an excellent platform for the development of immunoassays for measuring biomarkers in life science preclinical research. MSD assays have ultralow detection limits, provide up to five logs of linear dynamic range, use minimal sample, and handle difficult 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.

Advantages Over Traditional Immunoassays		
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.

96-Well, 1-Spot | 96-Well, Small Spot | 384-Well, 1-Spot

Figure 1. Spot patterns 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 molecules and reagents, including antibodies, carbohydrates, virus-like particles, cells, peptides, lysates, kinase substrates, or oligonucleotides. 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 (illustrated in Figure 2) and loads the plate into an MSD® imager. The imager 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) (as shown in Figure 2).[1] The imager measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

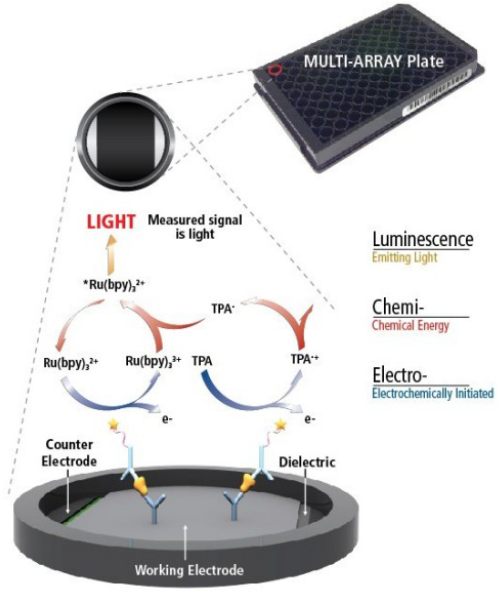


Figure 2. A top-view image of a well in the MSD MULTI-ARRAY plate depicting the electrochemiluminescence reaction and the working electrode, counter electrode, and dielectric.

[1] *Pyati R, Richter MM. ECL- Electrochemical Luminescence. Annu Rep Prog Chem Sect "C" (Physical Chemistry) 2007;103:12-78*

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 antispecies antibody.

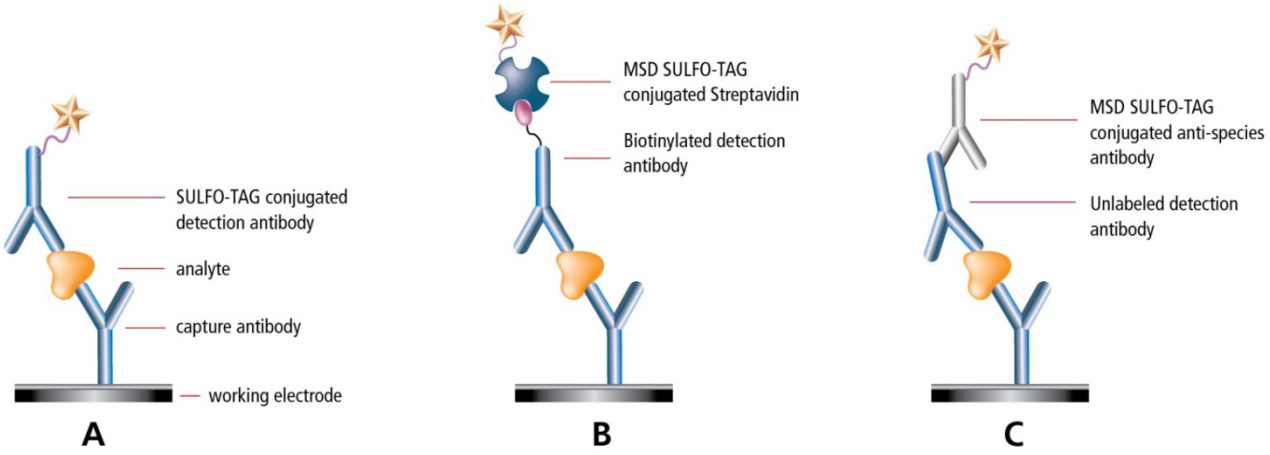


Figure 3. Immunoassay formats using capture antibodies on MSD MULTI-ARRAY plates.

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

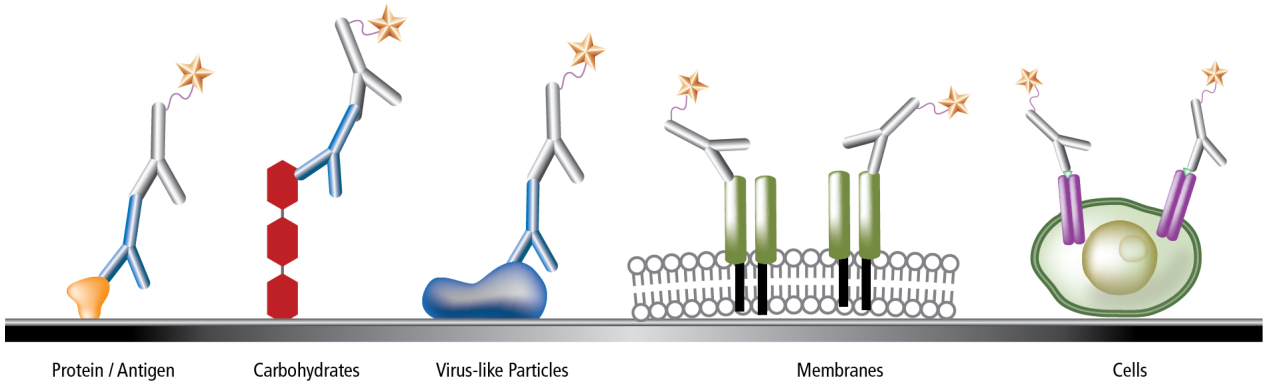


Figure 4. Examples of immunoassay formats using nonantibody molecules as capture reagents on MSD MULTI-ARRAY plates. In addition to sandwich immunoassays, other formats, such as direct binding or competitive assays, are also feasible.

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. It was measured using IgG as the capture reagent and SULFO-TAG conjugated protein A/G 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 and Spot Coating are included in the Plate Coating section of this insert.

Table 2. Binding.

		Binding Capacity (IgG)		
Plate Type	Surface Type	96-well, 1 Spot	96-well, Small Spot	384-well, 1 spot
High Bind Plate	Hydrophilic	5.0 pmol/well	1.25 pmol/well	1.0 pmol/well
Standard Plate	Hydrophobic	1.0 pmol/well	0.25 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. We recommend testing both plate types during assay development to determine the optimal type.

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.

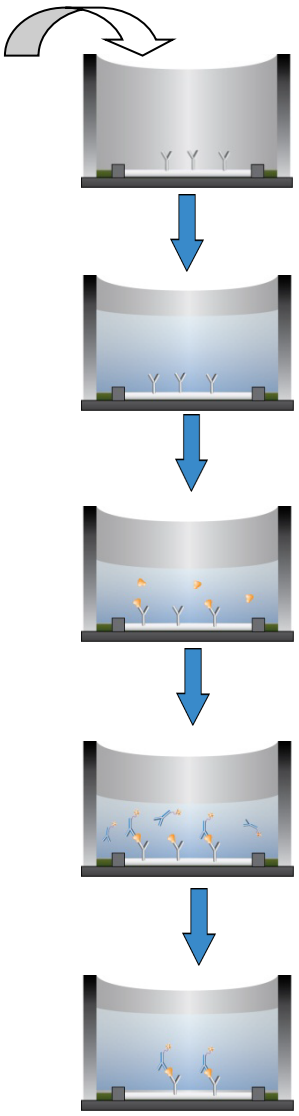
Table 3. Instruments.

MSD Instrument	Time and Cycle	Plate Type		
		96-well SECTOR[1] Plates	384-well SECTOR[1] Plates	96-well QuickPlex[1] Plates
MESO® SECTOR S 600	Read Cycle	6 sectors of 4×4-well arrays	6 sectors of 8x8 well arrays	N/A
	Read Time	1 min, 10 s	1 min, 10 s	
MESO SECTOR® S 600MM	Read Cycle	6 sectors of 4×4-well arrays	6 sectors of 8x8 well arrays	N/A
	Read Time	1 min, 10 s	1 min, 10 s	
SECTOR Imager 6000	Read Cycle	6 sectors of 4×4-well arrays	6 sectors of 8x8 well arrays	N/A
	Read Time	1 min, 10 s	1 min, 10 s	
SECTOR Imager 2400	Read Cycle	24 sectors of 2×2-well arrays	24 sectors of 4×4-well arrays	N/A
	Read Time	3 min, 30 s	3 min, 30 s	
MESO QuickPlex® SQ 120	Read Cycle	24 sectors of 2×2-well arrays	N/A	One well at a time
	Read Time	1 min, 30 s		2 min, 45 s
MESO QuickPlex SQ 120MM	Read Cycle	24 sectors of 2×2-well arrays	N/A	One well at a time
	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
	Read Time			2 min, 45 s

NA = not applicable

General Workflow

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



STEP 1: Coat Plate
Use spot coating or solution coating method.

Incubate according to coating protocol.

STEP 2: Wash and Block
Blocking is optional. A typical blocking solution is 0.5-5% (w/v) MSD Blocker A.

Incubate 1 hour with shaking or overnight.

STEP 3: Wash and Add Sample

Incubate 1–2 hours at room temperature with shaking.

STEP 4: Wash and Add Detection Reagent

Incubate 1–2 hours at room temperature with shaking.

STEP 5: Wash and Add Read Buffer

Analyze data with MSD imager.

Figure 5. Schematic diagram of the typical steps in an MSD assay.

Table 4. Recommended minimum volumes.

	Calibrator/Sample/Detection Reagent Volume	Blocking Solution/Read Buffer Volume
96-well Assay	25 µL/well	150 µL/well
384-well Assay	10 µL/well	40 µL/well

Plate Coating

MSD plates may be solution coated or spot coated. While both methods are feasible for coating antibodies, spot coating may lead to a more sensitive assay. Solution coating MSD plates is similar to coating ELISA plates, whereas, with spot coating coats, the working electrode only. Both methods are described in detail below. We recommend testing both coating methods during assay optimization.

Solution Coating of Antibodies

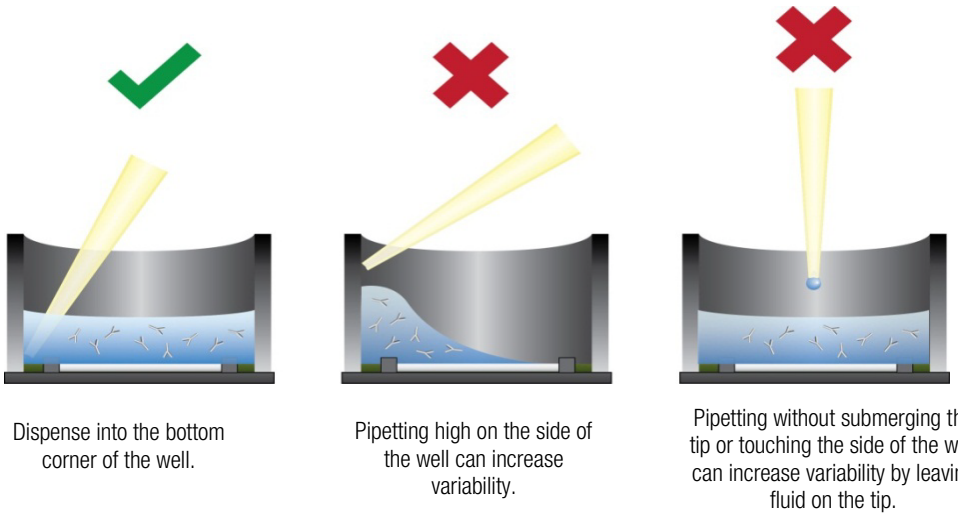


Figure 6. Appropriate solution-coating technique.

The technique for solution coating MSD MULTI-ARRAY plates is illustrated above. The protocol is the same for High Bind and Standard plates. Specifications for solution coating 96- and 384-well plates are provided in the table below.

Solution Coating Protocol

1. Prepare antibody solution in PBS. The recommended coating concentration range for solution coating antibodies is 1–20 µg/mL.
2. Add an appropriate volume of diluted antibody directly to the bottom corner of each well (Figure 6). 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 ºC. Do not shake the plate during incubation.

	Coating Buffer	Coating Volume
96-well	PBS	50 µL/well
384-well	PBS	15–25 µL/well

Spot Coating of Antibodies

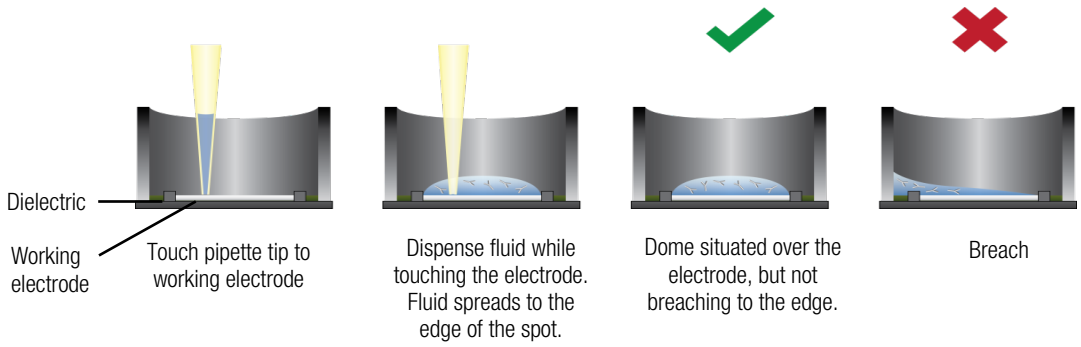


Figure 7. Appropriate spot-coating technique. The droplet of diluted antibody should be confined to the working electrode surface and not breach the dielectric barrier.

Standard and High Bind plates use different coating buffers because they have different surface hydrophobicity. When spot coating Standard plates, the coating buffer requires some surfactant to allow the solution to spread and cover the working electrode. When coated correctly, the dielectric prevents the fluid from spreading beyond the working electrode. However, if too much surfactant is used or too much fluid is dispensed, the coating solution will breach the working electrode and flow to the edge. This will lead to poor coating and more variable results. The parameters for spot coating Standard and High Bind plates are given in the table below; the general procedure is the same.

Spot Coating Protocol

1. Prepare antibody solution in the appropriate coating buffer. The recommended coating concentration range for spot coating antibodies is 4–40 µg/mL.
2. Carefully add the diluted antibody to the electrode spot in each well.
3. Incubate the plate at room temperature as specified in the table below. Do not shake the plate.

	Coating Buffer	Coating Volume/Well	Incubation Time
96-well Standard	PBS + 0.03% Triton X-100	5 µL	Overnight without sealing
96-well High Bind	PBS	5 µL	1 hour with plate sealed, or overnight without sealing
96-well, Small Spot Standard	PBS + 0.03% Triton X-100	1 µL	Overnight without sealing
96-well, Small Spot High Bind	PBS	1 µL	1 hour with plate sealed, or overnight without sealing
384-well Standard	PBS + 0.03% Triton X-100	1 µL	Overnight without sealing
384-well High Bind	PBS	1 µL	1 hour with plate sealed, or overnight without sealing

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 or when spot coating High Bind plates.
- Other coating buffers, including HEPES can be used; however, TBS has been shown to cause variability when used as a coating buffer. Coating buffer without capture reagent can be run as an assay background control.

Examples of Antibodies Coated on High Bind Plates

Four different mouse antibodies (IgG) against human cytokines were immobilized on High Bind plates by either solution coating (30 µL/well) or spot coating (5 µL/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 8). Spot vs. solution coating results for individual

antibodies are compared in Figure 9. The maximum signal is dependent on the binding of protein A/G to the antibody and the total amount of antibody immobilized.

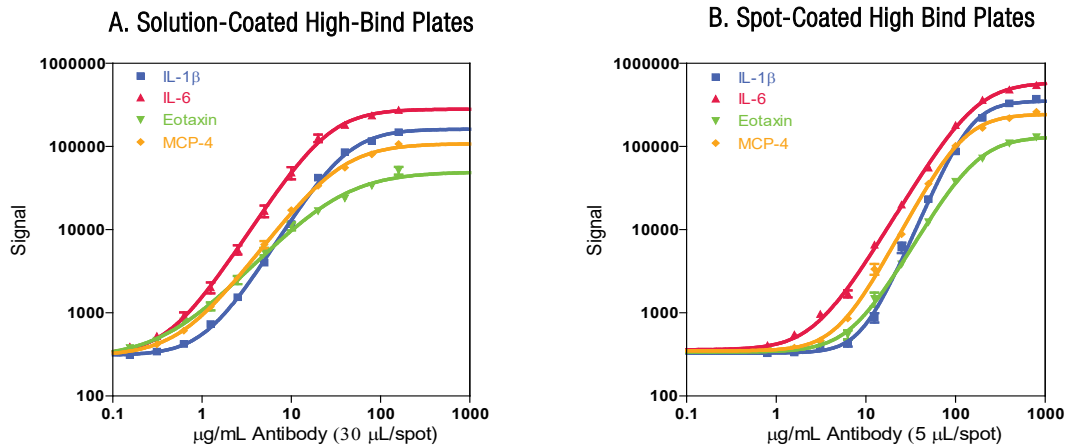


Figure 8. Signals from solution-coated (A) and spot-coated (B) antibodies on High Bind plates are compared for four different mouse antibodies raised against human cytokines.

	50% of Max Signal with Solution Coating			
	IL-1β	IL-6	Eotaxin	MCP-4
µg/mL	41	26	35	35
pmol/well	8.2	5.2	6.9	6.9

	50% of Max Signal with Spot Coating			
	IL-1β	IL-6	Eotaxin	MCP-4
µg/mL	149	167	169	122
pmol/well	5.0	5.6	5.6	4.1

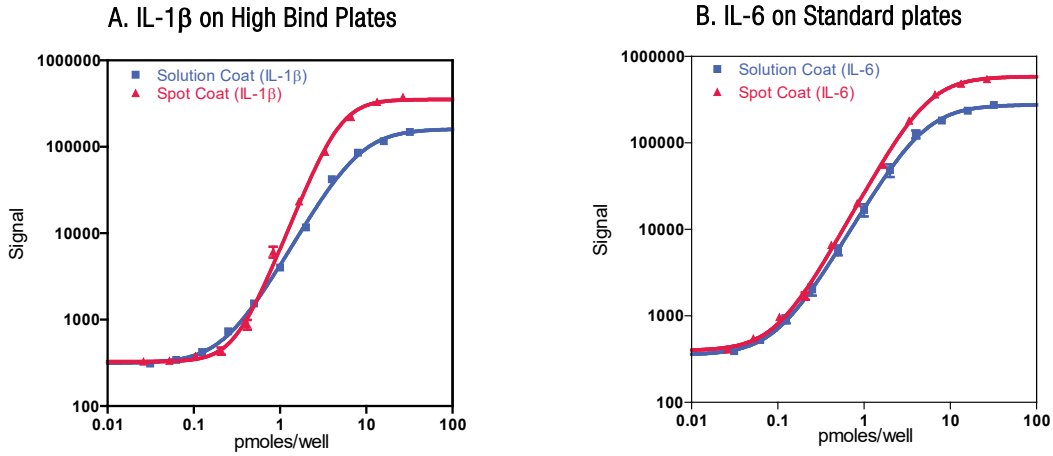


Figure 9. Comparison of solution-coated (blue) and spot-coated (red) capture antibodies shows that the spot-coated High Bind plates had 2- to 3-fold higher maximum signals compared to the solution-coated plates. This suggests that more antibodies can be immobilized through spot coating. Different antibodies have different signal profiles for solution vs. spot coating.

Examples of Antibodies Coated on Standard Plates

Four different mouse antibodies raised against human cytokines were immobilized on Standard plates by either solution coating or spot coating. 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 10). Spot vs. solution coating results for individual antibodies are compared in Figure 11. The maximum signal is dependent on the binding of protein A/G to the antibody and the total amount of antibody immobilized.

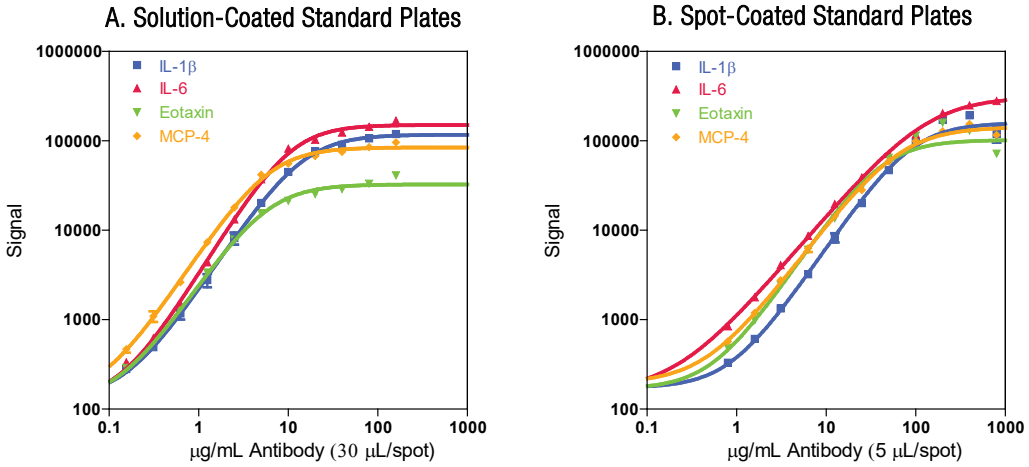


Figure 10. Signals from solution-coated (A) and spot-coated (B) antibodies on Standard plates are compared for four different mouse antibodies raised against human cytokines.

	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

	50% of Max Signal with Spot Coating			
	IL-1β	IL-6	Eotaxin	MCP-4
µg/mL	14	11	5.7	5.9
pmol/well	2.9	2.1	1.1	1.2

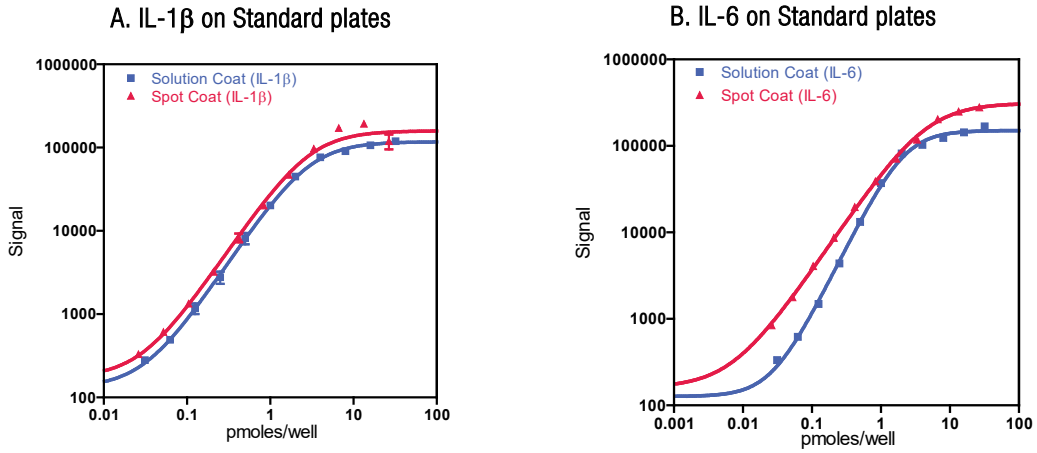


Figure 11. Comparison of solution-coated (blue) and spot-coated (red) capture antibodies shows that the spot-coated Standard plates had higher maximum signals compared to the solution-coated plates. This suggests that more antibody can be immobilized on the spot through spot coating. Different antibodies have different signal profiles for solution vs. spot coating.

Coating Non-Antibody Molecules

Antigens, peptides, carbohydrates, polysaccharides, oligonucleotides, and membranes have been successfully immobilized on MSD plates. A range of coating concentrations for nonantibody 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 ºC in a humidified CO_2 incubator.
2. Add 50 µL/well of blocking solution. Incubate for 30 minutes with gentle shaking.
3. Remove the cells and blocking solution using a multichannel pipette. Add 25 µL/well detection antibody solution. Incubate 1 hour with gentle shaking.
4. Wash twice with 300 µL/well PBS using a multichannel pipett. Add 150 µL/well read buffer 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.

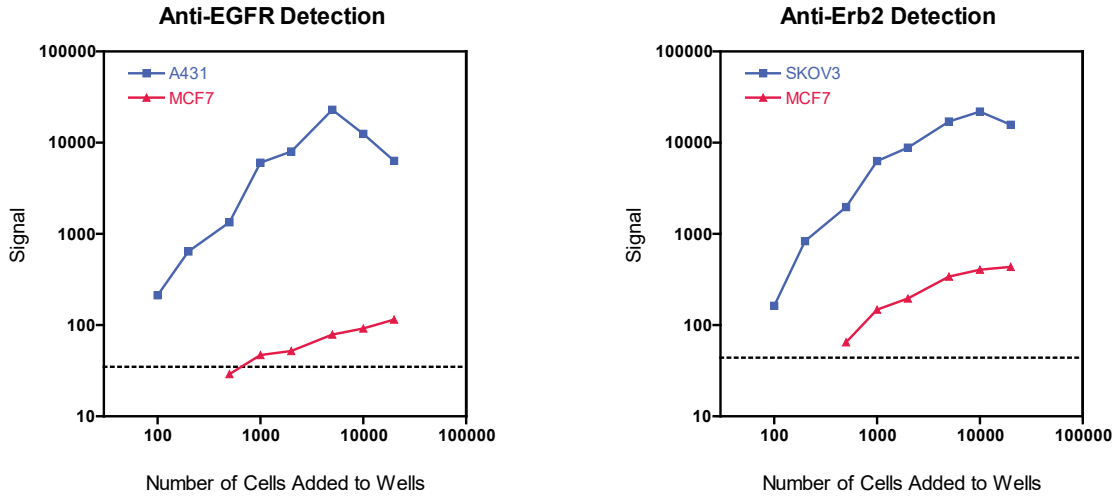


Figure 12. Cell binding assay on MSD High Bind plates.

Peptides

Peptides may be coated directly or first conjugated to carrier proteins such as BSA or ovalbumin and then captured on MSD plates. For peptides and small proteins, spot coating at 5 to 10 ng/spot has been shown to work well for different applications. The volumes specified for antibody coating may be used for nonantibody 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 13). 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.

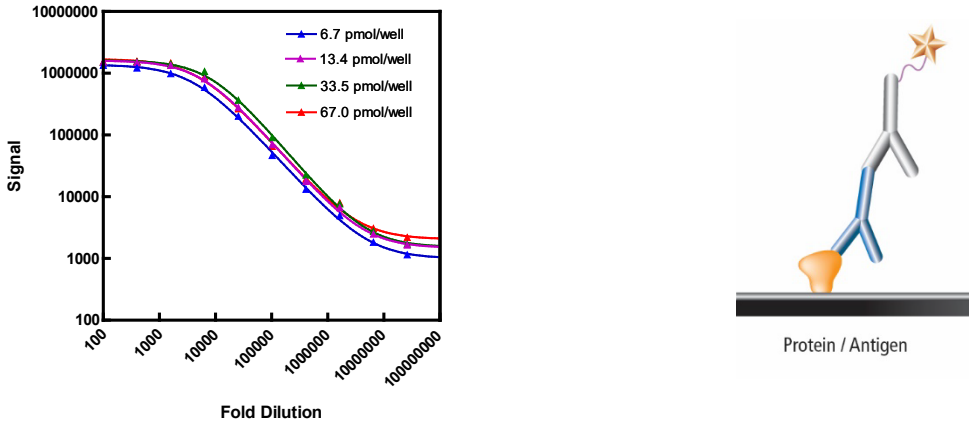


Figure 13. Titration of mouse antiserum on MSD plates coated with different concentrations of a peptide used as the capture reagent.

See references below for details on studies that have used MSD plates coated with nonantibody 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. J Gen Virol. 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. Clin Vaccine Immunol. 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. Clin Vaccine Immunol. 2009;16:387-96.
Cells	Lu, et al. A high throughput electrochemiluminescent cell-binding assay for therapeutic anti-CD20 antibody selection. J Immunol Methods. 2006;31;314:74-9.
Cells	Pang, et al. Improved detection of cell surface proteins using an electrochemiluminescent cell-binding assay. J Immunol Methods. 2010;31;362:176-9.
Antigens	Mao, et al. Spatially addressed combinatorial protein libraries for recombinant antibody discovery and optimization. Nat Biotechnol. 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? J Neurosci Methods. 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. Proteomics Clin Appl. 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. Either the primary detection antibody may be directly conjugated with MSD SULFO-TAG NHS-ester or a prelabeled secondary reporter, such as SULFO-TAG Streptavidin or SULFO-TAG antispecies antibody, which can be used coupled to an unlabeled detection reagent. The protocol for SULFO-TAG conjugation can be found at the www.mesoscale.com® website.

The following is an example of a typical assay format. Refer to the Appendix for Alternative Protocols.

STEP 1: Coat the Plate

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

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

1. Add 150 µL/well of blocking solution.
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 ºC 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 Calibrators/Controls, and Prepare Detection Antibody Solution.

1. Wash the plate three times with MSD Wash Buffer.
2. Dispense 25–50 µL/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 + 005% 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 µL/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. Please read the MSD Read Buffer Guide to select the appropriate read buffer for your application.

STEP 5: Wash and Read the Plate.

1. Wash the plate three times with MSD Wash Buffer.
2. Carefully add 150 µL/well of the selected read buffer, being careful to avoid making any bubbles. We recommend using the reverse pipetting technique.
3. Read the plate on an MSD imager.

Storage and Expiration

MSD's uncoated plates can be stored at room temperature (18–25 ºC). After opening the plate package, we recommend storing unused plates sealed in the original packaging to prevent exposure to dust and contaminants. The unopened 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.

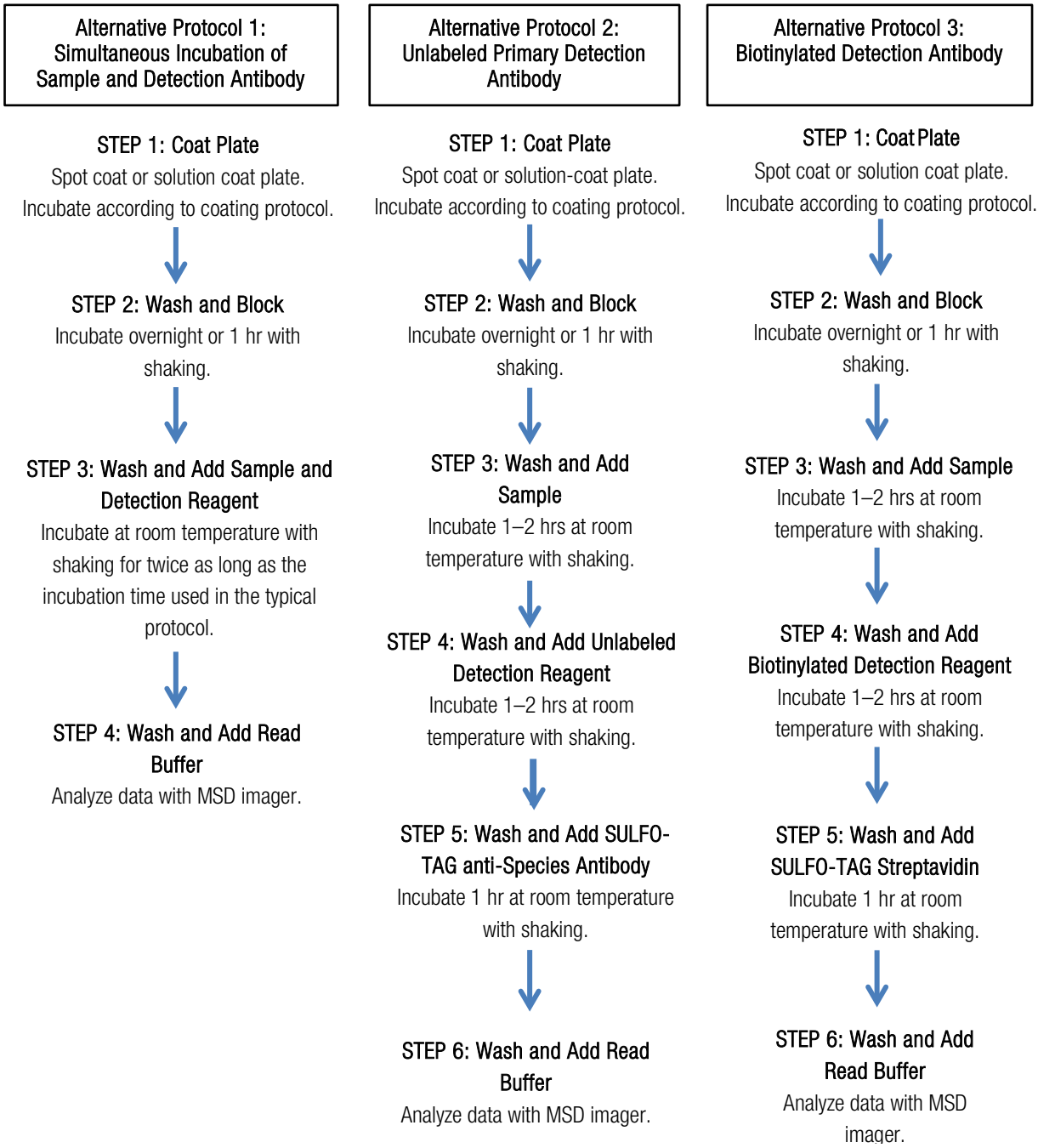
Product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service.

Best Practices and Technical Hints for Assays

- Dilute calibrators, samples, and controls in polypropylene microcentrifuge tubes or polypropylene container of sufficient volume. Use a fresh pipette tip for each dilution, and vortex after each dilution before proceeding.
- 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 1000 rpm. 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.
- Consistent incubation times will improve the reproducibility of test results.
- Avoid bubbles in wells at all pipetting steps because they may lead to variable results.
- Use reverse pipetting when necessary and do not blow out residual liquid to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Tap the plate on a paper towel to ensure the removal of residual fluid after washing.
- Read buffer should be at room temperature (20–26 ºC) 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.
- No shaking is necessary 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-airflow 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.
- Do not use MSD GOLD Read Buffer B on High Bind plates.
- 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.
- Ensure that diluents, wash buffer, and read buffer are equilibrated to room temperature before use. Mix well after thawing and before use. Plates should be brought to room temperature before opening the foil packet.
- Unless specified in the assay-specific protocol, assay incubation steps should be performed between 20–26 °C to achieve the most consistent signals between runs. Assays run above or below that range may be negatively affected.
- Ensure that all reagents are within their expiration date at the time of the test.
- Ensure that all equipment is serviced and calibrated on a routine basis.
- Sources of heat may introduce variability across the plate surface. This can include vents, plate shakers, direct sunlight, etc.
- For additional accuracy and precision, prewet pipette tips 3-5 times before transferring reagents and samples. Avoid pipetting bubbles while doing so.

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

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.

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

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.
- Positive and negative cell lysates may be used neat or diluted. A good starting concentration is 20 µg/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 (≥1000 counts) in the absence of sample, try using 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 ≥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.

When necessary, a 2X concentration of MSD Read Buffer T may be used to prevent the assay background signals from being too close to instrument background signals. If low specific signals are obtained with 1X read buffer, then 2X read buffer may be used to increase the signals and reduce interplate and inter-run 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.

1. Capture antibody titration
2. Detection antibody titration
3. Incubation format

Spot Coating of Capture Antibody	
Variable	**Range**
Capture antibody concentration	20, 10, 5, 0 µg/mL
Volume of capture antibody per well	5 µL
Detection antibody concentration	2, 1, 0.5, 0.25 µg/mL
Incubation format	Typical or simultaneous

Solution Coating of Capture Antibody	
Variable	**Range**
Capture antibody concentration	4, 2, 1, 0 µg/mL
Volume of capture antibody per well	25–30 µL
Detection antibody concentration	2, 1, 0.5, 0.25 µg/mL
Incubation format	Typical 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 (5 µL/spot for spot coating, 30 µL/well for solution coating)

Figure 14a. For titration of capture and detection antibodies

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µL capture Ab at 20 µg/mL for spot coating or 30 µL at 4 µg/mL for solution coating						5 µL capture Ab at 10 µg/mL for spot coating or 30 µL at 2 µg/mL for solution coating					
B												
C												
D												
E	5 µL capture Ab at 5 µg/mL for spot coating or 30 µL at 1 µg/mL for solution coating						0 µg/mL capture Ab					
F												
G												
H												

Step 2: Add sample (25 µL/well in appropriate diluent)

Figure 14b. 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 14c. 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 µL/well of antibody solution diluted in antibody diluent)

Figure 14d. Antibody titration

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

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

Step 1: Capture antibody titration (5 µL/spot for spot coating, 30 µL/well for solution coating)

Figure 15a. Titration of lysates/calibrator

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µL capture Ab at 20 µg/mL for spot coating or 30 µL at 4 µg/mL for solution coating			5 µL capture Ab at 10 µg/mL for spot coating or 30 µL at 2 µg/mL for solution coating			5 µL capture Ab at 5 µg/mL for spot coating or 30 µL at 1 µg/mL for solution coating			5 µL capture Ab at 2.5 µg/mL for spot coating or 30 µL at 0.5 µg/mL for solution coating or Uncoated control		
B												
C												
D												
E												
F												
G												
H												

Step 2: Sample addition (25 µL/well in appropriate diluent)

Figure 15b. For intracellular markers

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

Figure 15c. 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 (det Ab) titration (25 µL/well of antibody solution diluted in antibody diluent)

Figure 15d. 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 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- and interplate signal reproducibility. These include:

1. **Read buffer concentrations:** Higher concentrations of read buffer will lead to higher signals and less variability. Use deionized water for diluting read buffer, if dilution is required. Differences in the preparation of diluted read buffer or evaporation of read buffer stocks can lead to differences in absolute signal values between plates.
2. **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.
3. **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.
4. **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-related problems.
5. **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.

6. **Dissociation rates:** In MSD assays, the signal is generated from electrochemically stimulated SULFO-TAG conjugated molecules that are near (1 to 10 µm) 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 disassociate. 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_{off}); however, interactions with rapid dissociation 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.

Prototype Printing Service

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. Coating optimization packages are also available in which MSD provides plates manufactured using several coating conditions on different plate surfaces as a means of determining the optimal conditions for a given assay.

MSD provides on-site support for assay development through its scientific support team and field application scientists. Contact Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com.

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 should be tested during assay optimization.

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 (2.5-fold weight excess for antibodies) 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 antispecies antibodies (e.g., goat antirabbit or goat antimouse).

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 antispecies secondary antibody may be used. This can be done only when the capture and detection antibodies are from different species.

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 and the Spot 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 manufacturing 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. TBS is not recommended for spot coating since its buffering capacity may not be robust enough to maintain neutral pH during evaporation; therefore, pH extremes may adversely affect the integrity of the molecule being immobilized.

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 (≥40 µL/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 µL/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 antispecies 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. The following tables 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.

Plates

Table 5. Catalog numbers for 96-well uncoated SECTOR and QuickPlex plates.

	10 Plates	100 Plates	500 Plates
96-well Standard SECTOR Plates[1]	L15XA-3	L15XA-6	L15XA-7
96-well High Bind SECTOR Plates[1]	L15XB-3	L15XB-6	L15XB-7
96-well Small Spot Standard SECTOR Plates[1]	L45XA-3	L45XA-6	L45XA-7
96-well Small Spot High Bind SECTOR Plates[1]	L45XB-3	L45XB-6	L45XB-7
96-well Standard QuickPlex Plates[2]	L55XA-3	L55XA-6	L55XA-7
96-well High Bind QuickPlex Plates[2]	L55XB-3	L55XB-6	L55XB-7

1. 96-well SECTOR plates can be used on the MESO SECTOR S 600MM, MESO SECTOR S 600, MESO QuickPlex SQ 120MM, MESO QuickPlex SQ 120, SECTOR Imager 6000, and SECTOR Imager 2400 instruments.
2. 96-well Standard and High Bind QuickPlex plates can be used on the MESO QuickPlex SQ 120MM, MESO QuickPlex SQ 120, and MESO QuickPlex Q 60MM instruments.

Table 6. Catalog numbers for MA6000 384-well uncoated SECTOR plates.

	15 Plates	120 Plates	510 Plates
MA6000 384-well Standard SECTOR Plates[3]	L21XA-4	L25XA-6	L25XA-7
MA6000 384-well High Bind SECTOR Plates[3]	L21XB-4	L25XB-6	L25XB-7

3. MA6000 384-well SECTOR plates can be used on the MESO SECTOR S 600MM, MESO SECTOR S 600, and SECTOR Imager 6000 instruments.

Table 7. Catalog numbers for MA2400 384-well uncoated SECTOR plates.

	10 Plates	100 Plates	500 Plates
MA2400 384-well Standard SECTOR Plates[4]	L25XA-3	L25XA-6	L25XA-7
MA2400 384-well High Bind SECTOR Plates[4]	L25XB-3	L25XB-6	L25XB-7

4. MA2400 384-well SECTOR plates can only be used on the SECTOR Imager 2400 instrument.

Table 8. *Plates and reagents.*

Reagents	Catalog #	Description
ELISA Conversion Pack I	K15A01-1	Uncoated plates for immobilizing unlabeled capture antibodies
ELISA Conversion Pack II	K15A01-2	Plates precoated with anti-species antibodies for immobilizing antibodies from glycerol stocks or antibody stocks with high concentrations of carrier proteins
ELISA Conversion Pack III	K15A01-3	Plates precoated with avidin or streptavidin for immobilizing biotinylated antibodies
SULFO-TAG NHS-Ester	R91AN-3	Labeling reagent with an NHS-Ester functional group for conjugating molecules containing primary amines for use as detection reagents
SULFO-TAG Streptavidin	R32AD-5	Labeling reagent for use as a secondary reporter with biotinylated detection antibodies or other biotinylated detection reagents as primary detection reagents
SULFO-TAG Anti-Mouse Antibody (Goat)	R32AC-5	Labeling reagent for use as a secondary reporter with unlabeled mouse antibodies as primary detection reagents
SULFO-TAG Anti-Rabbit Antibody (Goat)	R32AB-5	Labeling reagent for use as a secondary reporter with unlabeled rabbit antibodies as primary detection reagents
Tris Wash Buffer (10X)	R61TX-2	10X concentrated Tris-buffered solution with surfactant for washing assay plates
Tris Lysis Buffer	R60TX-3	Cell lysis buffer for use in the preparation of lysates for intracellular signaling biomarker assays
Inhibitor Pack	R70AA-1	Protease and phosphatase inhibitors for use in intracellular signaling assays
Blocker A Kit	R93AA-2	A cocktail of proteins in a PBS-based buffer for blocking the nonspecific binding of proteins to the plate surface
Diluent 100	R50AA-4	A blend of stabilizers and protein in PBS for use as an antibody diluent

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.

For research use only.
Not for use in diagnostic procedures.

Plate Diagram