## MSD GOLD™ Streptavidin and Avidin Plates

<table>
<thead>
<tr>
<th></th>
<th>SECTOR® Plates</th>
<th>QUICKPLEX® Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well Streptavidin</td>
<td>L15SA</td>
<td>L55SA</td>
</tr>
<tr>
<td>96-well Small Spot Streptavidin</td>
<td>L45SA</td>
<td>N/A</td>
</tr>
<tr>
<td>96-well High Bind Avidin</td>
<td>L15AB</td>
<td>L55AB</td>
</tr>
</tbody>
</table>
MSD GOLD Streptavidin and Avidin Coated Plates

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
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## Ordering Information

### MSD Customer Service

Phone: 1-240-314-2795  
Fax: 1-301-990-2776  
Email: CustomerService@mesoscale.com

### MSD Scientific Support

Phone: 1-240-314-2798  
Fax: 1-240-632-2219 attn: Scientific Support  
Email: ScientificSupport@mesoscale.com
Introduction

MESO SCALE DISCOVERY’S MULTI-ARRAY® technology forms the basis of an immunoassay system for measuring biomarkers using highly sensitive electrochemiluminescence (ECL) detection. MSD GOLD plates and reagents utilize this technology by providing a rapid and convenient method for the development of new assays and the transfer of existing ELISAs to the MSD platform. MSD GOLD is a signature of our commitment to quality, consistency, and high performance. MSD GOLD designation certifies that the plates and reagents conform to rigorous specifications for uniformity and precision. These specifications are designed to meet the requirements of critical assays and clinical research for the drug safety and toxicology communities.

MSD GOLD Streptavidin- and Avidin-coated plates may be used for a range of applications including assay development, biomarker measurement, and immunogenicity evaluation. MSD GOLD Streptavidin plates are highly suited for use with homogenous assays or bridging assays, such as typical immunogenicity assays that require high free drug tolerance. Similar to all our MSD GOLD assay development tools, these plates are validated for lot-to-lot reproducibility and have low intra-plate and inter-plate %CVs. These attributes have resulted in the wide adoption of the MSD GOLD Streptavidin plates for immunogenicity as well as other assay development applications.

MSD assays follow a workflow similar to that of an ELISA. The main steps include coating the plates with capture reagent, adding samples/calibrators, adding detection reagent, reading the plate, and analyzing the data. Note that typical assay development on the MSD platform results in significant conservation of precious sample and antibodies with gain in sensitivity, dynamic range, and specificity.

Plate Types and Surfaces

MSD GOLD Streptavidin- and Avidin-coated plates are available in different spot formats as illustrated below. The surface types are different between the plates: Streptavidin plates have a standard, hydrophobic surface; High Bind Avidin plates have a hydrophilic surface. The surface type of the plate may affect the manner in which charged molecules interact or bind to the surface.

Choosing a Plate Type

Several parameters play a role in determining the best plate type for an assay. Some of them are discussed below.

Binding Capacity and Assay Performance

Selection of the right plate type is critical for assay development. The properties of the above listed plates are different and offer unique advantages. In general, avidin-coated plates have higher binding capacity due to their hydrophilic surface, but tend to offer lower signals and sensitivity. Alternatively, streptavidin-coated plates have relatively lower binding capacity, but provide higher assay signals and sensitivity. Small Spot Streptavidin Plates provide the highest assay signals and therefore superior sensitivity.
Streptavidin-coated plates frequently exhibit lower non-specific binding, especially with complex sample matrices. The binding capacity and properties of each plate type are provided below.

**Table 1. Binding capacity of MSD GOLD Streptavidin- and Avidin-coated plates**

<table>
<thead>
<tr>
<th>Plate Type</th>
<th>Surface Type</th>
<th>Binding Capacity (IgG)*</th>
<th>Assay Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Spot Streptavidin</td>
<td>Hydrophobic</td>
<td>0.075 pmol/well</td>
<td>+++</td>
</tr>
<tr>
<td>Streptavidin</td>
<td>Hydrophobic</td>
<td>0.3 pmol/well</td>
<td>++</td>
</tr>
<tr>
<td>High Bind Avidin</td>
<td>Hydrophilic</td>
<td>0.6 pmol/well</td>
<td>+</td>
</tr>
</tbody>
</table>

*The binding capacity was measured by titrating biotin-tagged IgG (BTI) in the plate (illustrated below). The amounts are based on picomoles per well of biotinylated material. These values are for IgGs and may vary for non-IgG proteins. For reference, 25 µL/well of 1 µg/mL of antibody is 0.167 pmol.

**Figure 2. Titration of biotin-tagged IgG (BTI) on MSD GOLD Streptavidin- and Avidin-coated plates**

For reproducible performance across different plate lots, MSD recommends coating the plates at a consistent concentration below the binding capacity suggested in Table 1.

**Amount of Coating Material**

In comparison to 96-well Streptavidin plates, the Small Spot Streptavidin plates have lower binding capacity and therefore will require relatively less coating material.
Instrument Compatibility

MSD offers specific Gold quality Streptavidin- and Avidin-coated plates that are compatible with SECTOR® and MESO® QuickPlex SQ 120 instruments. SECTOR plates can be used on both SECTOR Imagers and MESO QuickPlex® SQ 120 instruments. QUICKPLEX plates were designed to be read one well at a time by MESO QuickPlex SQ 120 instruments.

Table 2. Instrument compatibility of MSD GOLD Streptavidin- and Avidin-coated plates

<table>
<thead>
<tr>
<th>MSD Instrument</th>
<th>Plate Type</th>
<th>96-well SECTOR Plates</th>
<th>96-well QUICKPLEX Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Read Cycle</td>
<td>6 sectors of 4x4-well arrays</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Read Time</td>
<td>1 min, 10 sec</td>
<td>N/A</td>
</tr>
<tr>
<td>MESO SECTOR S 600</td>
<td>Read Cycle</td>
<td>6 sectors of 4x4-well arrays</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Read Time</td>
<td>1 min, 10 sec</td>
<td>N/A</td>
</tr>
<tr>
<td>SECTOR Imager 6000</td>
<td>Read Cycle</td>
<td>6 sectors of 4x4-well arrays</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Read Time</td>
<td>1 min, 10 sec</td>
<td>N/A</td>
</tr>
<tr>
<td>SECTOR Imager 2400</td>
<td>Read Cycle</td>
<td>24 sectors of 2x2-well arrays</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Read Time</td>
<td>3 min, 30 sec</td>
<td>N/A</td>
</tr>
<tr>
<td>MEO QuickPlex SQ 120</td>
<td>Read Cycle</td>
<td>24 sectors of 2x2-well arrays</td>
<td>One well at a time</td>
</tr>
<tr>
<td></td>
<td>Read Time</td>
<td>1 min, 30 sec</td>
<td>2 min, 45 sec</td>
</tr>
</tbody>
</table>

This document highlights how MSD GOLD Streptavidin- and Avidin-coated plates can be used to develop novel assays that take full advantage of MSD’s unique technology.

Example Assay Formats - This section provides the typical formats for assays developed using MSD GOLD Streptavidin- and Avidin-coated plates.

Product Options - This section provides the catalog numbers for the different plate packs and the Streptavidin Plate Training Pack.

Assay Workflows - This section provides typical workflows for some of the assays that can be developed.

Plate Coating and Typical Protocol - These sections provide a detailed description of plate coating and an optimized biomarker assay protocol.

Assay Performance - This section provides typical data obtained from multiple assays.

Appendix - This section provides best practices, conversion formulae, alternate protocols, and troubleshooting suggestions.
Example Assay Formats

Typical formats for assays developed using MSD GOLD Streptavidin- and Avidin-coated plates are similar to that of ELISAs using biotinylated capture reagents. For biomarker assays, a detection antibody conjugated with SULFO-TAG™ label (Figure 3A) or an unlabeled detection antibody followed by a SULFO-TAG conjugated anti-species antibody (Figure 3B) can be used as detection reagents. For serological assays, biotinylated antigen may be used for capturing serum antibodies and SULFO-TAG conjugated anti-species antibody can be used for detection (Figure 3C).

Streptavidin or avidin plates may also be used for the development of assays to test the immunogenicity of therapeutic antibodies. The bridging format for such an assay requires the drug to be conjugated with biotin to serve as the capture reagent (Figure 3D). The same drug conjugated with SULFO-TAG is the reporter in a bridging assay. Direct immunogenicity assays may also be developed using biotinylated protein drug as the capture reagent (Figures 3E and 3F). Additional information and technical notes for MSD immunogenicity assays are available at www.mesoscale.com.

Figure 3. Examples of assay formats on MSD GOLD Streptavidin- and Avidin-coated plates
Principle of ECL

The streptavidin- or avidin-coated spot within the MULTI-ARRAY MSD plate is a working electrode surface that adsorbs biotinylated capture reagent. The user can coat these spots with a number of different biotinylated capture materials, including antibodies, kinase substrates, or oligonucleotides. After coating, the user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG) over the course of one or more incubation periods. Analytes in the sample bind to the capture reagent immobilized on the electrode surface. Bound analytes then recruit detection antibodies from the solution holding them in close proximity to the electrode. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument. The instrument applies a voltage to the plate electrodes, causing the SULFO-TAG near the electrode to emit light through a series of reduction and oxidation reactions (Figure 4). The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

Figure 4. Image of a single well from an MSD MULTI-ARRAY plate depicting the electrochemiluminescence reaction and the working electrode, counter electrode, and dielectric.
Product Options

MSD offers a range of plates and reagents to enable assay development on our platform. MSD GOLD Streptavidin- and Avidin-coated plates are offered in different pack sizes. All-inclusive assay development training packs containing the components and guidance that may be necessary for developing biomarker, immunogenicity, or serology assays are also available.

Plates

Table 3. Catalog numbers of MSD GOLD assay development plates

<table>
<thead>
<tr>
<th>MSD GOLD Plates</th>
<th>1 Plate</th>
<th>5 Plates</th>
<th>30 Plates</th>
<th>120 Plates</th>
<th>510 Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSD GOLD 96-well Streptavidin SECTOR Plates</td>
<td>L15SA-1</td>
<td>L15SA-2</td>
<td>L15SA-5</td>
<td>L15SA-6</td>
<td>L15SA-7</td>
</tr>
<tr>
<td>MSD GOLD 96-well Streptavidin QUICKPLEX Plates</td>
<td>L55SA-1</td>
<td>L55SA-2</td>
<td>L55SA-5</td>
<td>L55SA-6</td>
<td>L55SA-7</td>
</tr>
<tr>
<td>MSD GOLD 96-well Small Spot Streptavidin SECTOR Plates</td>
<td>L45SA-1</td>
<td>L45SA-2</td>
<td>L45SA-5</td>
<td>L45SA-6</td>
<td>L45SA-7</td>
</tr>
<tr>
<td>MSD GOLD 96-well High Bind Avidin SECTOR Plates</td>
<td>L15AB-1</td>
<td>L15AB-2</td>
<td>L15AB-5</td>
<td>L15AB-6</td>
<td>L15AB-7</td>
</tr>
<tr>
<td>MSD GOLD 96-well High Bind Avidin QUICKPLEX Plates</td>
<td>L55AB-1</td>
<td>L55AB-2</td>
<td>L55AB-5</td>
<td>L55AB-6</td>
<td>L55AB-7</td>
</tr>
</tbody>
</table>

Note: SECTOR plates can be used on both SECTOR Imagers and MESO QuickPlex SQ 120 instruments. QUICKPLEX plates were designed to be read one well at a time by MESO QuickPlex SQ 120 instruments.

Assay Development Products

ELISA Conversion Packs:
- ELISA Conversion Pack I (Catalog No. K15A01-1) – includes uncoated plates for immobilizing unlabeled capture antibodies
- ELISA Conversion Pack II (Catalog No. K15A02-1) – includes plates pre-coated with anti-species antibodies for immobilizing antibodies from glycerol stocks or antibody stocks with high concentrations of carrier proteins
- ELISA Conversion Pack III (Catalog No. K15A02-3) – includes plates pre-coated with avidin or streptavidin for immobilizing biotinylated antibodies

Reagents:
- SULFO-TAG conjugated anti-species antibodies – for use as reporters with unconjugated detection antibodies

A complete list of assay development reagents, plates, buffers, diluents, and blockers is available at www.mesoscale.com.
Assay Workflows

**Biomarker Assay**

STEP 1: Add blocking solution and incubate for 1 hour with shaking (optional).

STEP 2: Wash. Coat plate with biotinylated antibody and incubate for 1–2 hours at room temperature with shaking or overnight at 2–8ºC.

STEP 3: Wash, add sample, and incubate for 1–2 hours with shaking.

STEP 4: Wash and add SULFO-TAG detection antibody. Incubate for 1 hour with shaking.

STEP 5: Wash, add read buffer, and analyze with MSD instrument.

**Serology Assay**

STEP 1: Add blocking solution and incubate for 1 hour with shaking (optional).

STEP 2: Wash. Coat plate with biotinylated antigen and incubate for 1–2 hours at room temperature with shaking or overnight at 2–8ºC.

STEP 3: Wash, add serum sample, and incubate for 1–2 hours with shaking.

STEP 4: Wash and add SULFO-TAG detection antibody. Incubate for 1 hour with shaking.

STEP 5: Wash, add read buffer, and analyze with MSD instrument.

**Bridging Immunogenicity Assay**

STEP 1: Combine biotinylated drug, SULFO-TAG conjugated drug, and sample in polypropylene plate. Incubate for 1–2 hours at room temperature with shaking or overnight at 2–8ºC.

STEP 2: During step 1 incubation, add blocking solution to the MSD plate and incubate for 1 hour with shaking.

STEP 3: Wash MSD plate. Transfer 50 µL/well assay solution from polypropylene plate to MSD plate. Incubate for 2 hours with shaking.

STEP 4: Wash, add read buffer, and analyze with MSD instrument.

**Figure 5.** Typical workflows for some of the assays that can be developed using MSD GOLD Streptavidin, Small Spot Streptavidin, and High Bind Avidin plates

**Recommended Minimum Volumes**

<table>
<thead>
<tr>
<th>Calibrator/Sample/Detection Reagent Volume</th>
<th>Blocking Solution/Read Buffer Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-50 µL/well</td>
<td>150 µL/well</td>
</tr>
</tbody>
</table>
Plate Coating

Coating with Antibodies

STEP 1: Block Plate (optional).

- Add 150 µL/well of MSD Blocker A solution (available for separate purchase; Catalog No. R93AA-2).
- Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour or overnight at 2-8°C (shaking not necessary for overnight incubation).
  
  **Note:** Blocking solutions containing biotin should be avoided as free biotin can interfere with the binding of biotinylated capture reagents to streptavidin- and avidin-coated plates.

STEP 2: Prepare Capture Antibody.

- Prepare 25 µL of antibody solution per well using antibody dilution buffer. MSD recommends Diluent 100 (Catalog No. R50AA-4), which contains a blend of stabilizers and Blocker A in PBS.
  
  **Note:** If required, capture antibody volume may be increased to 50 µL/well.
- The concentration of the capture reagent should be below the binding capacity of the plate. The recommended starting capture antibody concentrations for MSD GOLD Streptavidin plates, Small Spot Streptavidin plates, and High Bind Avidin plates are 1 µg/mL, 0.5 µg/mL, and 2 µg/mL, respectively.
- Prepare 3 mL of capture antibody solution per plate. If stock concentration of capture antibody is 50 µg/mL, and final concentration for use in the assay is 1 µg/mL, then for 1 plate, combine:
  - 60 µL of biotinylated capture antibody (50 µg/mL)
  - 2.94 mL of Diluent 100
- For detailed calculations on capture antibody concentration, please refer to the Appendix.

STEP 3: Wash and Add Capture Antibody.

- Wash the plate three times with 1X MSD Wash Buffer [Catalog No. for MSD Wash Buffer (20X) is R61AA-1].
- Dispense 25 µL/well of biotinylated capture antibody into the bottom of each well. Seal the plate and incubate at room temperature with shaking until binding equilibrium is achieved (usually 1 hour).
- Wash the plate three times with 1X MSD Wash Buffer. The plate is ready for use to run assays.

Coating with Non-Antibody Molecules

Non-antibody molecules, such as antigens and peptides, can also be immobilized on MSD GOLD Streptavidin- or Avidin-coated plates. If you want to optimize the coating concentrations, we recommend preparing the biotin non-antibody molecules at a range of concentrations that are below the binding capacity of the plate.
Typical Biomarker Assay Protocol

The following is an example of a typical biomarker assay format. For detailed instructions on developing an immunogenicity assay, please refer to our technical note on Bridging Immunogenicity Assays available at www.mesoscale.com. An example of an immunogenicity protocol is also provided in the Appendix of this document.

Conjugation of detection antibody: Before beginning the assay, the detection antibody must be conjugated with SULFO-TAG label, a necessary component of all MSD assays. Either the primary detection reagent can be directly conjugated with MSD SULFO-TAG NHS-ester or a pre-labeled secondary reporter such as SULFO-TAG anti-species antibody can be coupled to an unlabeled detection reagent. The protocol for SULFO-TAG conjugation can be found at www.mesoscale.com.

Prepare Plate: Use plate prepared as described in the Plate Coating section.

**STEP 1: Prepare and Add Calibrators/Controls.**
- Prepare calibrators and controls during the plate blocking step.
- Add 50 µL of diluted calibrators or controls to the bottom of each well. **(Note: As low as 25 µL/well may be used to conserve reagent.)**
- Seal the plate and incubate at room temperature for 1 hour. The exact time required will vary by application and should be determined experimentally.

**STEP 2: Wash. Prepare and Add Detection Antibody.**
- You will need at least 3 mL of detection antibody solution for each 96-well plate. In a 15 mL tube, combine antibody dilution buffer and SULFO-TAG conjugated detection antibody. MSD Diluent 100 may be used as the antibody dilution buffer.
- Wash the plate three times with 1X MSD Wash Buffer.
- Add 25 µL/well of detection antibody solution. **(Note: If required, detection antibody volume may be increased to 50 µL/well.)**
- Seal the plate and incubate at room temperature until the binding equilibrium is achieved. This usually takes 1 hour, but the exact time required will vary by application and should be determined experimentally.

**STEP 3: Wash. Prepare Read Buffer and Read Plate.**
- Prepare the read buffer during this time.
  **Note:** MSD offers two read buffer products:
  1. MSD GOLD Read Buffer is part of our MSD GOLD portfolio and is validated for reproducibility of signals across plate lots. MSD GOLD Read Buffer should be used at the supplied concentration without any dilution.
  2. MSD Read Buffer T is provided at a 4X stock concentration. Use deionized water to dilute Read Buffer T to a 1X or 2X concentration, but not higher than 2X. Use of 2X Read Buffer T generates greater signals in the assay and reduces inter-plate and inter-run variability.
- Wash the plate three times with 1X MSD Wash Buffer.
- Carefully add 150 µL/well of undiluted MSD GOLD Read Buffer or diluted Read Buffer T. Use reverse pipetting techniques to avoid introducing bubbles.
- Read the plate on an MSD instrument.
Example Assay Performance

MSD has optimized a wide range of assays on both streptavidin- and avidin-coated plates. Representative data from four assays are shown below. For all the assays tested, the same antibodies and reagents were used on the different plate types. Results may differ depending on reagent, protocol, and assay optimization.

Typical Calibration Curves

The calibration curves below compare assay performance on Streptavidin plates (red curve), Small Spot Streptavidin plates (blue curve), and High Bind Avidin plates (green curve). Representative calibration curves from four assays are shown below.

![Typical Calibration Curves](image)

**Figure 6.** Representative calibration curves of assays tested on MSD GOLD Streptavidin- and Avidin-coated plates
**Sensitivity**

Assay sensitivity was compared on MSD GOLD Streptavidin- and Avidin-coated plates. For each assay shown below, we measured the lower limit of detection (LLOD), which is the calculated concentration corresponding to the signal 2.5 standard deviations above the background. Overall, it was observed that the assays run on Small Spot Streptavidin plates yielded lower detection limits in comparison to those tested on the other plate types (Table 4).

**Table 4. Comparison of LLOD (pg/mL) between different MSD GOLD Streptavidin- and Avidin-coated plates**

<table>
<thead>
<tr>
<th>Plate Type</th>
<th>Assay</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well Small Spot Streptavidin Plate</td>
<td></td>
<td>0.030</td>
<td>0.115</td>
<td>0.111</td>
<td>0.054</td>
</tr>
<tr>
<td>96-well Streptavidin Plate</td>
<td></td>
<td>0.63</td>
<td>1.39</td>
<td>1.04</td>
<td>0.25</td>
</tr>
<tr>
<td>96-well High Bind Avidin Plate</td>
<td></td>
<td>3.59</td>
<td>5.07</td>
<td>2.64</td>
<td>1.80</td>
</tr>
</tbody>
</table>

**Reproducibility**

**Assay Reproducibility**

As part of validation studies, three plates each from three different lots of MSD GOLD Small Spot Streptavidin plates were tested. Intra-plate and inter-lot %CVs of calibrators and controls were evaluated. Representative data from four assays are shown in Table 5 below.

Calibrators within the quantifiable range of each assay were evaluated. The average intra-plate and inter-plate CVs for both the calibrator signals and the back-fitted calculated concentrations were less than 15% in all the lots tested (data not shown). Reproducibility of controls at three different levels is shown in the table below. The average intra-plate and inter-lot CVs for all four assays were below 15%.

**Table 5. Representative assay reproducibility results from four assays tested on MSD GOLD Small Spot Streptavidin plates**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Control</th>
<th>Plate Lot 1 (n=3 plates)</th>
<th>Plate Lot 2 (n=3 plates)</th>
<th>Plate Lot 3 (n=3 plates)</th>
<th>Inter-Lot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ave. Conc. (pg/mL)</td>
<td>Ave. Intra-Plate %CV</td>
<td>Ave. Conc. (pg/mL)</td>
<td>Ave. Intra-Plate %CV</td>
</tr>
<tr>
<td>IL-1β</td>
<td></td>
<td>High</td>
<td>723</td>
<td>13.3</td>
<td>707</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mid</td>
<td>70</td>
<td>8.8</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>7.0</td>
<td>9.2</td>
<td>6.6</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td>High</td>
<td>348</td>
<td>11.8</td>
<td>394</td>
</tr>
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<td></td>
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<td>36</td>
<td>3.5</td>
<td>33</td>
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<tr>
<td></td>
<td></td>
<td>Low</td>
<td>3.4</td>
<td>6.6</td>
<td>3.3</td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td>High</td>
<td>319</td>
<td>12.8</td>
<td>372</td>
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<td>32</td>
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<td>776</td>
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<td>Mid</td>
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<td>2.5</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>5.6</td>
<td>2.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>
Plate Reproducibility

Reproducibility of plates was measured by testing MSD GOLD plates with a constant amount of BTI. MSD GOLD Streptavidin plates were tested at 0.2 pmol/well and MSD GOLD Small Spot Streptavidin plates were tested at 0.05 pmol/well. The mean signal and %CV is calculated for each plate (intra-plate %CV) and across plates (inter-plate %CV). Mean intra-plate CVs must be less than 6% with no plate having an intra-plate CV greater than 12%.

The plot below shows the reproducibility data from 286 lots of 96-well Streptavidin plates manufactured between 10/2010 and 01/2017 (Fig. 7a). All plate lots met the quality specifications with a mean intra-plate CV of less than 6%. The results from 10,715 plates tested after the specifications were established in 2010 are shown in Fig. 7b. Over 99.9% of the plates have an intra-plate CV of <8% as per the quality control release specifications. There are no plates with an intra-plate CV greater than 12%. The reproducibility of Small Spot Streptavidin plates is illustrated in Fig. 7c. No plates had an intra-plate CV higher than 12%.

In addition, plates were tested for consistency in ECL signals obtained in typical curves from BTI titration. The signals between different plate lots are within +/- 15% of each other when normalized to the reference lot (data not shown).

96-well, Streptavidin Plate

Fig. 7a

96-well, Small Spot Streptavidin Plate

Fig. 7c

Figure 7. Representative reproducibility of MSD GOLD 96-well Streptavidin-coated plates when tested with a constant concentration of BTI on whole plates.
Effect of Plate Binding Capacity on an Immunogenicity Assay

Effective assessment of immunogenicity requires an assay platform that has high precision, minimal matrix effects, and low variability. A common source of assay variability is excessive biotinylated capture antibody. For a more robust and reproducible assay, it is recommended to coat the plate at a concentration that is below the binding capacity. We titrated a biotinylated capture antibody on Streptavidin plates from multiple lots and tested them using a bridging immunogenicity assay. Results from three plate lots are presented below.

Within the quantitative range of the assay, signals are highly consistent provided the amount of capture material is at or below the specified binding capacity of the plates. A hook effect is observed with MSD GOLD 96-well Small Spot Streptavidin plates at capture concentrations >0.11 pmol, whereas with MSD GOLD 96-well Streptavidin plates, the signals plateau at capture concentrations of 0.33 pmol.

Table 6. Titration of biotinylated drug on different lots of MSD GOLD Streptavidin-coated plates

### MSD GOLD 96-well Small Spot Streptavidin Plates

<table>
<thead>
<tr>
<th>Concentration of ADA (ng/ml)</th>
<th>Representative signal from one plate lot</th>
<th>% Signal CV of three plate lots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biotin Drug (pmol)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>0.11</td>
</tr>
<tr>
<td>3,000</td>
<td>397,724</td>
<td>459,171</td>
</tr>
<tr>
<td>1,000</td>
<td>139,329</td>
<td>182,532</td>
</tr>
<tr>
<td>100</td>
<td>16,200</td>
<td>20,345</td>
</tr>
<tr>
<td>10</td>
<td>1,740</td>
<td>2,245</td>
</tr>
<tr>
<td>1</td>
<td>255</td>
<td>299</td>
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<td>0.1</td>
<td>80</td>
<td>89</td>
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<td>0.01</td>
<td>64</td>
<td>66</td>
</tr>
<tr>
<td>0</td>
<td>59</td>
<td>60</td>
</tr>
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</table>

### MSD GOLD 96-well Streptavidin Plates

<table>
<thead>
<tr>
<th>Concentration of ADA (ng/ml)</th>
<th>Representative signal from one plate lot</th>
<th>% Signal CV of three plate lots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biotin Drug (pmol)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>0.11</td>
</tr>
<tr>
<td>3,000</td>
<td>258,140</td>
<td>249,047</td>
</tr>
<tr>
<td>1,000</td>
<td>85,367</td>
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<td>65</td>
<td>64</td>
</tr>
<tr>
<td>0</td>
<td>64</td>
<td>64</td>
</tr>
</tbody>
</table>
Multiplexing with MSD

**Typical Calibration Curves**

Assays developed on MSD Streptavidin or Avidin-coated plates can be easily converted to multiplex format using our U-PLEX® assay platform. U-PLEX assays use biotinylated capture reagents similar to those used on streptavidin- and avidin-coated plates. Calibration curves and sample quantification data from a set of four assays are shown below. Similar quantification of samples is observed on both U-PLEX and MSD GOLD Small Spot Streptavidin plates.

![Calibration Curves](image)

*Figure 8. Comparison of calibration curves of assays tested on Small Spot Streptavidin plates and U-PLEX plates.*
Comparison of Sample Quantification

Eighteen human serum and 19 human plasma samples spiked with calibrators were tested on MSD GOLD Small Spot Streptavidin plates and U-PLEX plates (multiplex). The dilution corrected sample concentration measurements from the multiplexed assays on the U-PLEX platform were compared to those obtained on the Small Spot Streptavidin plates. In general, the signals on U-PLEX plates are higher than those shown on Small Spot Streptavidin plates. However, the sample quantification between the two platforms is highly correlated as shown in Figure 9.

<table>
<thead>
<tr>
<th>Plate Type</th>
<th>Statistic</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>IL-8</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well Small Spot Streptavidin Plate</td>
<td>r² Value</td>
<td>0.96</td>
<td>0.95</td>
<td>0.91</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.98</td>
</tr>
<tr>
<td>U-PLEX Plate</td>
<td>r² Value</td>
<td>1.04</td>
<td>1.06</td>
<td>1.09</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*Figure 9. Correlation of sample quantification between assays tested on U-PLEX plates and MSD GOLD Streptavidin plates*
Plate Stability

MSD GOLD Streptavidin, Small Spot Streptavidin, and High Bind Avidin-coated plates can be stored at 2-8°C or at room temperature. The plates have a shelf life of 30 months from the date of manufacture when stored at 2-8°C and the expiration date provided on the product label is for 2-8°C storage. Stability data also verify that the plates are stable for 24 months from the date of manufacture when stored at room temperature. If plates are stored at room temperature, it is recommend to perform the appropriate studies under your experimental conditions to ensure the same level of performance.

Real time stability testing was performed on Streptavidin plates at 2-8°C over a period of 45 months and at room temperature over 24 months. The average laboratory temperature recorded during the room temperature study was 22.3°C, with minimum and maximum recorded temperatures of 16°C and 28.8°C, respectively. At regular intervals, binding capacity was measured to assess the performance of the plates. As shown below, the signals at 0.1, 0.2, and 0.3 picomoles of IgG were within 10% of the mean signal and usually well within ±15% of specification (dotted lines) during the 45-month/2-8°C stability study (Figure 10, top). The plot for room temperature stability (bottom) shows that the signals at 0.2 and 0.02 picomoles of IgG are within ±15% of specification (dotted lines) over the 24-month period. The high control (0.2 picomoles) has been plotted against the left y-axis and the low control (0.02 picomoles) has been plotted against the right y-axis.

Figure 10. Real time stability of MSD GOLD Streptavidin plates

References

Appendix

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 or at www.mesoscale.com.

Best Practices and Technical Hints

General Assay Techniques

• Avoid bubbles in wells during all pipetting steps. Bubbles introduced when adding read buffer may interfere with signal detection.
• Use reverse pipetting when necessary to avoid introduction of bubbles. For empty wells, pipette to the bottom corner touching the pipette tip on the bottom.
• Shaking should be vigorous with a rotary motion between 500 and 1,000 rpm.
• When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
• Gently tap the plate on a paper towel to remove residual fluid after washing.

Preparation of Calibrators and Samples

• Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
• If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.

Preparation of Detection Antibodies

• 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).
• You may adjust volumes proportionally when preparing detection antibody solution
• Do not use detection reagents containing biotin on the streptavidin/avidin plates.

Reading Plates

• Remove all plate seals prior to reading the plate.
• Make sure that the read buffer is at room temperature when added to the plate.
• If you plan to only coat and use partial plates, use the sector map in the instrument or software manual to select the wells to be read. After reading a partial plate, remove fluid, reseal unused sectors, return plate to its original packaging with the desiccant, and seal.
• To improve inter-plate precision, keep time intervals consistent between adding read buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding read buffer.
• Do not shake the plate after adding read buffer.
Capture Antibody Calculation for Coating Plate

1. Converting pmol of biotinylated protein/well to µg/mL concentration

\[
\text{µg/mL protein} = \frac{\text{pmol of biotinylated protein per well} \times \text{molecular weight of protein (Da)}}{\text{µL biotinylated protein added per well} \times 1,000}
\]

The following example determines the concentration of a 150 kD biotinylated antibody in µg/mL, such that 0.25 picomole is added to each well in a volume of 25 µL.

- picomole per well=0.25
- Antibody molecular weight (Da)=150,000
- Volume of biotinylated antibody added to well (µL)=25

\[
\text{µg/mL protein} = \frac{0.25 \times 150,000}{25 \times 1,000} = 1.5 \text{ µg/mL}
\]

2. Converting µg/mL biotinylated protein to picomole concentration

\[
\text{pmol protein} = \frac{\text{µg/mL concentration of protein} \times \text{volume (µL)} \times 1,000}{\text{protein molecular weight (Da)}}
\]

- Antibody molecular weight (Da)=150,000
- Volume (µL)=25
- Concentration of protein (µg/mL)=1

\[
\text{pmol protein} = \frac{1 \times 25 \times 1,000}{150,000} = 0.167 \text{ pmol}
\]

3. Converting nM biotinylated protein to µg/mL concentration

\[
\text{µg/mL protein} = \frac{nM \text{ concentration of protein} \times \text{molecular weight of protein (Da)}}{1,000,000}
\]

- Antibody molecular weight (Da)=150,000
- Concentration of protein (nM)=15

\[
\text{µg/mL protein} = \frac{15 \times 150,000}{1,000,000} = 2.2 \text{ µg/mL}
\]
Alternative Protocol for Bridging Immunogenicity Assay

Below is an example protocol for a bridging immunogenicity assay. Detailed application notes and guidance on developing bridging assays are available at www.mesoscale.com.

STEP 1: Prepare Reagents.

 Biotinylated drug and SULFO-TAG-conjugated drug

In a bridging immunogenicity assay, the anti-drug antibody (from serum/plasma) bridges a biotinylated capture drug and a SULFO-TAG conjugated detection drug.

MSD Biotin Conjugation Pack (included in the MSD GOLD Streptavidin Plate Training Pack) or any commercial biotinylation reagent such as Pierce EZ-Link Sulfo-NHS-LC-Biotin or Sulfo-NHS-LC-LC-Biotin may be used to conjugate the drug with biotin. Typical starting conjugation ratios are 5:1 or 10:1 biotin:drug. The efficiency of biotinylation will depend on the number of available lysine residues, the concentration of the drug, and the pH of the conjugation reaction. If precipitation occurs upon conjugating with biotin, use a lower conjugation ratio. Unlike many other applications, only a few biotin labels per drug molecule are required in this format; typically, 2–4 biotin labels per drug molecule are sufficient. After conjugation, any unconjugated biotin should be removed by purification. Desalting columns, dialysis, or buffer exchange using microconcentrators are suitable for this purpose.

For preparing SULFO-TAG conjugated drug, follow the protocol outlined in the MSD SULFO-TAG NHS-Ester application note, which can be found online at www.mesoscale.com.

 Anti-Drug Antibody Samples

Prepare a dilution series of anti-drug antibody in normal serum or plasma. Recommended test concentrations of anti-drug antibody are 10,000, 2,500, 625, 156, 39, 9.8, 2.4, and 0 ng/mL. Each well should receive 25 µL of anti-drug antibody sample. Adjust the range of test concentrations, if required.

STEP 2: Prepare Master Mix.

 Prepare a solution containing biotinylated drug and SULFO-TAG conjugated drug. The optimum concentrations of biotinylated drug and SULFO-TAG conjugated drug should be determined empirically.

 Add 50 µL of this solution to each well of a 96-well polypropylene plate.

 Add 25 µL/well of diluted samples from STEP 1(b).

 Seal the plate and incubate for 1–2 hours at room temperature with shaking or overnight at 2-8ºC.

STEP 3: Block MSD Plate.

 During the incubation of the Master Mix solution, add 150 µL/well of blocking solution to an MSD GOLD Streptavidin, Small Spot Streptavidin, or a High Bind Avidin plate.

 Seal the plate with an adhesive plate seal and incubate for 1 hour with shaking at room temperature.

STEP 4: Wash. Add Master Mix.

 Wash the MSD plate three times with 1X MSD Wash Buffer.

 Transfer 50 µL of Master Mix from each well of the polypropylene plate to corresponding wells on the MSD plate.

 Seal the plate with an adhesive plate seal and incubate for 1 hour with shaking at room temperature.
STEP 5: Wash. Read Plate.

- Wash the plate three times with PBS-T.
- Add 150 µL/well of undiluted MSD GOLD Read Buffer or diluted Read Buffer T. Use reverse pipetting techniques to avoid introducing bubbles.
- Read the plate on an MSD instrument.

**Note:** Do not exceed the recommended amounts of biotinylated drug specified for the plate type. For Streptavidin plates, do not exceed 0.3 picomoles of biotinylated drug per well. For MSD GOLD High Bind Avidin plates, do not exceed 0.6 picomoles of biotinylated drug per well.

For example, if the Master Mix uses 25 µL of biotinylated drug at 2 µg/mL (corresponding to 0.33 picomoles), 25 µL of SULFO-TAG conjugated drug, and 25 µL of sample, then when 50 µL of the Master Mix is transferred to the Streptavidin plate, only 0.22 picomoles (two thirds of the original quantity of biotinylated drug) is transferred to the plate.

If the assay is being developed for a non-antibody protein therapeutic, the concentration of biotinylated and SULFO-TAG conjugated drug should be adjusted according to the molecular weight of the drug. As an example, for a 75 kD protein therapeutic (half the molecular weight of a 150 kD antibody), the recommended concentrations of SULFO-TAG conjugated and biotinylated drug would be 50% of the concentrations above.

Please refer to the **Capture Antibody Calculation for Coating Plate** section for conversion of µg/mL to picomoles.

**Optimization - Antibody Concentrations**

An important step in assay optimization is selection of the capture antibody and detection antibody concentrations. The decision on antibody concentration will depend on your desired assay performance, background, and material cost. For capture antibodies, MSD recommends considering the **binding capacity** when coating the plate. Higher capture antibody concentration will increase assay signals until the concentration surpasses the plate **binding capacity**. Increasing the amount of detection antibodies will also increase signals but may lead to high background especially in assays where detection antibodies non-specifically interact with the coating material. To reduce assay variability, optimize the detection antibody concentration to where you observe minimal change with changes in antibody concentration (typically >kD). It is often better to reduce the number of SULFO-TAG labels on the detection antibody to reduce signals.

You can test antibodies at multiple concentrations and decide on the final concentrations based on the assay performance. We recommend testing at least three concentrations of capture and detection antibodies.

**Table 7. Suggestions for capture and detection antibody titration for assay development on MSD GOLD Streptavidin- and Avidin-coated Plates**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture antibody concentrations (Small Spot Streptavidin plates)</td>
<td>0.5, 0.25, 0.125, 0 µg/mL (25 µL per well)</td>
</tr>
<tr>
<td>Capture antibody concentrations (Streptavidin plates)</td>
<td>2, 1, 0.5, 0 µg/mL (25 µL per well)</td>
</tr>
<tr>
<td>Capture antibody concentrations (High Bind Avidin plates)</td>
<td>4, 2, 1, 0 µg/mL (25 µL per well)</td>
</tr>
<tr>
<td>Detection antibody concentration</td>
<td>2, 1, 0.5, 0.25 µg/mL (50 µL per well)</td>
</tr>
</tbody>
</table>
Optimization - Assay Protocol

In general, there are three incubation steps that should be considered for optimization: plate coating, sample incubation, and detection antibody incubation. To test the impact of protocols on assay performance, the incubation time can be varied from 30 minutes to overnight for each step. Sufficient incubation time will allow molecular interactions to reach equilibrium, resulting in both higher signals and improved reproducibility. However, you may choose to shorten the incubation time to make the protocols more convenient.

Some of the suggested factors for assay protocols optimization are as follows:

- **Typical Step-Wise Assay Protocol**: Every protocol step is followed by washing the plate.
- **Alternate Protocol - Extended Sample Incubation**: Incubating samples overnight at 2-8°C may improve sensitivity for some assays.
- **Alternate Protocol - Single Wash (Homogenous)**: For tissue culture samples, the protocol may be streamlined by adding capture antibody, calibrator/sample, and detection antibody to your plate in one step and incubate all three components simultaneously.

### Table 8. Suggestions for protocol optimization for assay development on MSD GOLD Streptavidin- and Avidin-coated Plates

<table>
<thead>
<tr>
<th></th>
<th>Typical Step-Wise Assay protocol</th>
<th>Extended Sample Incubation</th>
<th>Single Wash (Homogenous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture Antibody Incubation</td>
<td>1 hr</td>
<td>1 hr</td>
<td></td>
</tr>
<tr>
<td>Sample/Calibrator Incubation</td>
<td>Wash</td>
<td>Wash</td>
<td>3 hrs</td>
</tr>
<tr>
<td>Detection Antibody Incubation</td>
<td>1 hr</td>
<td>1 hr</td>
<td>Wash</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wash</td>
<td></td>
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Plate Diagram