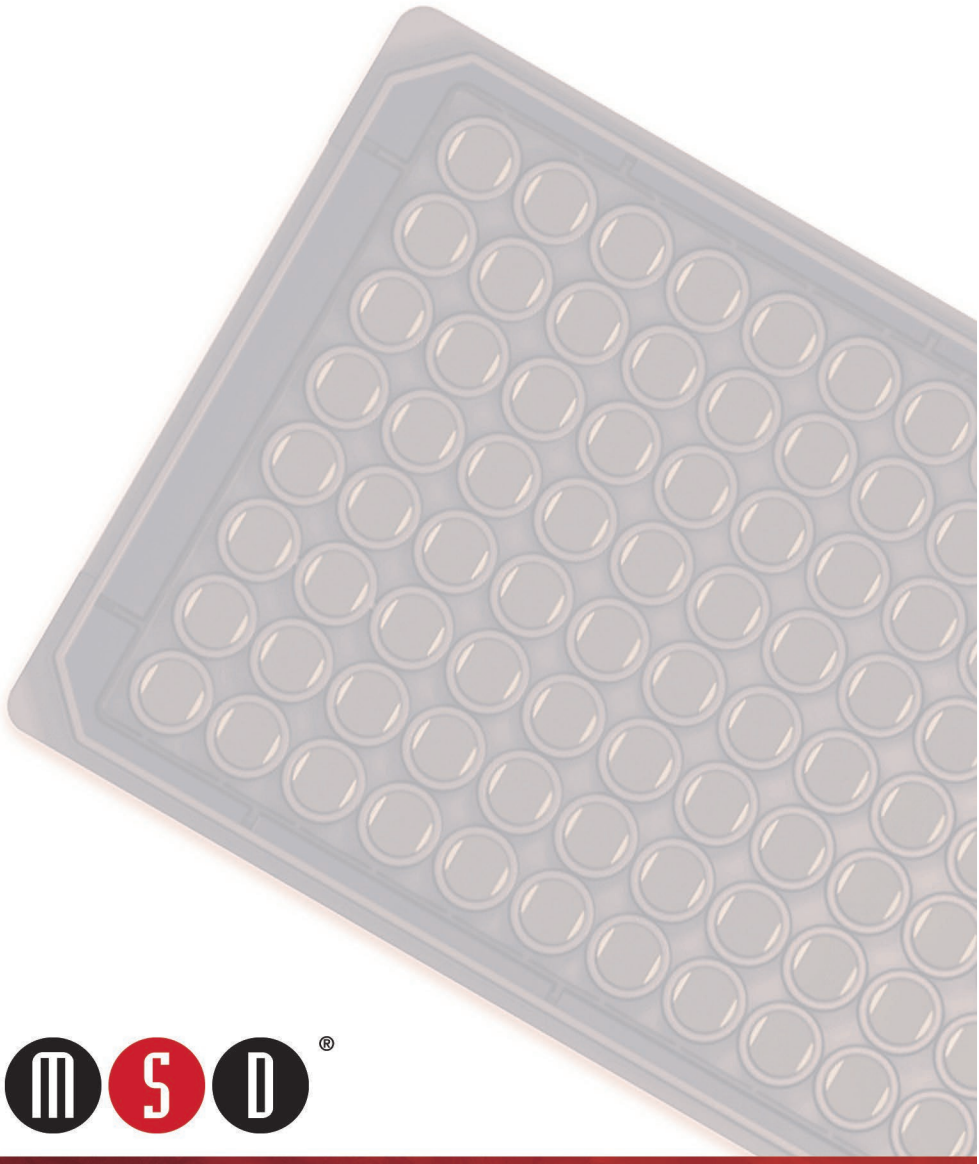
MSD® SECTOR and QuickPlex Plates

Streptavidin Coated Plates



www.mesoscale.com®

MSD Plates

Streptavidin Plates

	SECTOR™ Plate	QuickPlex™ Plate	QuickPlex Ultra™ Plate
MSD GOLD™ 96-well 1-Spot Streptavidin	L15SA	L55SA	
MSD GOLD 96-well Small Spot Streptavidin	L45SA		
96-well 1-Spot Streptavidin			L1BSA
96-well Small Spot Streptavidin			L4BLA

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

MESO SCALE DISCOVERY® SECTOR and QuickPlex plates are the basis of an assay system for measuring biomarkers using highly sensitive electrochemiluminescence (ECL) detection. MSD plates use this technology to provide a rapid and convenient method for the development of new assays and the transfer of existing ELISAs to the MSD platform. Streptavidin-coated plates are available in both SECTOR and QuickPlex plate formats. These are used for a range of applications, including assay development, biomarker measurements, and immunogenicity evaluations. Streptavidin-coated plates are highly suited for use with homogenous assays or bridging assays, such as typical immunogenicity assays that require tolerance to high levels of free drugs.

MSD GOLD is a signature of our commitment to quality, consistency, and high performance. The MSD GOLD designation certifies that the plates conform to rigorous specifications for uniformity and precision. These specifications are designed to meet the requirements of critical assays and research for the drug safety and toxicology communities. Similar to all our MSD GOLD assay development tools, MSD GOLD designated streptavidin-coated plates are validated for lot-to-lot reproducibility and have low intra-plate and inter-plate coefficients of variation percentages (%CVs). These attributes have led to the widespread adoption of 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 samples and gains in sensitivity, dynamic range, and specificity.

Plate Types and Surfaces

Streptavidin plates are available in 96-well 1-Spot and 96-well Small Spot formats (Figure 1).

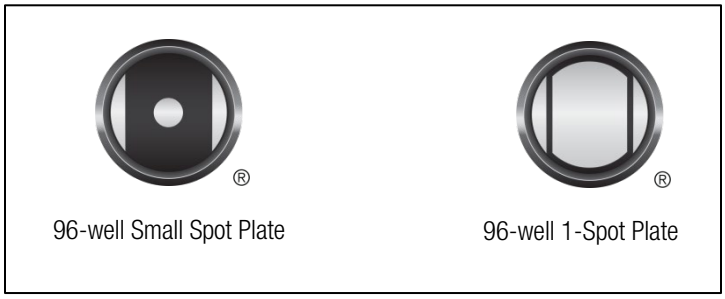


Figure 1. Spot patterns of MSD streptavidin plates.

Choosing a Plate Type

Binding Capacity and Assay Performance

Selection of the right plate type can be critical for successful assay development. The properties of coated plates differ and offer unique advantages. Small spot streptavidin-coated plates provide the highest assay signals and, therefore, superior sensitivity. Streptavidin-coated plates frequently exhibit lower nonspecific binding, especially with complex sample matrices. The binding capacity and properties of the two spot types are provided in Table 1.

Table 1. Binding capacities

Plate Type	Assay Sensitivity	Binding Capacity (IgG)*
Small Spot Streptavidin	+++	0.075 pmol/well
1-Spot Streptavidin	++	0.3 pmol/well

* The binding capacity was measured by titrating biotin-tagged IgG (BTI) in the plates (illustrated below). 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.

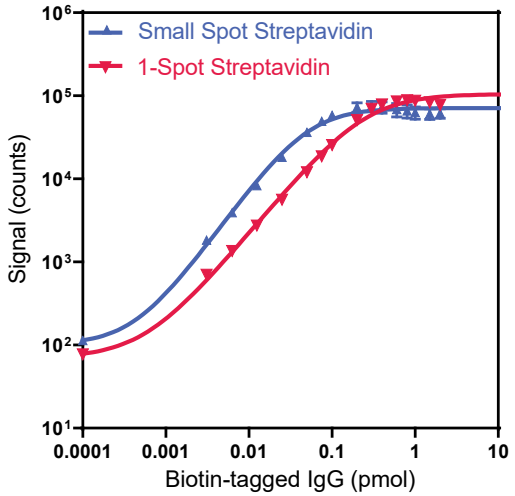


Figure 2. Titration of BTI on MSD streptavidin plates.

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 1-spot streptavidin-coated plates, the small spot streptavidin-coated plates have lower binding capacity and therefore require less coating material.

Instrument Compatibility

Streptavidin plates are compatible with MSD instruments according to Table 2. SECTOR plates are read multiple wells at a time; QuickPlex plates are read one well at a time.

Table 2. Instrument compatibility

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

NA = not applicable

Example Assay Formats

Typical formats for assays developed using streptavidin plates are similar to ELISAs using biotinylated capture reagents. For biomarker assays, a detection antibody conjugated with a 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 a detection reagent. For serology assays, biotinylated antigens may be used to capture antibodies, and SULFO-TAG conjugated anti-species antibodies can be used for detection (Figure 3C).

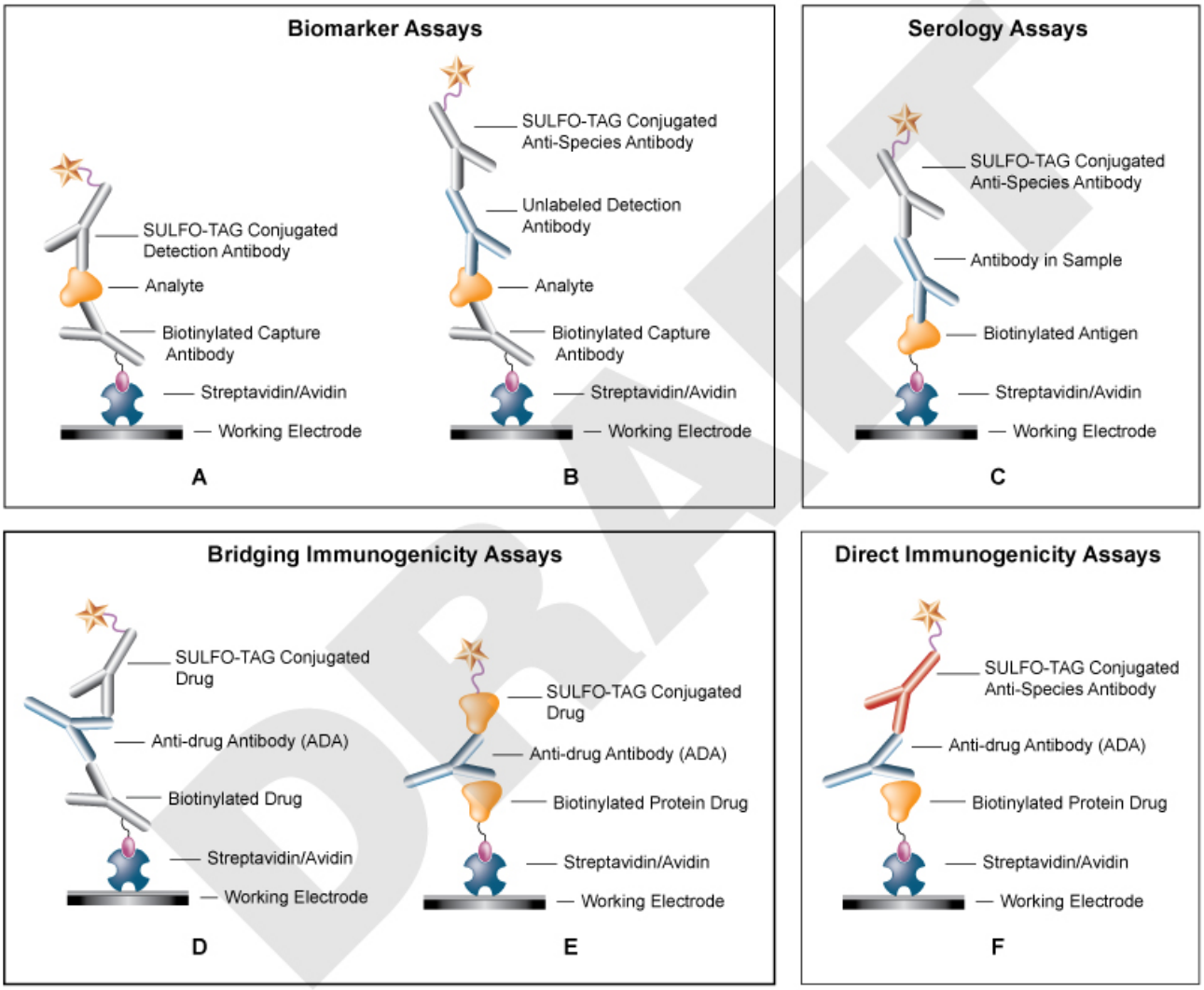


Figure 3A-F. Examples of biomarker and serology assay formats on MSD streptavidin plates.

Streptavidin plates may also be used for the development of assays to test the immunogenicity of protein therapeutics including antibodies. The bridging format for such assays require the drug to be conjugated with biotin to serve as the capture reagent (Figures 3D and 3E). The same drug conjugated with SULFO-TAG is the reporter in a bridging assay. Direct immunogenicity assays

may also be developed using biotinylated protein drugs as the capture reagent (Figures 3E and 3F). Additional information for MSD immunogenicity assays is available at https://www.mesoscale.com/en/technical_resources/technical_literature.

Principle of ECL

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

Product Options

MSD offers a range of plates and reagents to enable assay development on our platform. Streptavidin plates are offered in different pack sizes (Table 3).

Plates

Table 3. Catalog numbers of MSD assay development SECTOR and QuickPlex streptavidin plates.

MSD GOLD Plates	1 Plate	5 Plates	30 Plates	120 Plates	510 Plates
MSD GOLD 96-well 1-Spot Streptavidin SECTOR Plates[1]	L15SA-1	L15SA-2	L15SA-5	L15SA-6	L15SA-7
MSD GOLD 96-well Small Spot Streptavidin SECTOR Plates[1]	L45SA-1	L45SA-2	L45SA-5	L45SA-6	L45SA-7
MSD GOLD 96-well 1-Spot Streptavidin QuickPlex Plates[2]	L55SA-1	L55SA-2	L55SA-5	L55SA-6	L55SA-7
MSD Plates	**1 Plate**	**5 Plates**	**30 Plates**	**120 Plates**	**510 Plates**
MSD 96-well 1-Spot Streptavidin QuickPlex Ultra Plates[3]	L1BSA-1	L1BSA-2	L1BSA-5	L1BSA-6	L1BSA-7
MSD 96-well Small Spot Streptavidin QuickPlex Ultra Plates[3]	L4BLA-1	L4BLA-2	L4BLA-5	L4BLA-6	L4BLA-7

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

Recommended Minimum Volumes

Table 4. Minimum volumes recommended for 96-well plates.

Calibrator/Sample/Detection Reagent Volume	Blocking Solution/Read Buffer Volume	Minimum Wash Volume
25–50 µL/well	150 µL/well	150 µL/well

Biomarker Assays

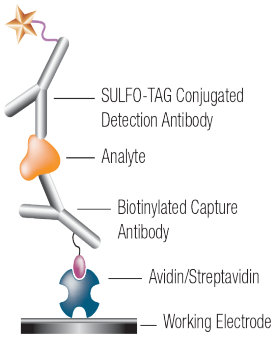


Plate Coating

STEP 1: Add blocking solution and incubate for 1 hour with shaking at room temperature or overnight at 2–8 °C.

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

Assay Protocol

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

STEP 4: Wash and add SULFO-TAG detection antibody. Incubate for 1–2 hours with shaking at room temperature.

STEP 5: Wash, and add read buffer.

Serology Assays

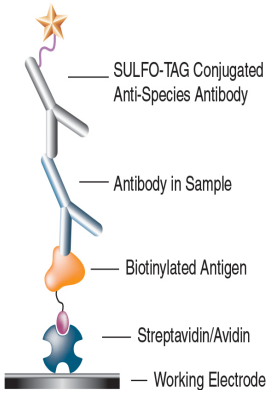


Plate Coating

STEP 1: Add blocking solution and incubate for 1 hour with shaking at room temperature or overnight at 2–8 °C.

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

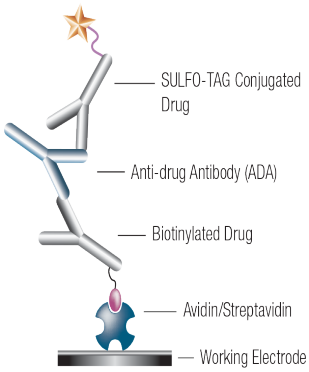
Assay Protocol

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

STEP 4: Wash and add SULFO-TAG detection antibody. Incubate for 1–2 hours with shaking at room temperature.

STEP 5: Wash, and add read buffer.

Bridging Immunogenicity Assays



Homogenous 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 2–8 °C.

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

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

STEP 4: Wash, and add read buffer.

Figure 4. Typical workflows for some of the assays that can be developed using MSD streptavidin plates.

Plate Coating

Coating with Antibodies

STEP 1: Block Plate.

- ❑ 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 because free biotin interferes with the binding of biotinylated capture reagents to streptavidin-coated plates.

STEP 2: Prepare Capture Antibody.

- ❑ Prepare 25 µL of capture antibody 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 antibody should be below the binding capacity of the plate (Table 1). The recommended starting capture antibody concentrations are 1 µg/mL for 1-Spot Streptavidin plates and 0.3 µg/mL for Small Spot Streptavidin plates in a 25 µL coating volume.
- ❑ 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] 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) or overnight at 2–8 °C.
- ❑ 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 streptavidin plates. If you want to optimize the coating concentrations, we recommend preparing the biotinylated reagents at a range of concentrations below the plate's binding capacity. Please refer to Table 1 for plate binding capacities and to the Appendix for calculations.

Typical Biomarker Assay Protocol

The following is an example of a typical biomarker assay protocol. For detailed instructions on developing an immunogenicity assay, please refer to the example of an immunogenicity protocol provided in the Appendix of this document or the Bridging Immunogenicity Assays Guidelines for Assay Development at www.mesoscale.com

Conjugation of detection antibody: Before beginning the assay, the detection antibody must be conjugated with the 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 prelabeled secondary reporter such as SULFO-TAG anti-species antibody can be bound to an unlabeled detection reagent. The protocol for SULFO-TAG conjugation can be found at www.mesoscale.com/en/support/product_information.

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

STEP 1: Prepare and Add Samples.

- ❑ Prepare calibrators and controls during the plate-blocking step.
- ❑ Add 50 µL of diluted calibrators controls, or samples to the bottom of each well.

Note: Volume as low as 25 µL/well may be used to conserve reagent.

- ❑ Seal the plate and incubate at room temperature with shaking 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 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.

Note: If required, detection antibody volume may be increased to 50 µL/well.

- ❑ 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 required will vary by application and should be determined experimentally.

STEP 3: Wash, Prepare Read Buffer, and Read Plate.

- ❑ If needed, prepare the read buffer during this time.

Note: MSD offers multiple read buffer products such as the following.

- MSD GOLD Read Buffers are part of our MSD GOLD portfolio and are validated for the reproducibility of signals across plate lots. MSD GOLD Read Buffers should be used at the supplied concentration without any dilution.
- MSD Read Buffer T (4X) is provided at a 4X stock concentration. Use deionized water to dilute Read Buffer T (4X) to a 2X concentration.

- ❑ Wash the plate three times with 1X MSD Wash Buffer.
- ❑ Carefully add 150 µL/well of an undiluted MSD GOLD Read Buffer or 2X MSD 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-coated plates. Representative data from four assays are shown below (Figure 5). For all the assays tested, the same antibodies and reagents were used on the different plate types. Results may differ depending on the reagent, protocol, and assay optimization.

Typical Calibration Curves

The calibration curves below compare assay performance on 1-spot streptavidin plates (red curve) and small spot streptavidin plates (blue curve). Representative calibration curves from four assays are shown below.

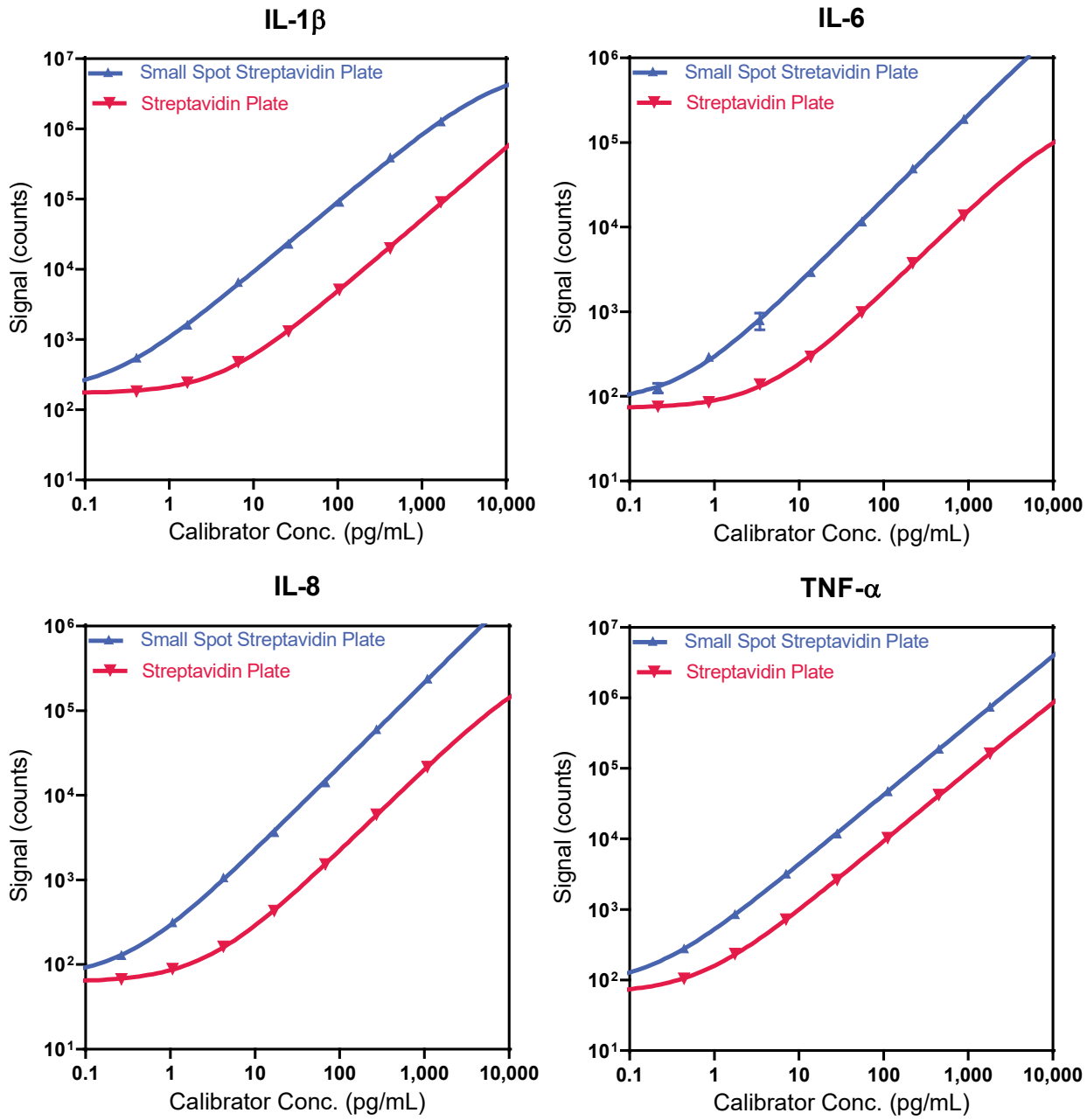


Figure 5. Representative calibration curves of assays tested on MSD streptavidin plates.

Sensitivity

Assay sensitivity was compared between streptavidin 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, we observed that the assays run on small spot streptavidin plates yielded lower detection limits compared to those tested on 1-spot plates (Table 5).

Table 5. Comparison of LLOD (pg/mL) between different MSD streptavidin plates.

	Assay			
Plate Type	IL-1β	IL-6	IL-8	TNF-α
96-well Small Spot Streptavidin Plate	0.030	0.115	0.111	0.054
96-well 1-Spot Streptavidin Plate	0.63	1.39	1.04	0.25

Reproducibility

Assay Reproducibility

As part of validation studies of MSD GOLD plates, three plates, each from three different lots of small spot streptavidin plates, were tested. Intraplate and interlot %CVs of calibrators and controls were evaluated. Representative data from four assays are shown in Table 6 below.

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

Table 6. Representative assay reproducibility results from four assays tested on MSD GOLD small spot streptavidin plates.

		Plate Lot 1 (n = 3 plates)		Plate Lot 2 (n = 3 plates)		Plate Lot 3 (n = 3 plates)		Interlot	
Assay	Control	Avg Conc (pg/mL)	Avg Intra-plate %CV	Avg Conc (pg/mL)	Avg Intra-plate %CV	Avg Conc (pg/mL)	Avg Intra-plate %CV	Avg Inter-lot Conc (pg/mL)	Interlot %CV
IL-1β	High	723	13.3	707	11.3	752	5.1	727	3.1
	Mid	70	8.8	67	5.4	69	13.3	69	2.0
	Low	7.0	9.2	6.6	8.9	7.1	9.3	6.9	3.7
IL-6	High	348	11.8	394	9.0	404	6.2	382	7.8
	Mid	36	3.5	33	7.5	37	6.1	36	5.8
	Low	3.4	6.6	3.3	5.5	3.8	6.7	3.5	7.4
IL-8	High	319	12.8	372	6.3	356	5.0	349	7.8
	Mid	32	6.7	33	6.2	33	9.1	33	2.0
	Low	3.0	8.8	3.3	11.6	3.3	6.4	3.2	6.2
TNF-α	High	784	3.1	776	2.1	738	5.7	766	3.2
	Mid	71	2.5	66	3.5	66	3.9	67	4.3
	Low	5.6	2.5	5.3	3.9	5.4	4.4	5.5	3.3

Avg = average; Conc = concentration; %CV = coefficient of variation percentage; Mid = middle

Plate Reproducibility

Reproducibility data was generated by testing MSD GOLD streptavidin-coated plates with a constant amount of biotinylated SULFO-TAG labeled IgG (BTI): streptavidin plates were tested at 0.2 pmol/well, and small spot streptavidin plates were tested at 0.05 pmol/well. The mean signal and %CV were calculated for each plate (intraplate %CV) and across plates (interplate %CV). Mean intraplate CVs of MSD GOLD plates must be less than 6%, with no plate having an intraplate CV greater than 12%.

The plots below (Figure 6) show the reproducibility data from 407 lots of 96-well streptavidin-coated plates manufactured between 10/2010 and 11/2018 (Figure 6A). All plate lots met the quality specifications with a mean intraplate CV of less than 6%. The results from 14,917 plates tested after the specifications were established in 2010 are shown in Figure 6B. Over 99.9% of the plates have an intraplate CV of <8% as per the quality control release specifications. There are no plates with an intraplate CV greater than 12%. The reproducible behavior of the small spot streptavidin plates is illustrated in Figures 6C and 6D. The mean intraplate %CVs from 51 small spot streptavidin plate lots tested between 11/2014 and 3/2018 are shown in Figure 6C. The individual plate results (1,919 plates) from each lot are shown in Figure 6D. No plates had an intraplate 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).

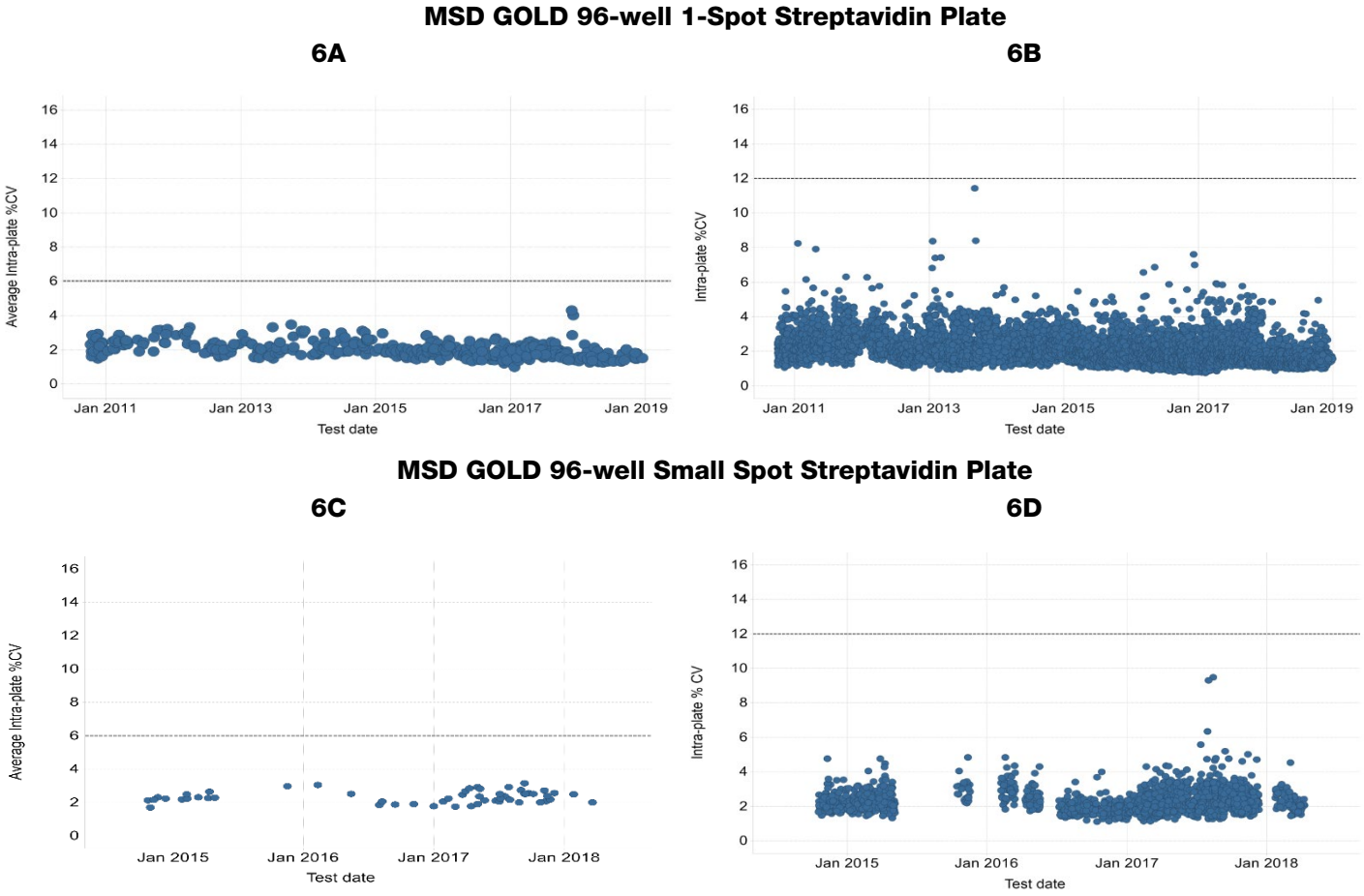


Figure 6. Representative reproducibility data of MSD GOLD 96-well streptavidin-coated plates when tested with a constant concentration of BTI.

Plate Stability

Streptavidin-coated plates can be stored at 2–8 °C or 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, we recommend performing the appropriate studies under your experimental conditions to ensure the same level of performance.

Real-time stability testing was performed on streptavidin-coated plates stored at 2–8 °C over 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 (Figure 7), 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 7, 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.

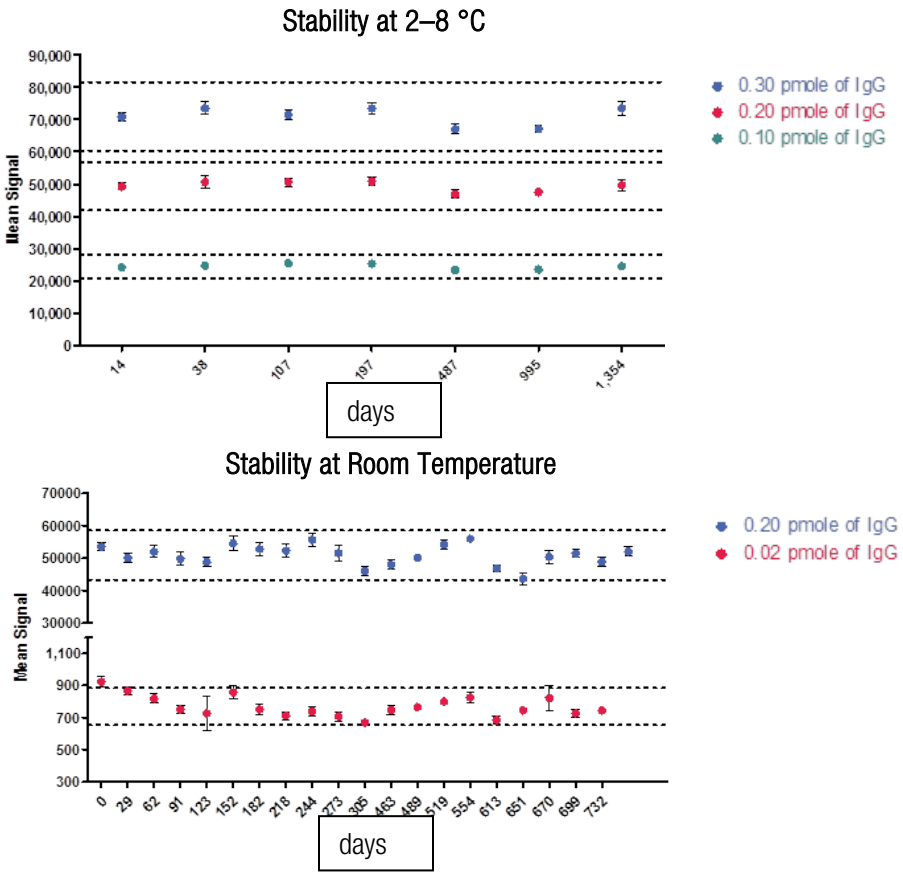


Figure 7. Real-time stability (days) of MSD GOLD 96-well 1-spot streptavidin plates.

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, we recommend coating the plate at a concentration that is below the binding capacity (Table 1). We titrated a biotinylated capture antibody on streptavidin-coated plates from multiple lots and tested them using a bridging immunogenicity assay. Results from three plate lots are presented in Table 7.

Within the quantitative range of the assay, signals were highly consistent, provided the amount of capture material was at or below the specified binding capacity (Table 1) 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 96-well streptavidin plates, the signals plateaued at capture concentrations of 0.33 pmol (Table 7).

Table 7. Titration of biotinylated drug on different lots of MSD GOLD streptavidin plates.

MSD GOLD 96-well Small Spot Streptavidin Plates

Representative Signal from One Plate Lot				
Conc. of ADA (ng/ml)	Biotin Drug (pmol)			
	0.33	0.11	0.037	0.012
3,000	397,724	459,171	264,197	95,333
1,000	139,329	182,532	101,846	36,460
100	16,200	20,345	11,016	3,971
10	1,740	2,245	1,214	442
1	255	299	192	104
0.1	80	89	75	64
0.01	64	66	62	58
0	59	60	57	57

%CV of Signal of Three Plate Lots			
Biotin Drug (pmol)			
0.33	0.11	0.037	0.012
5.2	2.8	4.2	3.2
11.5	2.8	9.4	10.4
13.5	3.0	10.4	10.8
15.8	4.5	3.6	6.5
8.7	1.4	6.4	6.3
1.6	0.7	1.8	5.2
5.2	8.5	6.3	15.9
7.9	3.8	2.9	12.1

MSD GOLD 96-well 1-Spot Streptavidin Plates

Representative Signal from One Plate Lot				
Conc. of ADA (ng/ml)	Biotin Drug (pmol)			
	0.33	0.11	0.037	0.012
3,000	258,140	249,047	126,737	46,552
1,000	85,367	88,591	45,567	14,047
100	10,103	9,703	5,010	1,811
10	1,085	1,033	544	236
1	180	176	119	79
0.1	75	76	69	62
0.01	65	64	58	60
0	64	64	63	61

%CV of Signal of Three Plate Lots			
Biotin Drug (pmol)			
0.33	0.11	0.037	0.012
1.8	2.4	7.0	9.8
5.7	4.2	8.8	3.0
2.3	2.5	6.4	6.0
1.6	4.8	6.8	4.5
2.5	1.9	4.8	3.5
6.3	2.4	2.9	0.9
5.0	5.4	5.0	3.1
7.2	2.5	1.6	3.9

Multiplexing with MSD

Assays developed on MSD small spot streptavidin-coated plates can be easily converted to multiplex format using our 10-spot U-PLEX® assay platform.

Typical Calibration Curves

U-PLEX assays use biotinylated capture reagents similar to those used on streptavidin-coated plates (Figure 8). 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 small spot streptavidin plates.

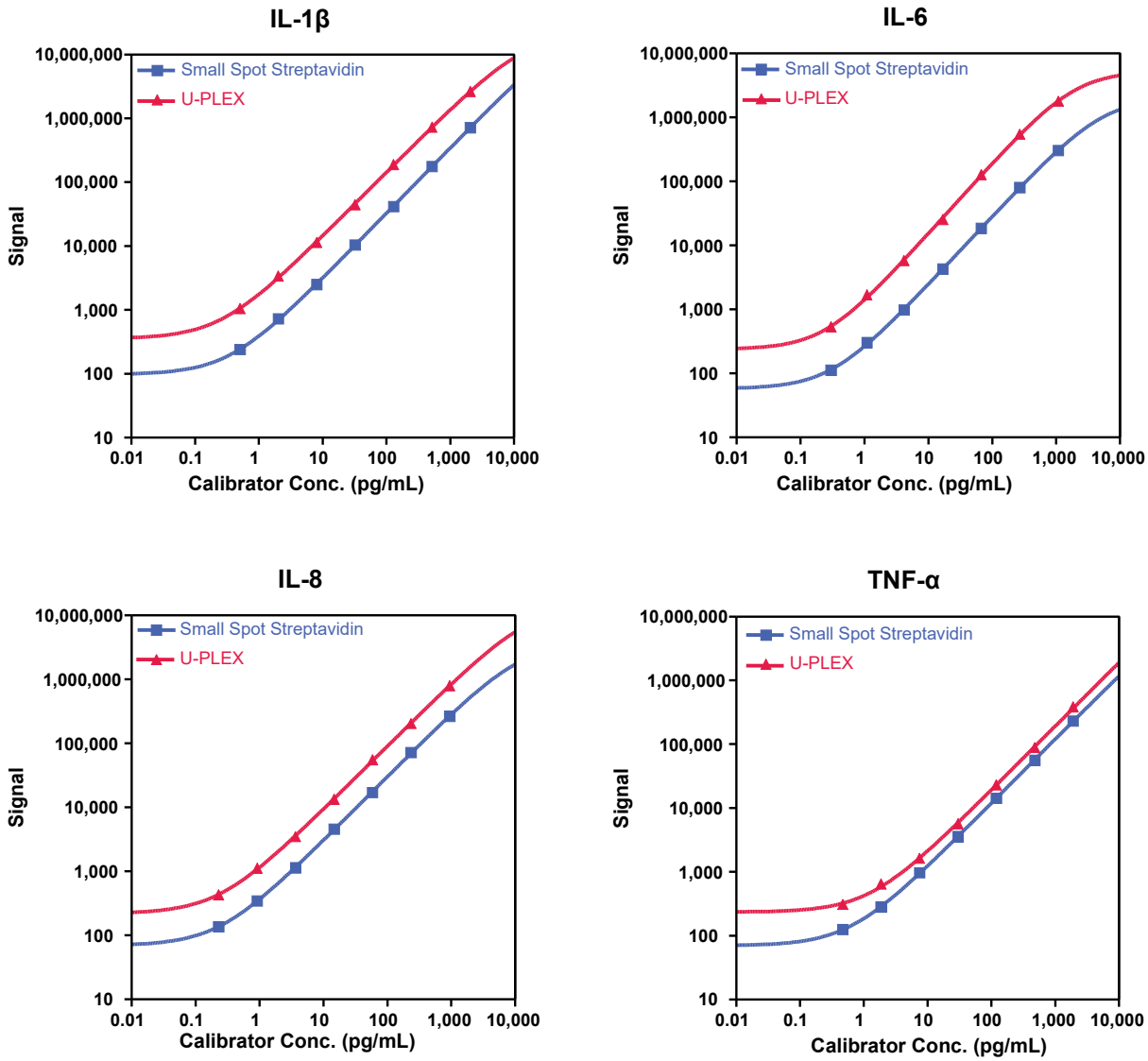


Figure 8. *Comparison of calibration curves of assays tested on MSD small spot streptavidin plates and U-PLEX plates.*

Comparison of Sample Quantification

Eighteen human serum and 19 human plasma samples spiked with calibrators were tested on 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.

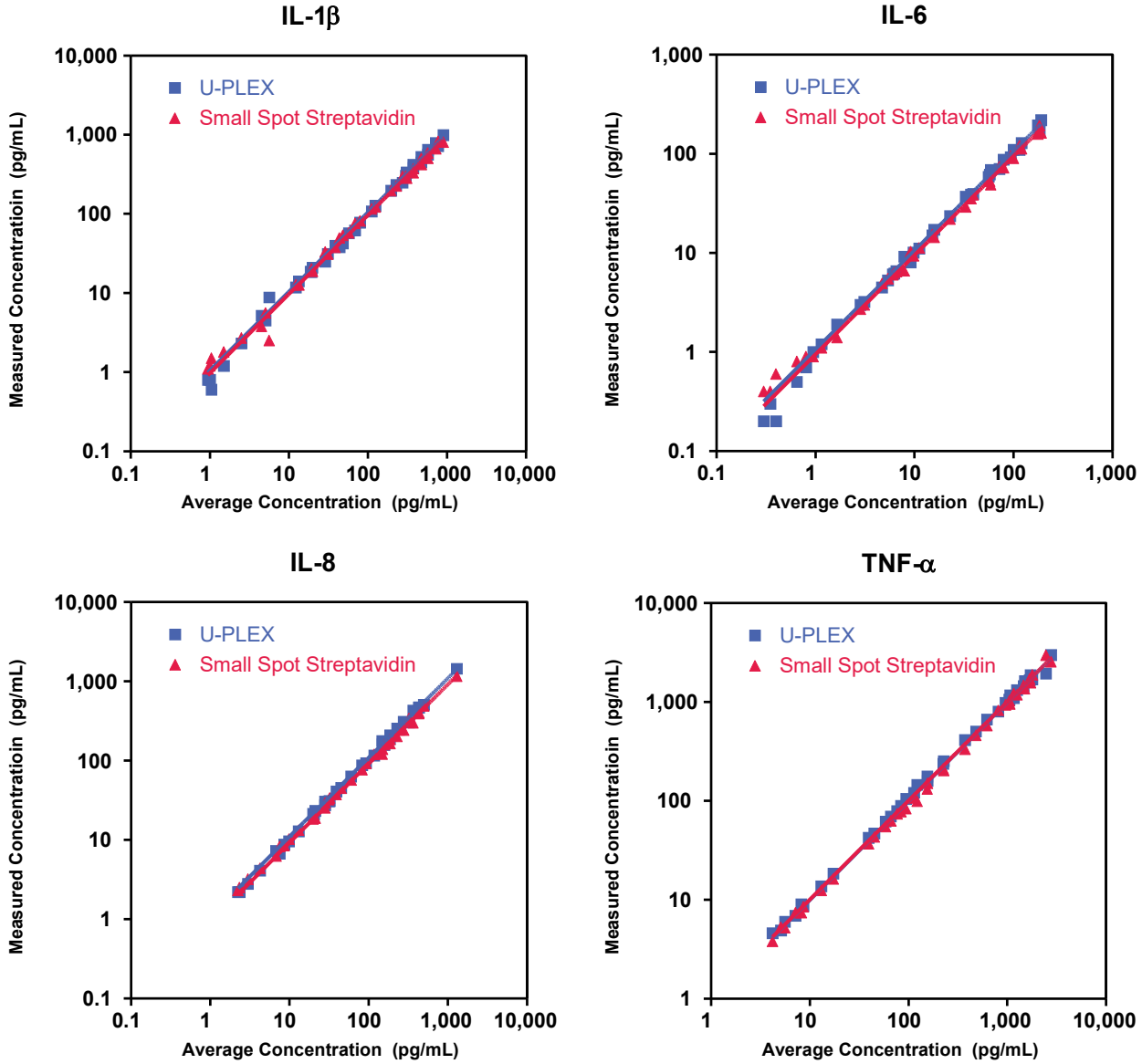


Plate Type	Statistic	IL-1β	IL-6	IL-8	TNF-α
96-well Small Spot Streptavidin Plate	r^2 Value	0.96	0.95	0.91	1.01
	Slope	0.99	0.99	1.00	0.98
U-PLEX Plate	r^2 Value	1.04	1.06	1.09	0.99
	Slope	0.99	0.99	1.00	0.98

Figure 9. Correlation of sample quantification between assays tested on U-PLEX plates and MSD GOLD small spot streptavidin plates.

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/en/support/product_information/search_msds.

Best Practices and Technical Hints

General Assay Techniques

- 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 reach room temperature before opening the foil packet.
- Avoid bubbles in wells during all pipetting steps, as they may lead to variable results. Bubbles introduced when adding the read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner. Do not touch the pipette tip to the bottom of the wells when pipetting into the MSD plate.
- 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 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 it from airflow, and add solutions to the plate as soon as possible.
- 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 impacted.
- Tap the plate on a paper towel to remove residual fluid after washing.
- 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.
- Use a new adhesive plate seal for all incubation steps. Avoid reusing plate seals.

Preparation of Calibrators and Samples

- Prepare calibrators, samples, and controls in polypropylene microcentrifuge tubes or polypropylene containers of sufficient volume. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.

Preparation of detection antibodies

- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates need not be shielded from light except for direct sunlight.
- You may adjust volumes proportionally when preparing detection antibody.
- Do not use detection reagents containing biotin on the streptavidin plates.

Reading Plates

- Remove all plate seals before reading the plate.
- Make sure that the read buffer is at room temperature (20–26 ºC) 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 the plate to its original packaging with the desiccant, and seal.
- Keep time intervals consistent between adding the read buffer and reading the plate to improve inter-plate precision. It is recommended that an MSD instrument be prepared to read a plate before adding a read buffer.
- Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
- Do not shake the plate after adding the read buffer.

Capture Antibody Calculation for Coating Plates

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

$$\mu g/mL\ protein = \frac{pmol\ of\ biotinylated\ protein\ per\ well \times molecular\ weight\ of\ protein\ (Da)}{}$$

The following example determines the concentration of a 150 kDa biotinylated antibody in µg/mL, such that 0.25 pmol 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

µg/mL protein = 0.25 × 150,000/(25 × 1,000) = 1.5 µg/mL

2. Converting µg/mL biotinylated protein to picomole concentration

$$pmol\ protein = \frac{\mu g/mL\ concentration\ of\ protein \times volume\ (\mu L) \times 1{,}000}{protein\ molecular\ weight\ (Da)}$$

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

pmol protein = 1 × 25 × 1,000/150,000 = 0.167 pmol

3. Converting nM biotinylated protein to µg/mL concentration

$$\mu g/mL\ protein = \frac{nM\ concentration\ of\ protein \times molecular\ weight\ of\ protein\ (Da)}{1{,}000{,}000}$$

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

µg/mL protein = 15 × 150,000/1,000,000 = 2.2 µg/mL

Alternative Protocol for Bridging Immunogenicity Assay

Below is an example protocol for a bridging immunogenicity assay. For more detail, refer to the Bridging Immunogenicity Assays Guidelines for Assay Development available at www.www.mesoscale.com.

STEP 1: Prepare Reagents.

- ❑ Biotinylated Drug and SULFO-TAG Conjugated Drug
  - In a bridging immunogenicity assay, the antidrug antibody (from serum/plasma) bridges a biotinylated capture drug and a SULFO-TAG conjugated detection drug.
  - 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 micro concentrators are suitable for this purpose.
  - For preparing the SULFO-TAG conjugated drug, follow the protocol outlined in the note (using the recommended challenge ratio for immunogenicity applications), MSD SULFO-TAG NHS-Ester application, which can be found online at www.mesoscale.com/en/technical_resources/technical_literature/technical_notes_search.
- ❑ Antidrug Antibody Samples
  - Prepare a dilution series of antidrug antibody in normal serum or plasma. Recommended test concentrations of antidrug antibody are 10,000, 2,500, 625, 156, 39, 9.8, 2.4, and 0 ng/mL. Each well should receive 25 µL of antidrug 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 (antidrug antibody samples, above).
- ❑ Seal the plate and incubate for 1–2 hours at room temperature with shaking or overnight at 2–8 °C without shaking.

STEP 3: Block MSD Plate.

- ❑ During the incubation of the Master Mix solution, add 150 µL/well of blocking solution to a streptavidin 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 an undiluted MSD GOLD Read Buffer B or 2X MSD 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 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 drugs should be adjusted according to the molecular weight of the drug. As an example, for a 75 kDa protein therapeutic (half the molecular weight of a 150 kDa 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 of this Appendix for the conversion of µg/mL to picomoles.

Optimization of Antibody Concentrations

An important step in assay optimization is the 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 (Table 1) when coating the plate. Higher capture antibody concentration will increase assay signals until the concentration surpasses the plate binding capacity (Table 1). Increasing the amount of detection antibodies will also increase signals but may lead to high background, especially in assays where detection antibodies nonspecifically 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 8).

Table 8. Suggestions for capture and detection antibody titration for assay development on MSD streptavidin plates.

Variable	Test conditions
Capture antibody concentrations (Small Spot Streptavidin plates)	0.5, 0.25, 0.125, 0 µg/mL (25 µL per well)
Capture antibody concentrations (1-Spot Streptavidin plates)	2, 1, 0.5, 0 µg/mL (25 µL per well)
Detection antibody concentration	2, 1, 0.5, 0.25 µg/mL (50 µL per well)

Optimization of Assay Protocol

Three incubation steps should be considered for optimization: plate coating incubation, 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 higher signals and improved reproducibility. However, you may shorten the incubation time to make the protocols more convenient.

Some suggested factors for assay protocol optimization are as follows (see Table 9).

- **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 a capture antibody, calibrator/sample, and detection antibody to your plate in one step and incubating all three components simultaneously.

Table 9. Suggestions for protocol optimization for assay development on MSD streptavidin plates.

Incubation Step	Typical Step-Wise Protocol	Extended Sample Incubation	Single Wash (Homogenous)
Capture Antibody Incubation	1 hr	1 hr	3 hr
	Wash	Wash	
Sample/Calibrator Incubation	1 hr	Overnight	
	Wash	Wash	
Detection Antibody Incubation	1 hr	1 hr	
	Wash	Wash	Wash

Plate Diagram