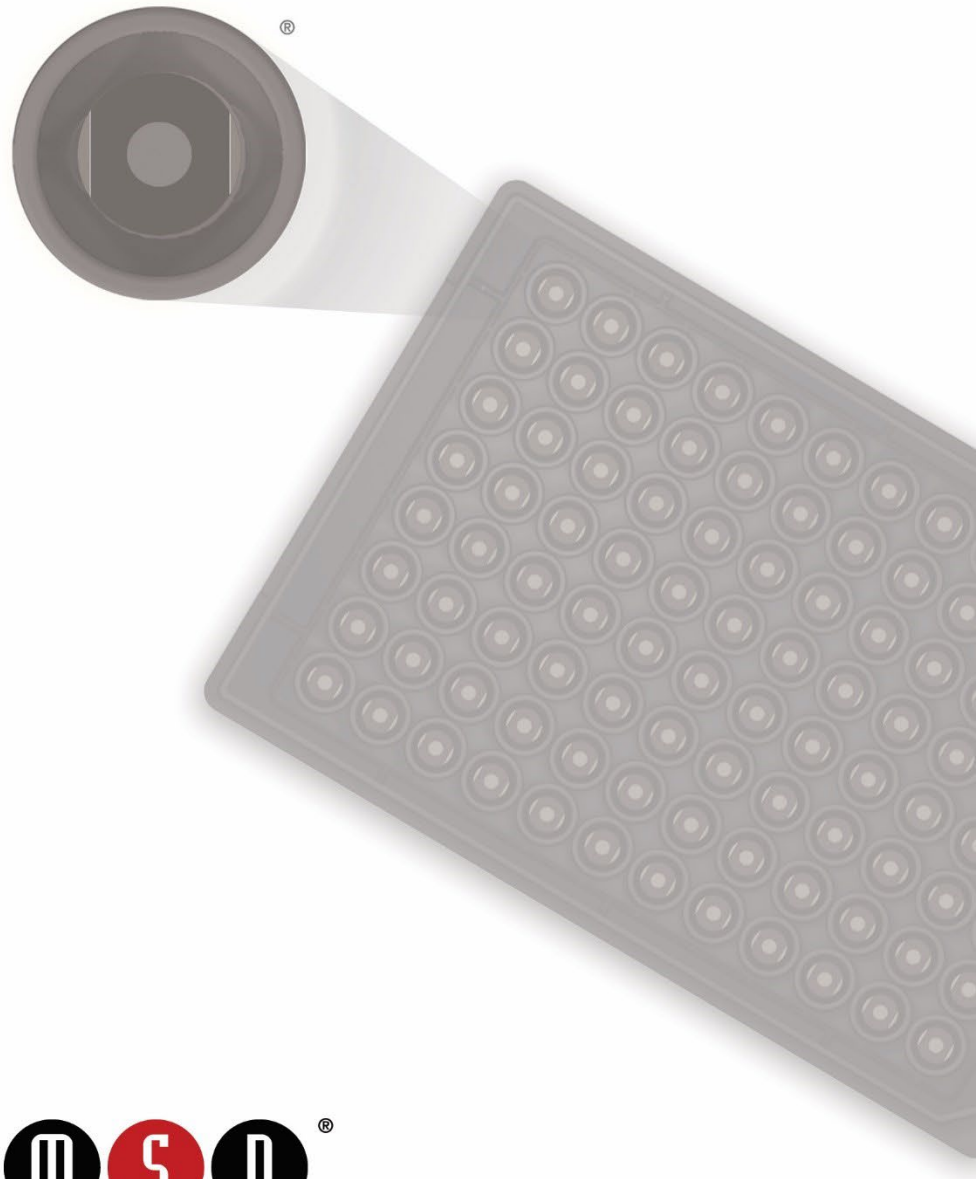
U-PLEX® Biomarker Group 3 (human)

Singleplex Assays



MSD U-PLEX Platform

U-PLEX Biomarker Group 3 (Human) Singleplex Assays

For use with serum, EDTA plasma, tissue lysates, and cell culture supernatants.

This product insert should be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Introduction

The U-PLEX Biomarker Group 3 (human) contains 21 analytes. A complete list of the entire U-PLEX menu can be found at *www.mesoscale.com/en/products_and_services/assay_kits/u-plex_gateway*.

A representative data set for each assay is presented in the product-specific datasheets. The datasheets are available at www.mesoscale.com/support/product_information.

Principle of the Assay

Singleplex assays are supplied on either 96-well (Figure 1), or 384-well plates. These plates provide high sensitivity and consistent performance. GOLD-branded plates also deliver excellent inter- and intra-lot uniformity.

Each singleplex assay is supplied with a biotinylated capture antibody that binds to streptavidin on the plate surface. Analytes in the sample bind to the capture antibody. Detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD SULFO TAG™) bind to the analytes to complete the sandwich immunoassay. Once the immunoassay is complete, the plate is loaded into an MSD instrument where a voltage applied to the plate causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample.

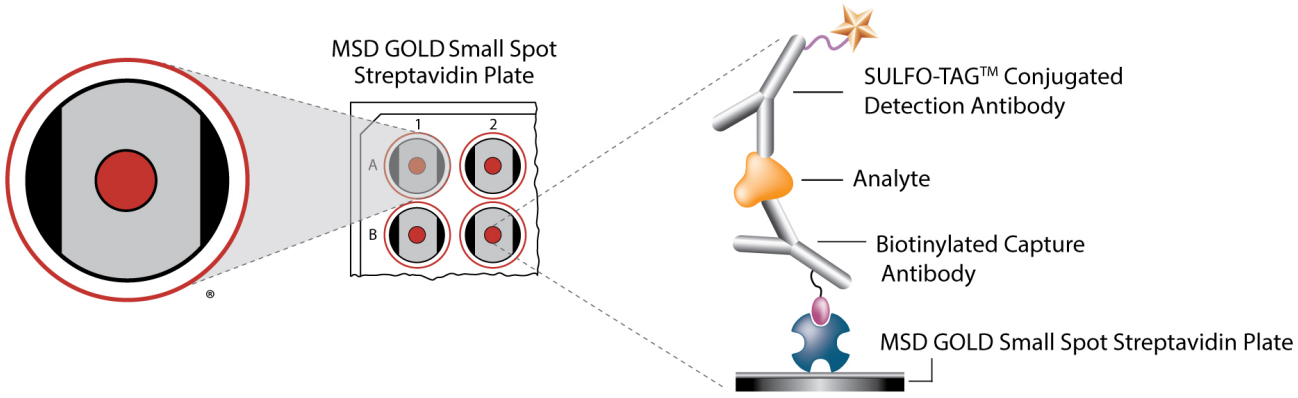

Figure 1. *A U-PLEX singleplex assay on a streptavidin plate.*

Components

Table 1 lists the components provided with U-PLEX Biomarker Group 3 (human) Singleplex Assays.

U-PLEX Singleplex Assays are also available with 384-well SECTOR plates. See Appendix B for details.

Table 1. Reagents that are supplied with all U-PLEX Biomarker Group 3 (human) 96-well Singleplex Assays

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 plate	5 plates	25 plates	
MSD GOLD 96-Well Small Spot Streptavidin SECTOR Plate	2–8 °C	L45SA-1	—	1 plate	5 plates	25 plates	96-well plate, foil sealed, with desiccant.
Diluent 100	2–8 °C	R50AA-2	200 mL	1 bottle	3 bottles	3 bottles	Diluent for capture antibody and samples
		R50AA-3	1,000 mL	—	—	2 bottles	
Diluent 12	≤–10 °C	R50JA-3	50 mL	1 bottle	3 bottles	—	Diluent for samples and calibrator
		R50JA-2	200 mL	—	—	3 bottles	
Diluent 11	≤–10 °C	R55BA-5	10 mL	1 bottle	—	—	Diluent for detection antibody
		R55BA-3	50 mL	—	1 bottle	5 bottles	
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent reaction
		R60AM-2	90 mL	—	1 bottle	5 bottles	

RT = room temperature
Dash (—) = not applicable

Assay-Specific Reagents

U-PLEX Antibody Set

You will receive a U-PLEX Antibody Set containing a biotinylated capture antibody and a SULFO-TAG conjugated detection antibody (Table 2).

Table 2. Contents of U-PLEX Antibody Set

Name	Storage	Size	Quantity Supplied			Description
			1 Plate	5 Plates	25 Plates	
U-PLEX Analyte-Specific Antibody Set	2–8 °C	1-Plate	1	—	—	Set containing biotinylated capture antibody and SULFO-TAG conjugated detection antibody
		5-Plate	—	1	5	

Dash (—) = not applicable

U-PLEX Calibrators

Biomarker Group 3 calibrators (Table 3) are lyophilized multi-analyte blends.

Individual analyte concentrations are provided in the lot-specific certificates of analysis (COA). Assays include one vial of the appropriate calibrator for each assay plate.

Table 3. Analytes included in the calibrator blends available for U-PLEX Biomarker Group 3 (human)

Name	Storage	Catalog No.	Analytes
Calibrator 24	2–8 °C	C0351-2	DPPIV, ICAM-1, SAA, SHBG, VCAM-1
Calibrator 25	2–8 °C	C0352-2	CA1, Complement factor D, CRP, Cystatin C, Factor VII, NGAL/LCN2, sTfR-1
Calibrator 26	2–8 °C	C0353-2	A2M, Adiponectin, ApoA1, ApoC3, Complement C9, RBP4, Serpin A1
Human Clusterin Calibrator	≤–70 °C	C01B9-2	Clusterin
Human vWF Calibrator	2–8 °C	C01C9-2	vWF

*Calibrator 23 is a liquid-blend calibrator that can be blended with other calibrators once thawed.
^These calibrators are used in two assays.

Instrument Compatibility

MSD offers U-PLEX assays designed for use on specific instrument platforms (Table 4).

Table 4. Instrument compatibility

Instrument	Assays on 96-well SECTOR plates	Assays on 96-well QuickPlex plates	Assays on 384-well SECTOR plates
MESO QuickPlex Q 60MM	—	Y	—
MESO® QuickPlex SQ 120	Y	—	—
MESO QuickPlex® SQ 120MM	Y	—	—
MESO SECTOR S 600MM	Y	—	Y
MESO SECTOR® S 600	Y	—	Y

Y = compatible
Dash (—) = not applicable

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene tubes for preparing dilutions
- ❑ Liquid-handling equipment suitable for dispensing 10 to 150 µL/well into a 96-well or 384-well microtiter plate
- ❑ Plate-washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm (1,500 rpm for 384-well plates)
- ❑ MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) for plate washing. The standard protocol uses a minimum of 200 mL for a 96-well plate and 415 mL for a 384-well plate. Automated plate washers may need overage added to these volumes.
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer

Safety

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

Additional product-specific safety information is available in the applicable safety data sheet (SDS), which can be obtained from MSD Customer Service or at the www.mesoscale.com® website.

Assay Protocol (96-well plates)

Please read the entire detailed Reagent Preparation instructions and the Best Practices (Appendix A) before starting work.

STEP 1: Coat Plates

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X Wash Buffer.
- ❑ Add 200 µL of biotinylated capture antibody to 3.3 mL of Diluent 100. Mix by vortexing.
- ❑ Add 25 µL of the biotinylated antibody solution to each well of the provided MSD plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and shake for 1 hour at room temperature or overnight at 2–8 °C.
- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use.

STEP 2: Add Samples and Calibrators

- ❑ Add 50 µL of prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X Wash Buffer.
- ❑ Add 50 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

STEP 4: Wash and Read

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

Reagent Preparation

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

Prepare Samples

Sample Dilution, 4,000-fold

Based on in-house testing of normal samples, a 4,000-fold dilution is recommended for CA1, Clusterin, Complement factor D, CRP, Cystatin C, DPPIV, Factor VII, ICAM-1, NGAL/LCN2, SAA, SHBG, sTfR-1, VCAM-1 and vWF before loading onto the plate (Table 5). See Table 5 for recommended Calibrators.

Table 5. Dilute samples 4,000-fold

Analytes			
CA1	Clusterin	Complement factor D	CRP
Cystatin C	DPPIV	Factor VII	ICAM-1
NGAL/LCN2	SAA	SHBG	sTfR-1
VCAM-1	vWF	—	

Dash (—) = not applicable

A two-step dilution procedure is encouraged. First, dilute the sample 100-fold by adding 10 µL of samples to 990 µL of Diluent 100. Dilute the sample one more time 40-fold by adding 10 µL of diluted sample to 390 µL of Diluent 12. The sample is now diluted 4,000-fold (Figure 3; Table 6).

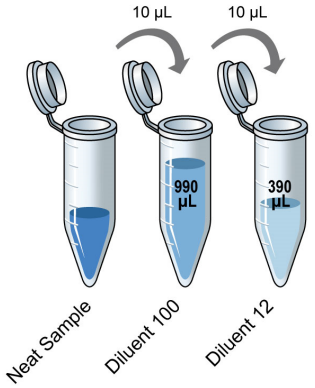

Figure 3. Dilution schema for preparation of samples diluted 4,000-fold.

Table 6. Dilution for optimal sample analysis

Dilution Step	Tube No.	Dilution Fold	Source	Source Volume	Diluent (µL)	Diluent Type	Total Volume (µL)
1	1	100	Neat Sample	10	990	Diluent 100	1,000
2	2	40	From tube 1	10	390	Diluent 12	400

Sample Dilution, 200,000-fold

Based on in-house testing of normal samples, a 200,000-fold dilution is recommended for A2M, Adiponectin, Apo1, ApoC3, Complement 9, RBP4, and SerpinA1 before loading onto the plate (Table 7). See Table 5 for recommended Calibrators.

Table 7. Dilute samples 200,000-fold

Analytes			
A2M	Adiponectin	Apo1	ApoC3
Complement C9	RBP4	Serpin A1	—

Dash (—) = not applicable

A three-step dilution procedure is encouraged. First, dilute the sample 100-fold by adding 10 µL of sample to 990 µL of Diluent 100. Dilute the sample again 100-fold by adding 10 µL of diluted sample to 990 µL of Diluent 100. Dilute the sample one more time 20-fold by adding 10 µL of diluted sample to 190 µL. The sample is now diluted 200,000-fold (Figure 4; Table 8).

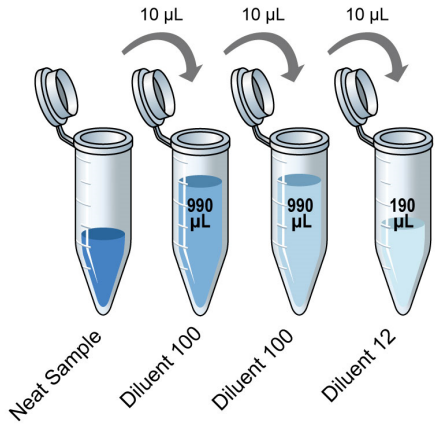

Figure 4. Dilution schema for preparation of samples diluted 200,000-fold.

Table 8. Dilution for optimal sample analysis

Dilution Step	Tube No.	Dilution Fold	Source	Source Volume	Diluent (µL)	Diluent Type	Total Volume (µL)
1	1	100	Neat Sample	10	990	Diluent 100	1,000
2	2	100	From tube 1	10	990	Diluent 100	1,000
3	3	20	From tube 2	10	190	Diluent 12	200

Prepare Calibration Standards

For Lyophilized Calibrators

Bring Calibrators to room temperature. Reconstitute lyophilized Calibrators by adding 250 µL of Assay Diluent to the glass vial. This will result in a 10X concentrated stock of the Calibrator. Invert the reconstituted Calibrator at least 3 times. Do not vortex at this point. Let the reconstituted solution equilibrate at room temperature for 15–30 minutes and then vortex briefly. The Calibrator is now ready for use.

For Liquid Calibrators:

Thaw the stock Calibrator(s) and keep it on ice. Once thawed, the Calibrator is ready to use. Keep dilution(s) at room temperature.

Dilutions

The following instructions are for the preparation of 7 Calibrator Standard solutions plus a Zero Calibrator Standard for use in an 8-point standard curve (Figure 5; Table 9).

Important: Change pipette tips and vortex calibrators after each dilution step. Calibrators are typically run in duplicate. There is a sufficient volume of each dilution to run up to 6 replicates using this process.

- ❑ Prepare Calibrator Standard 1 by adding 25 µL of the reconstituted Calibrator to 225 µL of Assay Diluent (Figure 5). Mix by vortexing.
- ❑ For Calibrator Standard 2, add 75 µL of Calibrator Standard 1 to 225 µL of Assay Diluent.
- ❑ Repeat 4-fold serial dilutions to generate a total of 7 Calibrator Standards (Table 9). Mix by vortexing between each serial dilution.
- ❑ Use Assay Diluent as Calibrator Standard 8 (zero Calibrator).

Note: For the lot-specific concentration of Calibrators in the blend, refer to the COA supplied with the assay. You can also find a copy of the COA at www.mesoscale.com.

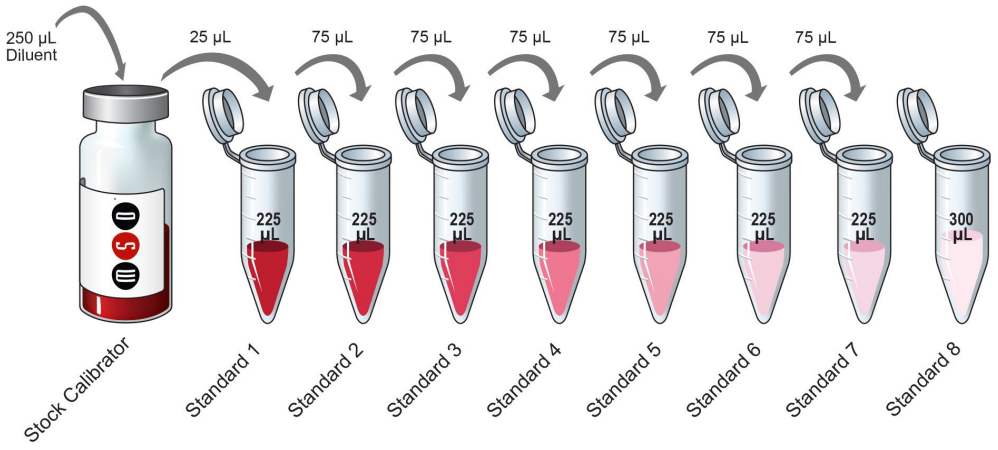

Figure 5. Dilution schema for U-PLEX calibrator standards for singleplex assays.

Table 9. Serial dilution to generate the standard curve

Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Reconstituted Calibrator (µL)	Assay Diluent (µL)	Total Volume (µL)
1	1	Stock Calibrator vial	25	225	250
2	2	From tube 1	75	225	300
3	3	From tube 2	75	225	300
4	4	From tube 3	75	225	300
5	5	From tube 4	75	225	300
6	6	From tube 5	75	225	300
7	7	From tube 6	75	225	300
8 (zero Calibrator)	8	—	0	300	300

Dash (—) = not applicable

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X for 96-well assays. Prepare the detection antibody solution immediately before use.

For one plate, combine:

- ❑ 60 µL of the supplied 100X detection antibody
- ❑ 5,940 µL of Diluent 11

Wash Buffer

Prepare a 1X working solution of MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) by diluting the 20X stock with deionized water. 1X MSD Wash Buffer can be stored at room temperature for up to two weeks. MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) is ordered separately.

Read Buffer

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

Appendix A

Alternative Assay Protocols

The suggestions below may be useful for simplifying the protocol.

- ❑ Alternate Protocol 1, Co-incubation: Co-incubating samples and detection antibody solution may improve the sensitivity for some assays. Note that the use of the co-incubation protocol may result in sample concentrations that vary from concentrations obtained with the standard protocol. If this protocol is chosen, we recommend that this protocol be used for the entirety of the research project.
- ❑ Alternate Protocol 2, Shortened Incubation: Some 384-well assays may achieve acceptable performance with shorter incubations. Consider reducing the incubation time of samples in the plate and the incubation time of detection antibody.
- ❑ Alternate Protocol 3, Reduced Wash: For cell culture supernatants, you may simplify the protocol by eliminating one of the washes in each step.

Best Practices

- Equilibrate all assay components to room temperature before use. Mix well. Bring plates to room temperature before opening the packet.
- Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is very important when adding Read Buffer at the final step prior to plate reading the plate.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm (1,000 to 1,500 rpm for 384-well plates) depending on the shaker design and orbit. Keep the shaking speed and model the same for long-term studies.
- Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
- Avoid excessive drying of the plate during washing steps, especially if working inside a laminar flow hood or another 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.
- Use a new adhesive plate seal for all incubation steps. Avoid re-using plate seals.
- Dispense reagents and wash fluids at the side of the well towards the bottom corner.
- Remove the plate seal before reading the plate in the instrument.
- Keep time intervals consistent between the addition of Read Buffer and reading the plate to improve inter-plate precision. Prepare an MSD instrument before adding Read Buffer.
- Do not shake the plate after adding Read Buffer.
- Do not obscure or damage the plate barcode; it is required for the plate reader.
- Only use the reagents provided with this kit.
- Reconstituted or thawed calibrators should be used immediately. If storage is necessary, divide into suitably sized aliquots, and store immediately at ≤–70 °C.

Appendix B

Components for 384-well Assays

Table 10. Reagents that are supplied with all U-PLEX Biomarker Group 3 (human) 384-well Singleplex Assays

Reagent	Storage	Catalog No.	Size	Quantity Supplied		Description
				5 Plates	25 Plates	
MSD 384-well Streptavidin SECTOR Plate	2–8 °C	L21SA-1	—	5 plates	25 plates	384-well plate, foil sealed, with desiccant
Diluent 100	2–8 °C	R50AA-3	1,000 mL	varies by assay		Diluent for biotinylated capture antibody and sample dilution
Diluent 12	≤–10 °C	R50JA-2	200 mL	varies by assay		Diluent for samples and Calibrators
Diluent 11	≤–10 °C	R55BA-3	50 mL	2 bottles	10 bottles	Diluent for detection antibody
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1 bottle	5 bottles	Buffer to catalyze the electrochemiluminescent reaction

Dash (—) = not applicable
RT = room temperature

Reagent Preparation for 384-well Plates

Important: Upon the first thaw, aliquot diluents into suitably sized aliquots before refreezing.

Coat 384-well Plate

- ❑ Add 240 µL of biotinylated capture antibody to 11.76 mL of Diluent 100. Mix by vortexing.
- ❑ Add 25 µL of the above solution to each well of the provided plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with shaking at room temperature for 2 hours.
- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use. Plates may be sealed and stored overnight at 4 °C.

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 0.5X for 384-well assays. Prepare the detection antibody solution immediately before use.

- ❑ For one plate, combine:
  - 60 µL of the supplied 100X detection antibody
  - 11.94 mL of Diluent 11

Assay Protocol (384-well plates)

Important: Please read the entire detailed Reagent Preparation instructions and the Best Practices (Appendix A) before starting work.

STEP 1: Add Samples and Calibrators

- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of the prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer.
- ❑ Add 40 µL of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer is not required before reading the plate.

Plate Diagrams

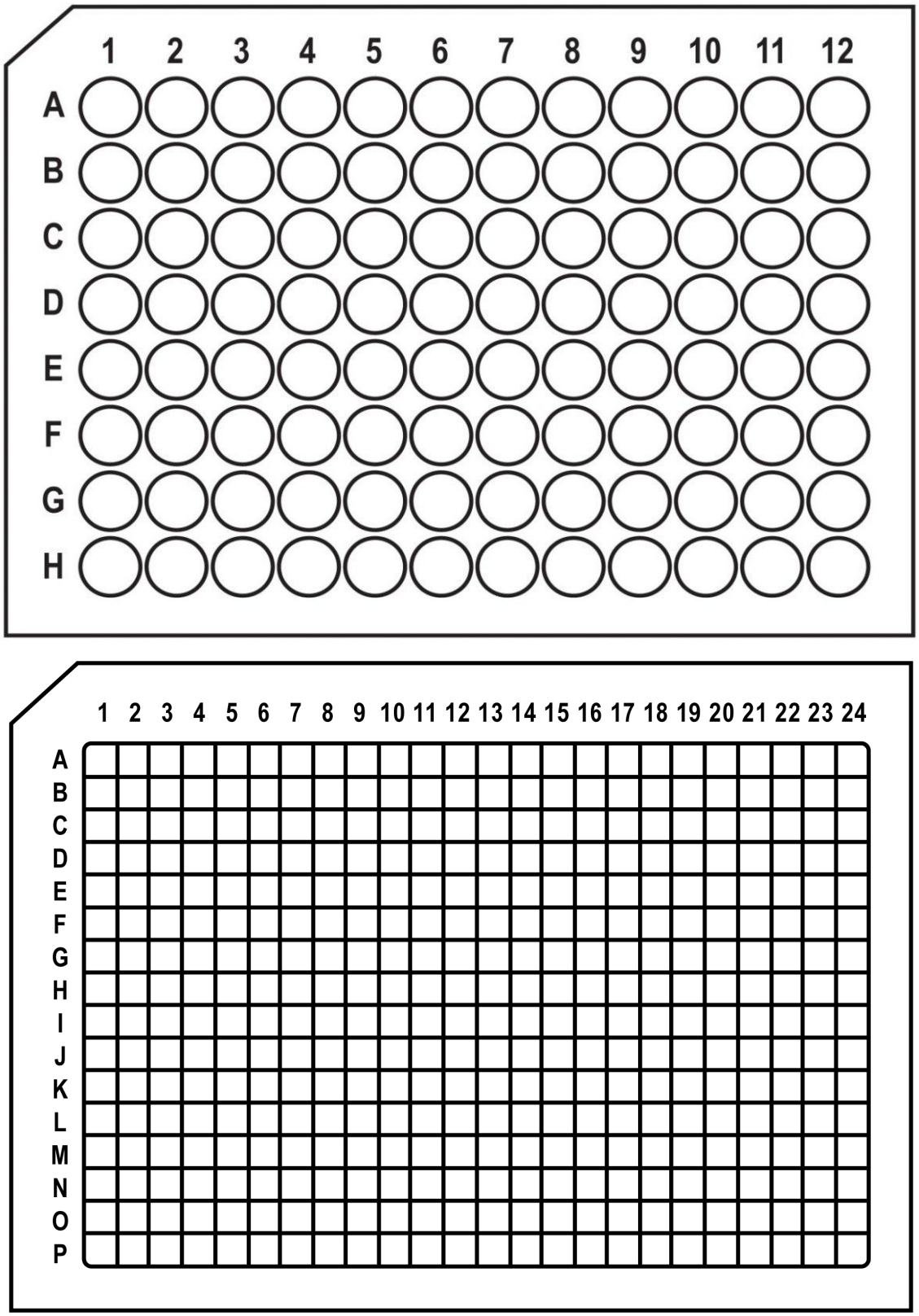

Figure 3. *Plate diagrams. Similar plate layouts can be created in Excel and easily imported into DISCOVERY WORKBENCH® software.*