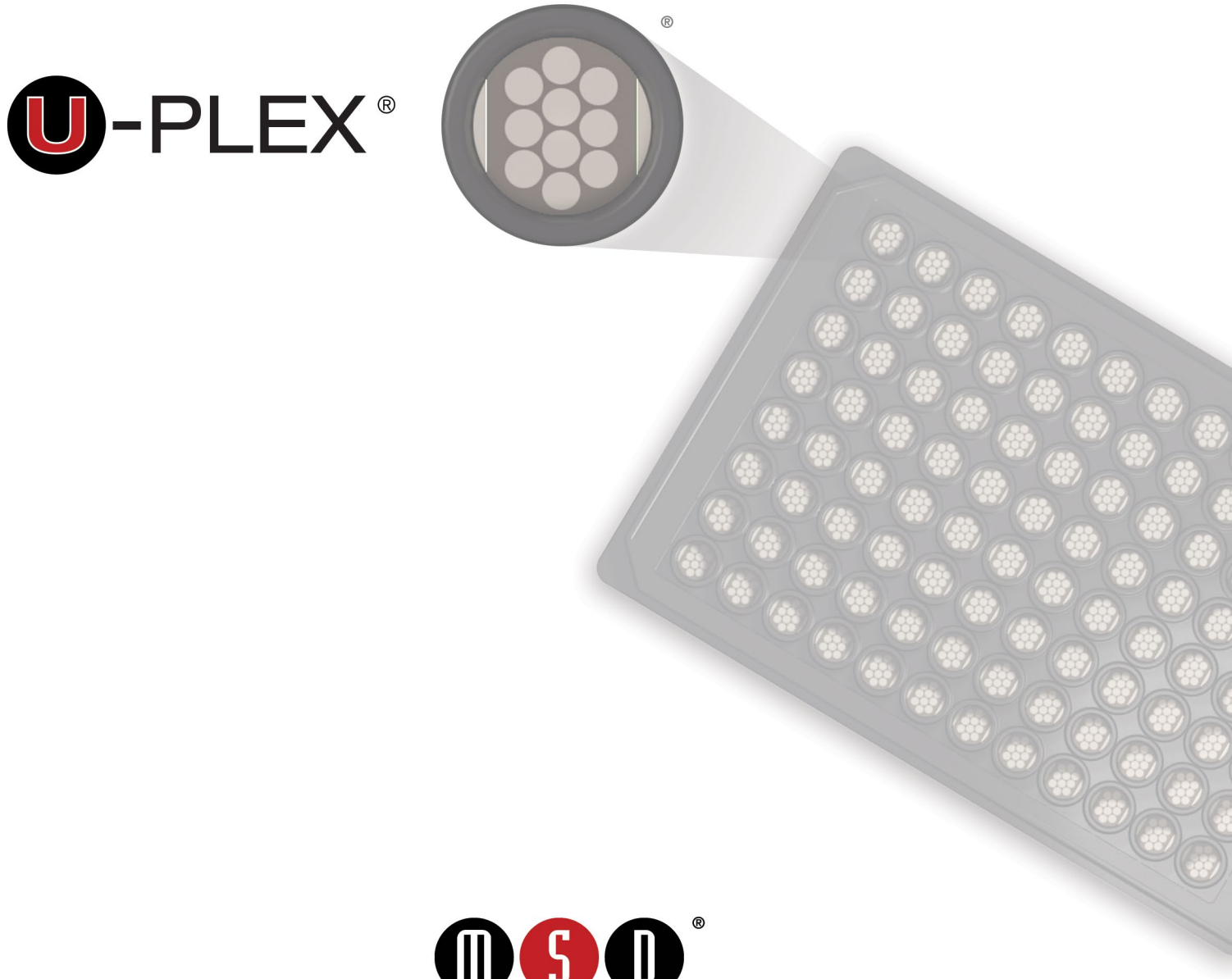
U-PLEX® Biomarker Group 1 (Human)

Singleplex Assays



MSD U-PLEX Platform

U-PLEX Biomarker Group 1 (Human) Singleplex Assays

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

Catalog numbers of U-PLEX Biomarker Group 1 (human) Singleplex Assays are provided in Table 8 on page 15.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

The MSD U-PLEX platform combines high sensitivity and a rapid read time (less than 2 minutes) with the flexibility to easily design and build custom assays and efficiently transition from singleplex to multiplex assays. U-PLEX Singleplex assays have high sensitivity, provide up to 5 logs of linear dynamic range, and use minimal sample volume.

The U-PLEX Biomarker Group 1 (human) contains 71 analytes (Table 1) that are important in many biological processes.

A representative data set for each of the assays in U-PLEX Biomarker Group 1 (human) is presented in the product-specific datasheets available at *www.mesoscale.com/support/product_information*.

Table 1. Assays included in U-PLEX Biomarker Group 1 (human)

Assays			
CTACK (CCL27)	IL-2	IL-17C	M-CSF (CSF1)
ENA-78 (CXCL5)	IL-2Rα	IL-17D	MDC (CCL22)
Eotaxin (CCL11)	IL-3	IL-17E/IL-25	MIF
Eotaxin-2 (CCL24)	IL-4	IL-17F	MIP-1α (CCL3)
Eotaxin-3 (CCL26)	IL-5	IL-18	MIP-1β (CCL4)
EPO	IL-6	IL-21	MIP-3α (CCL20)
FLT3L	IL-7	IL-22	MIP-3β (CCL19)
Fractalkine (CX3CL1)	IL-8 (CXCL8)	IL-23	MIP-5 (CCL15)
G-CSF (CSF2)	IL-9	IL-27	SDF-1α (CXCL12)
GM-CSF (CSF3)	IL-10	IL-29/IFN-λ1	TARC (CCL17)
GRO-α (CXCL1)	IL-12/IL-23p40	IL-31	TNF-α
I-309 (CCL1)	IL-12p70	IL-33	TNF-β
IFN-α2a	IL-13	IP-10 (CXCL10)	TPO
IFN-β	IL-15	I-TAC (CXCL11)	TRAIL
IFN-γ	IL-16	MCP-1 (CCL2)	TSLP
IL-1α	IL-17A	MCP-2 (CCL8)	VEGF-A
IL-1β	IL-17A/F	MCP-3 (CCL7)	YKL-40
IL-1RA	IL-17B	MCP-4 (CCL13)	

Principle of the Assay

Singleplex assays are supplied on MSD GOLD™ Small Spot Streptavidin 96-well or MSD Streptavidin 384-well plates (Figure 1). These plates provide high sensitivity, consistent performance, and excellent inter- and intralot 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 reagents. 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 electrodes 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.

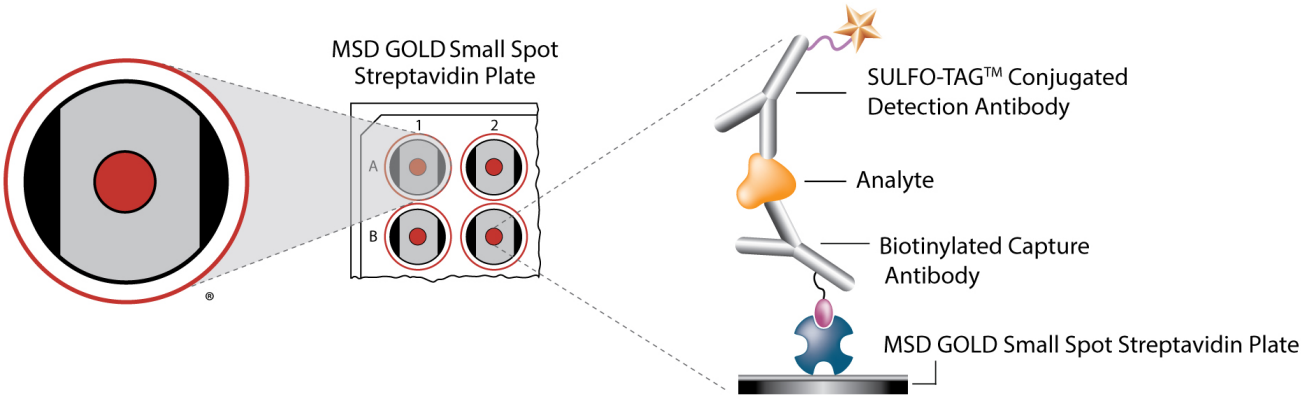

Figure 1. U-PLEX immunoassay on a U-PLEX 10-Assay Plate.

Components

Table 2, Table 3, and Table 5 list the components provided with U-PLEX Biomarker Group 1 (human) Singleplex Assays. U-PLEX Singleplex Assays are available with either SECTOR™ or QuickPlex® 96-well plates or SECTOR 384-well plates (Table 4).

Reagents Supplied With All U-PLEX Singleplex Assays

Table 2. Reagents that are supplied with all U-PLEX Biomarker Group 1 (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.
MSD GOLD 96-Well Small Spot Streptavidin QuickPlex Plate		L4BSA-1					
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Diluent for biotinylated capture antibody
Diluent 57	≤–10 °C	R50BZ-1	10 mL	1 bottle	—	—	Diluent for samples and Calibrator
		R50BZ-2	50 mL	—	1 bottle	5 bottles	
Diluent 3	≤–10 °C	R50AP-1	8 mL	1 bottle	—	—	Diluent for detection antibody
		R50AP-2	40 mL	—	1 bottle	5 bottles	
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent reaction to use at room temperature
		R60AM-2	90 mL	—	1 bottle	5 bottles	

RT = room temperature
Dash (—) = not applicable

Table 3. Reagents that are supplied with all U-PLEX Biomarker Group 1 (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-4	50 mL	2 bottles	10 bottles	Diluent for biotinylated capture antibody
Diluent 57	≤–10 °C	R50BZ-2	50 mL	2 bottles	10 bottles	Diluent for samples and Calibrators
Diluent 3	≤–10 °C	R50AP-2	40 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

RT = room temperature
Dash (—) = not applicable

Assay-Specific Reagents

U-PLEX Antibody Set

Based upon the analyte selected, you will receive a U-PLEX Antibody Set containing a biotinylated capture antibody and SULFO-TAG conjugated detection antibody. A complete list of all Antibody Sets available for U-PLEX Biomarker Group 1 (human) and their respective catalog numbers are provided in the Appendix (Table 9).

Table 4. Contents of U-PLEX Antibody Set

Name	Storage	Size	Quantity Supplied			Description
			1 Plate	5 Plates	25 Plates	
U-PLEX Human 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

Calibrators

Calibrators are multi-analyte blends, each containing multiple recombinant human proteins lyophilized in a buffered diluent.

Individual analyte concentrations are provided in the lot-specific certificates of analysis (COA). Based on the analyte selected, you will receive one of the following Calibrators.

Table 5. Analytes included in the Calibrator blends available for U-PLEX Biomarker Group 1 (human)

Name	Storage	Catalog No.	Size	Quantity Supplied			Analytes
				1 Plate	5 Plates	25 Plates	
Calibrator 1	2–8 °C	C0060-2	1 vial	1 vial	5 vials	25 vials	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, TNF-α, VEGF-A
Calibrator 2	2–8 °C	C0061-2	1 vial	1 vial	5 vials	25 vials	Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC
Calibrator 3	2–8 °C	C0062-2	1 vial	1 vial	5 vials	25 vials	G-CSF, IFN-α2a, IL-1α, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-18, TNF-β, TPO
Calibrator 4	2–8 °C	C0063-2	1 vial	1 vial	5 vials	25 vials	CTACK, ENA-78, Fractalkine, I-TAC, MIP-3α, MIP-3β, SDF-1α
Calibrator 6	2–8 °C	C0072-2	1 vial	1 vial	5 vials	25 vials	IL-17A/F, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-29/IFN-λ1, IL-31, IL-33, TSLP
Calibrator 9	2–8 °C	C0090-2	1 vial	1 vial	5 vials	25 vials	EPO, FLT3L, IFN-β, IL-1RA, IL-2Rα, IL-3, IL-9, IL-17B, IL-17C, IL-17D
Calibrator 10	2–8 °C	C0091-2	1 vial	1 vial	5 vials	25 vials	Eotaxin-2, GRO-α, I-309, MCP-2, MCP-3, M-CSF, MIF, MIP-5, TRAIL, YKL-40

Instrument Compatibility

MSD offers U-PLEX Singleplex Assays designed for use on specific instrument platforms (Table 6).

Table 6. Instrument compatibility

Instrument	Assays on 96-well SECTOR Plate (K151XXK -1/-2/-4)	Assays on 96-well QuickPlex Plate (K151XXK -21/-22/-24)	Assays on 384-well SECTOR Plate (K251XXK -2/-4)
MESO® QuickPlex SQ 120	Y	—	—
MESO QuickPlex® SQ 120MM	Y	—	—
MESO SECTOR® S 600	Y	—	Y
MESO SECTOR S 600MM	Y	—	Y
MESO QuickPlex Q 60MM	—	Y	—

Dash (—) = not applicable

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge 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 415 mL of 1X Wash Buffer for a 384-well plate and 130 mL for a 96-well plate. Automated plate washers may need overage added to these volumes.
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer
- ❑ MSD Diluent 100 (50 mL, catalog number R50AA-4) may be needed to dilute samples.

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.

Best Practices

- Bring frozen diluents to room temperature in a 20–26 °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 before use. Plates should be brought to room temperature before opening the foil packet.
- To avoid cross-contamination between vials, open vials for one protocol step at a time. Use filtered pipette tips and use a fresh pipette tip for each reagent addition.
- MSD assays are tested and characterized between 21–26 °C; testing outside this temperature range may result in increased variability.
- Prepare calibrators, samples, and controls in a polypropylene container of sufficient volume.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates should not be exposed to direct sunlight.
- To ensure that all lyophilized powder is reconstituted, it is recommended that vials be inverted 3 times to distribute the diluent inside the vial. Then vortex the vial with 3 short pulses (upright, inverted, upright) after the solution sits at room temperature for the recommended amount of time in the product protocol.
- Ensure that all reagents are within their expiration date at the time of the test.
- For additional accuracy and precision, pre-wet pipette tips before transferring reagents and samples. Avoid pipetting bubbles while doing so.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm for 96-well plates and 1,000–1,500 rpm for 384-well plates. Binding reactions may reach equilibrium sooner if you use shaking at the middle of the range or above. For long-term studies, the shaking speed and shaker model should be kept consistent.
- Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
- Consistent incubation times will improve the reproducibility of test results.
- Ensure that all necessary instruments, equipment, and reagents for the next step are prepared before washing the plates to prevent the plates from drying out.
- 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.
- Avoid creating bubbles in wells during all pipetting steps as they may lead to variable results.
- Use reverse pipetting when necessary and do not blow out residual liquid to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Dispense reagents and wash fluids at the side of the well towards the bottom corner away from the coated spots.
- Protect plates from sources of heat such as vents, sunlight, etc., which may introduce variability across the plate surface. Some models of shakers generate heat that may affect plates on the platform.
- Ensure that all equipment is serviced and calibrated on a routine basis.
- Remove the plate seal before reading the plate.

- Read Buffer should be at room temperature (20–26 ºC) before adding it to the plate.
- Keep time intervals consistent between the addition of Read Buffer and reading the plate to improve interplate precision. It is recommended that an MSD instrument be prepared to read a plate before adding Read Buffer. Unless otherwise directed, read the plate as soon as possible after adding Read Buffer.
- Do not shake the plate after adding Read Buffer.
- Do not obscure or damage the plate barcode; it is required for the plate reader.
- Only use the Read Buffer and Wash Buffer recommended for use with this kit.
- For 384-well assays, the protocol assumes the use of automated plate washers that can begin to aspirate before the total 90 µL is dispensed. If this ability is not present, reduce the wash volume to 80 µL to avoid overflowing the wells.
- Aliquot and freeze Diluent 100 to prevent contamination after opening.

Reagent Preparation

Bring all reagents to room temperature and refer to the Best Practices section (page 9) before beginning the protocol.

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

To prepare MSD Wash Buffer and other supplemental reagents, please refer to the Additional Materials and Equipment section (page 8).

Coat 96-well Plate

- ❑ Add 200 µL of biotinylated capture antibody to 3.3 mL of Diluent 100. Mix by vortexing.
- ❑ Add 25 µL of the above solution to each well of the provided MSD GOLD Small Spot Streptavidin Plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with shaking at room temperature for 1 hour.
- ❑ 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. Plates may be sealed and stored overnight at 4 °C.

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 90 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use. It may be sealed and stored overnight at 4 °C.

Prepare Calibrator Standards

Bring the Calibrator vial(s) to room temperature. Reconstitute each vial of Calibrator by adding 250 µL of Assay Diluent to the glass vial. This will result in a 5X concentrated stock of the Calibrator, which will need to be diluted 5-fold (per the instructions given below) to generate the highest point in the standard curve (i.e., Calibrator Standard 1). Invert the reconstituted Calibrator at least 3

times. Do not vortex. Let the reconstituted solution equilibrate at room temperature for 15–30 minutes and then vortex briefly. The Calibrator is now ready for use. Keep the dilutions at room temperature.

Note: We recommend that reconstituted or thawed Calibrators be used immediately. If storage is necessary, divide into suitably sized aliquots (60 µL aliquots are recommended) and store immediately at ≤–70 °C.

The following instructions (Figure 2; Table 7) will enable you to prepare 7 Calibrator Standard solutions plus a zero Calibrator Standard for up to 6 replicates.

- ❑ Prepare Calibrator Standard 1 by adding 50 µL of the reconstituted Calibrator to 200 µL of Assay Diluent. Mix by vortexing.
- ❑ For Calibrator Standard 2, add 75 µL of Calibrator Standard 1 to 225 µL of Assay Diluent. Mix by vortexing.
- ❑ Repeat 4-fold serial dilutions 5 additional times to generate a total of 7 Calibrator Standards. 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 pack. You can also find a copy of the COA at www.mesoscale.com.

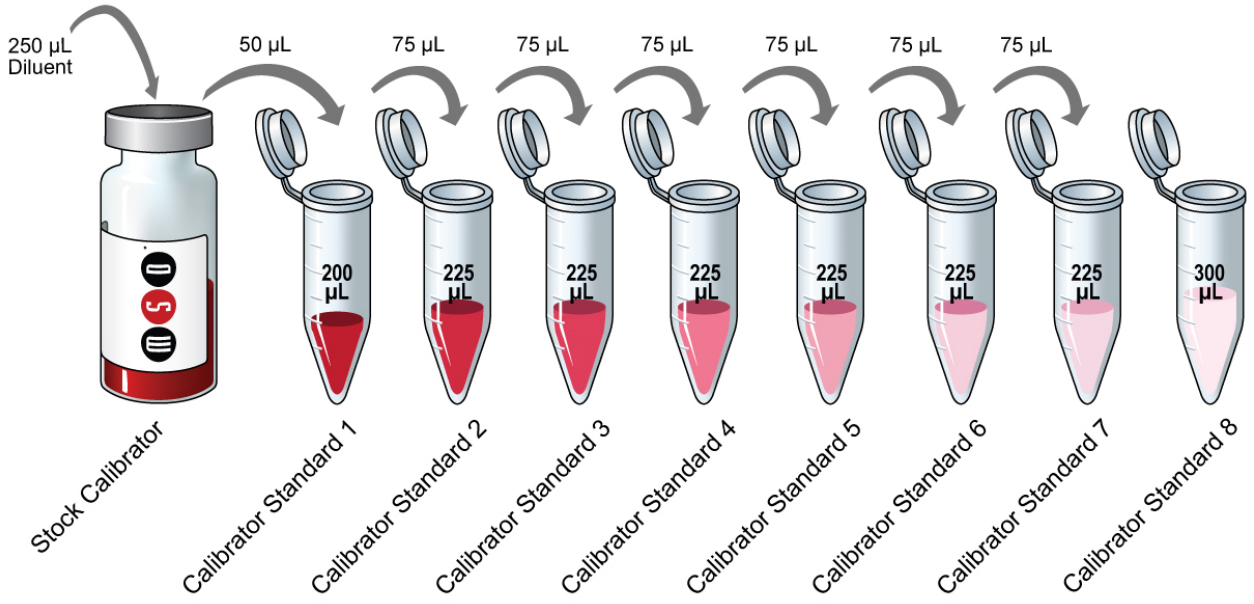

Figure 2. Dilution schema for Calibrator Standards for U-PLEX Biomarker Group 1 (human) Singleplex Assays.

Table 7. 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	50	200	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

Dilute Samples

Depending on the sample set under investigation, a dilution may be necessary. Assay Diluent may be used for sample dilution. The dilution factor for the given sample type may need to be optimized.

Note: For MIF, MIP-5, and YKL-40, the concentrations in normal serum and EDTA plasma may exceed the standard working range of the assays. Pre-assay dilution of samples may be required to generate optimal results. Refer to the product-specific datasheets for additional information. Diluent 100 may be used in place of Assay Diluent for samples that require high dilution.

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X for 96-well assays (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
- ❑ 5,940 µL of Diluent 3 (11.94 mL for 384-well assays)

Wash Buffer

Prepare a 1X working solution by diluting the 20X stock with deionized water. 1X MSD Wash Buffer can be stored at room temperature for up to two weeks.

Read Buffer

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

Assay Protocols

Note: Follow Reagent Preparation (above) before beginning this assay protocol.

96-well Plate Assays

STEP 1: Add Samples and Calibrators

- ❑ Add 25 µL of Assay Diluent to each well. Tap the plate gently on all sides.
- ❑ 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 1 hour.

STEP 2: Wash and Add Detection Antibody Solution

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

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD 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.

384-well Plate Assays

STEP 1: Add Samples and Calibrators

- ❑ 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 90 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 90 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.

Alternate 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 assays may achieve acceptable performance with shorter incubations. Consider reducing the incubation time of samples in the plate and of detection antibody each to 1 hour.
- ❑ **Alternate Protocol 3, Reduced Wash:** For cell culture supernatants, you may simplify the protocol by eliminating one of the wash steps. After incubating the Calibrator Standard or sample, add detection antibody solution to the plate without decanting or washing the plate.

Assay Performance

A representative data set for each assay is presented in the product-specific datasheets available at www.mesoscale.com. The data represent the performance of the assay tested in multiplex format on U-PLEX 96-well plates. The data were generated during the development of the assay and do not represent the product specifications. Under your experimental conditions, the assay may perform differently than the representative data shown.

Appendix

U-PLEX Singleplex Assays

Assays (Table 8) include Antibody Sets, plates, Diluents, Calibrators, and MSD GOLD Read Buffer B.

Table 8. Catalog numbers of U-PLEX Biomarker Group 1 (human) Singleplex Assays

Product	96-Well SECTOR plates (1/5/25 plates)	96-Well QuickPlex plates (1/5/25 plates)	384-Well SECTOR plates (5/25 plates)
U-PLEX Human CTACK Assay	K151VDK-1/-2/-4	K151VDK-21/-22/-24	K251VDK-2/-4
U-PLEX Human ENA-78 Assay	K151VEK-1/-2/-4	K151VEK-21/-22/-24	K251VEK-2/-4
U-PLEX Human Eotaxin Assay	K151UDK-1/-2/-4	K151UDK-21/-22/-24	K251UDK-2/-4
U-PLEX Human Eotaxin-2 Assay	K151XQK-1/-2/-4	K151XQK-21/-22/-24	K251XQK-2/-4
U-PLEX Human Eotaxin-3 Assay	K151UEK-1/-2/-4	K151UEK-21/-22/-24	K251UEK-2/-4
U-PLEX Human EPO Assay	K151VXK-1/-2/-4	K151VXK-21/-22/-24	K251VXK-2/-4
U-PLEX Human FLT3L Assay	K151XFK-1/-2/-4	K151XFK-21/-22/-24	K251XFK-2/-4
U-PLEX Human Fractalkine Assay	K151VCK-1/-2/-4	K151VCK-21/-22/-24	K251VCK-2/-4
U-PLEX Human G-CSF Assay	K151VGK-1/-2/-4	K151VGK-21/-22/-24	K251VGK-2/-4
U-PLEX Human GM-CSF Assay	K151UMK-1/-2/-4	K151UMK-21/-22/-24	K251UMK-2/-4
U-PLEX Human GRO-α Assay	K151UXK-1/-2/-4	K151UXK-21/-22/-24	K251UXK-2/-4
U-PLEX Human I-309 Assay	K151UYK-1/-2/-4	K151UYK-21/-22/-24	K251UYK-2/-4
U-PLEX Human IFN-α2a Assay	K151VHK-1/-2/-4	K151VHK-21/-22/-24	K251VHK-2/-4
U-PLEX Human IFN-β Assay	K151VIK-1/-2/-4	K151VIK-21/-22/-24	K251VIK-2/-4
U-PLEX Human IFN-γ Assay	K151TTK-1/-2/-4	K151TTK-21/-22/-24	K251TTK-2/-4
U-PLEX Human IL-1α Assay	K151UNK-1/-2/-4	K151UNK-21/-22/-24	K251UNK-2/-4
U-PLEX Human IL-1β Assay	K151TUK-1/-2/-4	K151TUK-21/-22/-24	K251TUK-2/-4
U-PLEX Human IL-1RA Assay	K151XPK-1/-2/-4	K151XPK-21/-22/-24	K251XPK-2/-4
U-PLEX Human IL-2 Assay	K151TVK-1/-2/-4	K151TVK-21/-22/-24	K251TVK-2/-4
U-PLEX Human IL-2Rα Assay	K151XGK-1/-2/-4	K151XGK-21/-22/-24	K251XGK-2/-4
U-PLEX Human IL-3 Assay	K151XMK-1/-2/-4	K151XMK-21/-22/-24	K251XMK-2/-4
U-PLEX Human IL-4 Assay	K151TWK-1/-2/-4	K151TWK-21/-22/-24	K251TWK-2/-4
U-PLEX Human IL-5 Assay	K151UOK-1/-2/-4	K151UOK-21/-22/-24	K251UOK-2/-4
U-PLEX Human IL-6 Assay	K151TXK-1/-2/-4	K151TXK-21/-22/-24	K251TXK-2/-4
U-PLEX Human IL-7 Assay	K151UPK-1/-2/-4	K151UPK-21/-22/-24	K251UPK-2/-4
U-PLEX Human IL-8 Assay	K151TYK-1/-2/-4	K151TYK-21/-22/-24	K251TYK-2/-4
U-PLEX Human IL-9 Assay	K151XKK-1/-2/-4	K151XKK-21/-22/-24	K251XKK-2/-4
U-PLEX Human IL-10 Assay	K151TZK-1/-2/-4	K151TZK-21/-22/-24	K251TZK-2/-4
U-PLEX Human IL-12/IL-23p40 Assay	K151UQK-1/-2/-4	K151UQK-21/-22/-24	K251UQK-2/-4
U-PLEX Human IL-12p70 Assay	K151UAK-1/-2/-4	K151UAK-21/-22/-24	K251UAK-2/-4
U-PLEX Human IL-13 Assay	K151UBK-1/-2/-4	K151UBK-21/-22/-24	K251UBK-2/-4
U-PLEX Human IL-15 Assay	K151URK-1/-2/-4	K151URK-21/-22/-24	K251URK-2/-4
U-PLEX Human IL-16 Assay	K151USK-1/-2/-4	K151USK-21/-22/-24	K251USK-2/-4
U-PLEX Human IL-17A Assay	K151UTK-1/-2/-4	K151UTK-21/-22/-24	K251UTK-2/-4
U-PLEX Human IL-17A/F Assay	K151VYK-1/-2/-4	K151VYK-21/-22/-24	K251VYK-2/-4
U-PLEX Human IL-17B Assay	K151XNK-1/-2/-4	K151XNK-21/-22/-24	K251XNK-2/-4

Product	96-Well SECTOR plates (1/5/25 plates)	96-Well QuickPlex plates (1/5/25 plates)	384-Well SECTOR plates (5/25 plates)
U-PLEX Human IL-17C Assay	K151WJK-1/-2/-4	K151WJK-21/-22/-24	K251WJK-2/-4
U-PLEX Human IL-17D Assay	K151XOK-1/-2/-4	K151XOK-21/-22/-24	K251XOK-2/-4
U-PLEX Human IL-17E/IL-25 Assay	K151VZK-1/-2/-4	K151VZK-21/-22/-24	K251VZK-2/-4
U-PLEX Human IL-17F Assay	K151WAK-1/-2/-4	K151WAK-21/-22/-24	K251WAK-2/-4
U-PLEX Human IL-18 Assay	K151VJK-1/-2/-4	K151VJK-21/-22/-24	K251VJK-2/-4
U-PLEX Human IL-21 Assay	K151WBK-1/-2/-4	K151WBK-21/-22/-24	K251WBK-2/-4
U-PLEX Human IL-22 Assay	K151WIK-1/-2/-4	K151WIK-21/-22/-24	K251WIK-2/-4
U-PLEX Human IL-23 Assay	K151WGK-1/-2/-4	K151WGK-21/-22/-24	K251WGK-2/-4
U-PLEX Human IL-27 Assay	K151WCK-1/-2/-4	K151WCK-21/-22/-24	K251WCK-2/-4
U-PLEX Human IL-29/IFN-λ1 Assay	K151WDK-1/-2/-4	K151WDK-21/-22/-24	K251WDK-2/-4
U-PLEX Human IL-31 Assay	K151WEK-1/-2/-4	K151WEK-21/-22/-24	K251WEK-2/-4
U-PLEX Human IL-33 Assay	K151WFK-1/-2/-4	K151WFK-21/-22/-24	K251WFK-2/-4
U-PLEX Human IP-10 Assay	K151UFK-1/-2/-4	K151UFK-21/-22/-24	K251UFK-2/-4
U-PLEX Human I-TAC Assay	K151UWK-1/-2/-4	K151UWK-21/-22/-24	K251UWK-2/-4
U-PLEX Human MCP-1 Assay	K151UGK-1/-2/-4	K151UGK-21/-22/-24	K251UGK-2/-4
U-PLEX Human MCP-2 Assay	K151XHK-1/-2/-4	K151XHK-21/-22/-24	K251XHK-2/-4
U-PLEX Human MCP-3 Assay	K151XIK-1/-2/-4	K151XIK-21/-22/-24	K251XIK-2/-4
U-PLEX Human MCP-4 Assay	K151UHK-1/-2/-4	K151UHK-21/-22/-24	K251UHK-2/-4
U-PLEX Human M-CSF Assay	K151XRK-1/-2/-4	K151XRK-21/-22/-24	K251XRK-2/-4
U-PLEX Human MDC Assay	K151UIK-1/-2/-4	K151UIK-21/-22/-24	K251UIK-2/-4
U-PLEX Human MIF Assay	K151XJK-1/-2/-4	K151XJK-21/-22/-24	K251XJK-2/-4
U-PLEX Human MIP-1α Assay	K151UJK-1/-2/-4	K151UJK-21/-22/-24	K251UJK-2/-4
U-PLEX Human MIP-1β Assay	K151UKK-1/-2/-4	K151UKK-21/-22/-24	K251UKK-2/-4
U-PLEX Human MIP-3α Assay	K151UZK-1/-2/-4	K151UZK-21/-22/-24	K251UZK-2/-4
U-PLEX Human MIP-3β Assay	K151VAK-1/-2/-4	K151VAK-21/-22/-24	K251VAK-2/-4
U-PLEX Human MIP-5 Assay	K151XSK-1/-2/-4	K151XSK-21/-22/-24	K251XSK-2/-4
U-PLEX Human SDF-1α Assay	K151VBK-1/-2/-4	K151VBK-21/-22/-24	K251VBK-2/-4
U-PLEX Human TARC Assay	K151ULK-1/-2/-4	K151ULK-21/-22/-24	K251ULK-2/-4
U-PLEX Human TNF-α Assay	K151UCK-1/-2/-4	K151UCK-21/-22/-24	K251UCK-2/-4
U-PLEX Human TNF-β Assay	K151UUK-1/-2/-4	K151UUK-21/-22/-24	K251UUK-2/-4
U-PLEX Human TPO Assay	K151VKK-1/-2/-4	K151VKK-21/-22/-24	K251VKK-2/-4
U-PLEX Human TRAIL Assay	K151XTK-1/-2/-4	K151XTK-21/-22/-24	K251XTK-2/-4
U-PLEX Human TSLP Assay	K151WHK-1/-2/-4	K151WHK-21/-22/-24	K251WHK-2/-4
U-PLEX Human VEGF-A Assay	K151UVK-1/-2/-4	K151UVK-21/-22/-24	K251UVK-2/-4
U-PLEX Human YKL-40 Assay	K151VLK-1/-2/-4	K151VLK-21/-22/-24	K251VLK-2/-4

U-PLEX Singleplex Antibody Sets

Antibody Sets (Table 9) include a biotinylated capture antibody and SULFO-TAG conjugated detection antibody.

Table 9. Catalog numbers of Antibody Sets available for U-PLEX Biomarker Group 1 (human)

Product	Catalog Numbers (1/5 Plate Size)
U-PLEX Human CTACK Antibody Set	B21VD-2/-3
U-PLEX Human ENA-78 Antibody Set	B21VE-2/-3
U-PLEX Human Eotaxin Antibody Set	B21UD-2/-3
U-PLEX Human Eotaxin-2 Antibody Set	B21XQ-2/-3
U-PLEX Human Eotaxin-3 Antibody Set	B21UE-2/-3
U-PLEX Human EPO Antibody Set	B21VX-2/-3
U-PLEX Human FLT3L Antibody Set	B21XF-2/-3
U-PLEX Human Fractalkine Antibody Set	B21VC-2/-3
U-PLEX Human G-CSF Antibody Set	B21VG-2/-3
U-PLEX Human GM-CSF Antibody Set	B21UM-2/-3
U-PLEX Human GRO-α Antibody Set	B21UX-2/-3
U-PLEX Human I-309 Antibody Set	B21UY-2/-3
U-PLEX Human IFN-α2a Antibody Set	B21VH-2/-3
U-PLEX Human IFN-β Antibody Set	B21VI-2/-3
U-PLEX Human IFN-λ Antibody Set	B21TT-2/-3
U-PLEX Human IL-1α Antibody Set	B21UN-2/-3
U-PLEX Human IL-1β Antibody Set	B21TU-2/-3
U-PLEX Human IL-1RA Antibody Set	B21XP-2/-3
U-PLEX Human IL-2 Antibody Set	B21TV-2/-3
U-PLEX Human IL-2Rα Antibody Set	B21XG-2/-3
U-PLEX Human IL-3 Antibody Set	B21XM-2/-3
U-PLEX Human IL-4 Antibody Set	B21TW-2/-3
U-PLEX Human IL-5 Antibody Set	B21UO-2/-3
U-PLEX Human IL-6 Antibody Set	B21TX-2/-3
U-PLEX Human IL-7 Antibody Set	B21UP-2/-3
U-PLEX Human IL-8 Antibody Set	B21TY-2/-3
U-PLEX Human IL-9 Antibody Set	B21XK-2/-3
U-PLEX Human IL-10 Antibody Set	B21TZ-2/-3
U-PLEX Human IL-12/IL-23p40 Antibody Set	B21UQ-2/-3
U-PLEX Human IL-12p70 Antibody Set	B21UA-2/-3
U-PLEX Human IL-13 Antibody Set	B21UB-2/-3
U-PLEX Human IL-15 Antibody Set	B21UR-2/-3
U-PLEX Human IL-16 Antibody Set	B21US-2/-3
U-PLEX Human IL-17A Antibody Set	B21UT-2/-3
U-PLEX Human IL-17A/F Antibody Set	B21VY-2/-3
U-PLEX Human IL-17B Antibody Set	B21XN-2/-3

Product	Catalog Numbers (1/5 Plate Size)
U-PLEX Human IL-17C Antibody Set	B21WJ-2/-3
U-PLEX Human IL-17D Antibody Set	B21XO-2/-3
U-PLEX Human IL-17E/IL-25 Antibody Set	B21VZ-2/-3
U-PLEX Human IL-17F Antibody Set	B21WA-2/-3
U-PLEX Human IL-18 Antibody Set	B21VJ-2/-3
U-PLEX Human IL-21 Antibody Set	B21WB-2/-3
U-PLEX Human IL-22 Antibody Set	B21WI-2/-3
U-PLEX Human IL-23 Antibody Set	B21WG-2/-3
U-PLEX Human IL-27 Antibody Set	B21WC-2/-3
U-PLEX Human IL-29/IFN-λ1 Antibody Set	B21WD-2/-3
U-PLEX Human IL-31 Antibody Set	B21WE-2/-3
U-PLEX Human IL-33 Antibody Set	B21WF-2/-3
U-PLEX Human IP-10 Antibody Set	B21UF-2/-3
U-PLEX Human I-TAC Antibody Set	B21UW-2/-3
U-PLEX Human MCP-1 Antibody Set	B21UG-2/-3
U-PLEX Human MCP-2 Antibody Set	B21XH-2/-3
U-PLEX Human MCP-3 Antibody Set	B21XI-2/-3
U-PLEX Human MCP-4 Antibody Set	B21UH-2/-3
U-PLEX Human M-CSF Antibody Set	B21XR-2/-3
U-PLEX Human MDC Antibody Set	B21UI-2/-3
U-PLEX Human MIF Antibody Set	B21XJ-2/-3
U-PLEX Human MIP-1α Antibody Set	B21UJ-2/-3
U-PLEX Human MIP-1β Antibody Set	B21UK-2/-3
U-PLEX Human MIP-3α Antibody Set	B21UZ-2/-3
U-PLEX Human MIP-3β Antibody Set	B21VA-2/-3
U-PLEX Human MIP-5 Antibody Set	B21XS-2/-3
U-PLEX Human SDF-1α Antibody Set	B21VB-2/-3
U-PLEX Human TARC Antibody Set	B21UL-2/-3
U-PLEX Human TNF-α Antibody Set	B21UC-2/-3
U-PLEX Human TNF-β Antibody Set	B21UU-2/-3
U-PLEX Human TPO Antibody Set	B21VK-2/-3
U-PLEX Human TRAIL Antibody Set	B21XT-2/-3
U-PLEX Human TSLP Antibody Set	B21WH-2/-3
U-PLEX Human VEGF-A Antibody Set	B21UV-2/-3
U-PLEX Human YKL-40 Antibody Set	B21VL-2/-3

Summary Protocols

Coat 96-well Plate

- ❑ Add 200 µL of biotinylated capture antibody to 3.3 mL of Diluent 100. Mix by vortexing.
- ❑ Add 25 µL of the above solution to each well of the provided MSD GOLD Small Spot Streptavidin Plate. Seal the plate with an adhesive plate seal and shake for 1 hour at room temperature.
- ❑ 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.

96-well Assay Protocol

STEP 1: Add Samples and Calibrators

- ❑ Add 25 µL of Assay Diluent to each well. Tap the plate gently on all sides.
- ❑ Add 25 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 1 hour.

STEP 2: 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 3: 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.

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. Seal the plate with an adhesive plate seal and shake for 2 hours at room temperature.
- ❑ Wash the plate 3 times with 90 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use and may be stored overnight at 4 °C.

384-well Assay Protocol

STEP 1: Add Samples and Calibrators

- ❑ Add 25 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 at room temperature.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 90 µL/well of 1X Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours at room temperature.

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with 90 µL/well of 1X 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

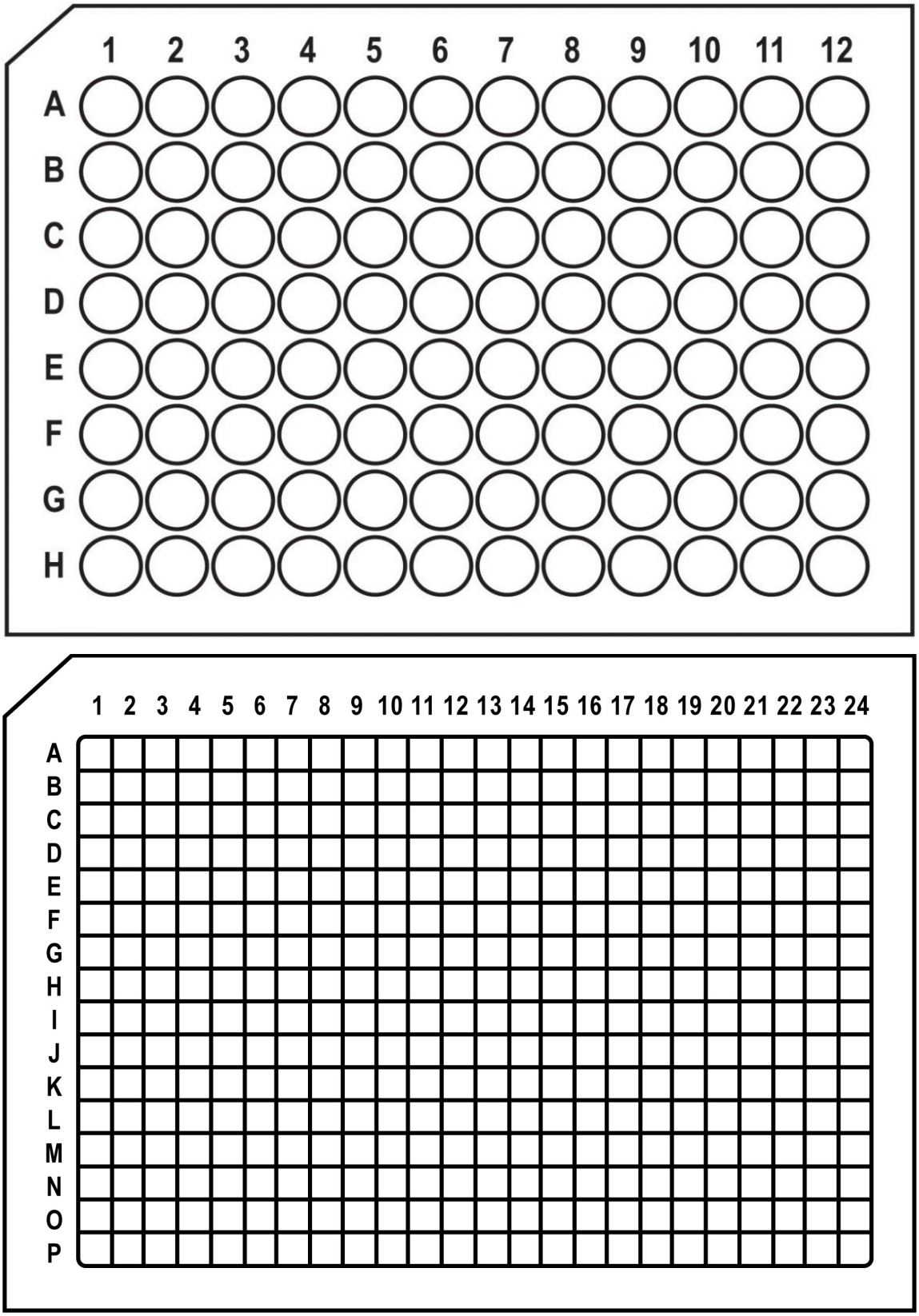

Figure 3. *Plate diagrams. Similar plate layouts can be created in Excel and easily imported into DISCOVERY WORKBENCH® software.*