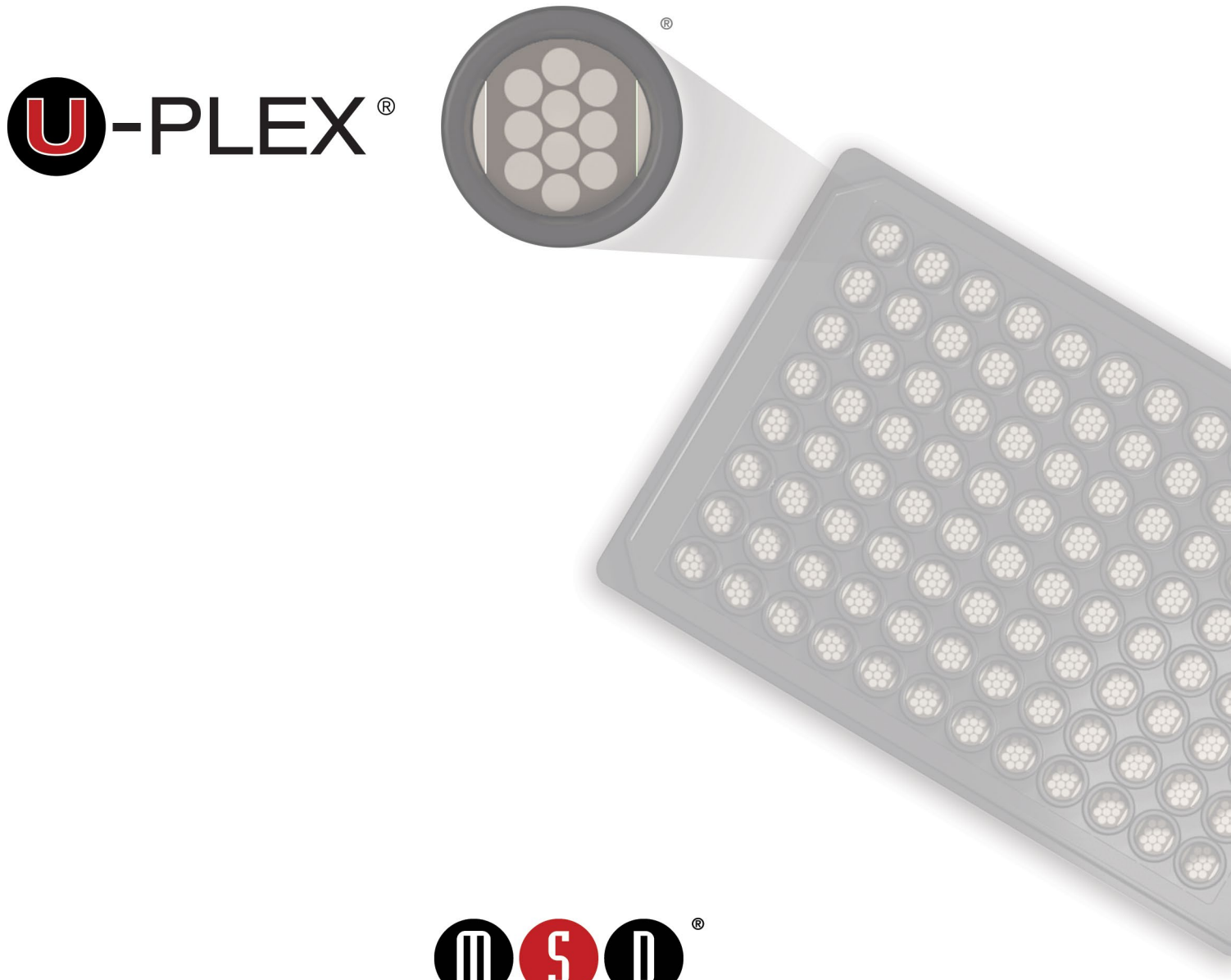
MSD® U-PLEX Platform

U-PLEX® Biomarker Group 1 (human)

Multiplex Assays



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MSD U-PLEX Platform

U-PLEX Biomarker Group 1 (human) Multiplex Assays

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

For use with:

U-PLEX Biomarker Group 1 (human) Assays (K15067M-1, 15067M-2, and K15067M-4)

U-PLEX Biomarker Group 1 (human) 384-well Assays (K25067M-2 and K25067M-4)

U-PLEX Biomarker Group 1 (human) Combos (catalog numbers are provided in Table 9 on page 18)

This package 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

U-PLEX technology allows the creation of custom multiplex assays for any combination of analytes by using U-PLEX plates and unique Linkers.

The U-PLEX assay menu is organized into groups, which include a broad menu of analytes assembled by species and analytical compatibility. For ultimate flexibility, custom combinations can be created from a selection of MSD U-PLEX assays, your own antibodies, or a combination of both.

This product insert is for U-PLEX custom multiplex assays that contain a combination of assays from the U-PLEX Biomarker Group 1 (human), including those with open spots to enable you to use your own antibody pairs.

A representative data set for each assay is presented in the product-specific datasheets available at the www.mesoscale.com® website. The performance of MSD assays may vary when tested in combination with your other assays. The data presented in the datasheets were generated during the development of the assays and do not represent the product specifications.

Principle of the Assay

Biotinylated capture antibodies are coupled to U-PLEX Linkers, which self-assemble onto unique spots on the U-PLEX plate. 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 (Figure 1). Once the sandwich immunoassay is complete, the U-PLEX 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.

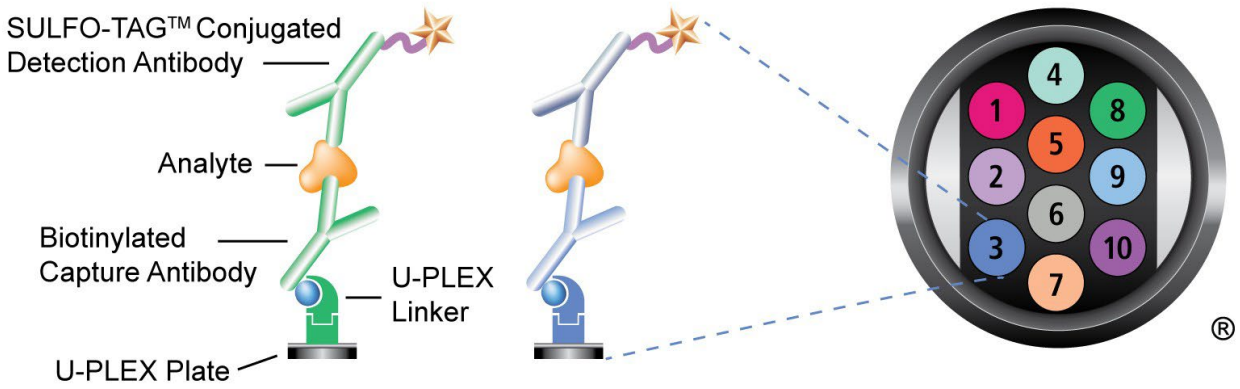


Figure 1. U-PLEX immunoassay on a U-PLEX 96-well 10-Assay Plate. U-PLEX 384-well plates are similar.

Components

Tables 1 and 2 list the components provided with U-PLEX Biomarker Group 1 (human) multiplex assays. You will only receive components relevant to the assays that you order.

Table 1. List of reagents that are supplied with all U-PLEX Biomarker Group 1 (human) 96-well Assays

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 Plate	5 Plates	25 Plates	
Diluent 57	≤–10 °C	R50BZ-1	10 mL	1 bottle	—	—	Diluent for samples and Calibrators
		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	
Stop Solution	2–8 °C	R50AO-1	40 mL	1 bottle	1 bottle	5 bottles	Biotin-containing buffer to stop Linker-antibody coupling reaction
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

Table 2. Reagents that are supplied with all U-PLEX Biomarker Group 1 (human) 384-well Assays

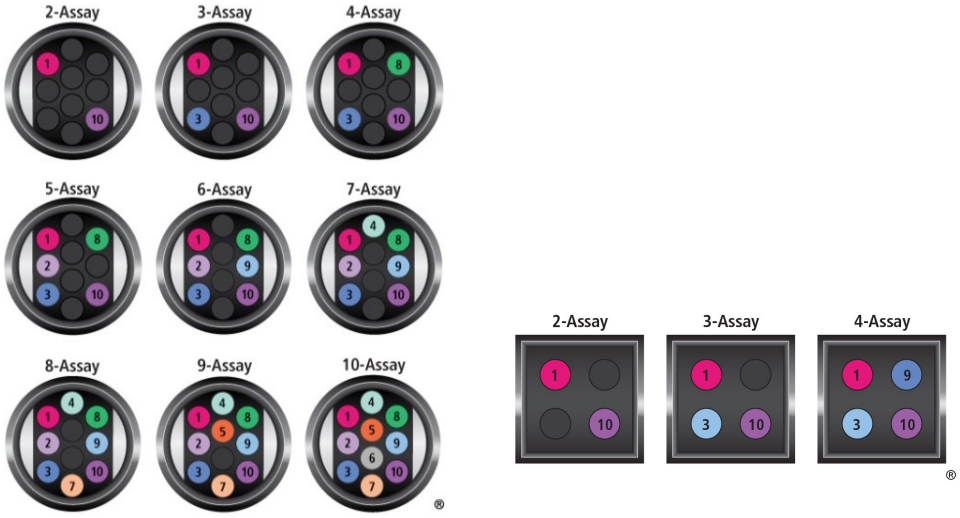
Reagent	Storage	Catalog No.	Size	Quantity Supplied		Description
				5 Plates	25 Plates	
Diluent 57	≤–10 °C	R50BZ-2	50 mL	2 bottles	10 bottles	Diluent for samples and Calibrators
Diluent 3	≤–10 °C	R50AP-2	40 mL	2 bottles	10 bottles	Diluent for detection antibody
Stop Solution	2–8 °C	R50AO-1	40 mL	2 bottles	10 bottles	Biotin-containing buffer to stop Linker-antibody coupling reaction
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1 bottle	5 bottles	Buffer to catalyze the electrochemiluminescent reaction

RT = room temperature

Assay-Specific Reagents

U-PLEX Plates

U-PLEX plates are provided in a sealed foil pouch with desiccant. The spots correspond to unique U-PLEX Linkers. The number and layout of the active spots on the plate depend on the plate well density (96 vs 384) and the number of assays to be multiplexed (Figure 2). For example, if 4 assays are being multiplexed, either the U-PLEX 96-well or 384-well 4-Assay plate will be provided.



A. 96-well spot maps *B. 384-well spot maps*

Figure 2. *Spot Map of the different U-PLEX multiplex plates showing the placement of Linkers within a well. The colored spots represent the active U-PLEX binding spots. **(A)** 96-well plates; **(B)** 384-well plates.*

Linkers

Based upon the number of assays you select for multiplexing, you will receive the corresponding number of unique Linkers. Each Linker has a biotin-binding domain that couples to the biotinylated capture antibody, as well as a domain that binds to its matching spot on the U-PLEX plate. The Linkers are color-coded and numbered with the spot to which they attach on the plate. 1-Plate packs (only available with 96-well plates) include 300 µL of each Linker. 5-Plate packs include 1.8 mL of each Linker. 25-Plate packs include 5 vials of 1.8 mL of each Linker.

We recommend recording which antibody is coupled to each Linker when performing the coupling step (as described in the Reagent Preparation section).

U-PLEX Antibody Sets

Based upon the analytes selected, you will receive U-PLEX Antibody Sets (Table 3) containing the biotinylated capture antibody and the SULFO-TAG™ conjugated detection antibody. The biotinylated capture antibody is provided at a ready-to-use concentration and the SULFO-TAG conjugated detection antibody is provided at a 100X concentration (for a 96-well plate). A complete list of all Antibody Sets available for U-PLEX Biomarker Group 1 (human) and their respective catalog numbers is provided in Appendix A (Table 10).

Table 3. *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

Calibrators

Calibrators contain analytes that may be either lyophilized or frozen. Analyte concentrations are provided in the lot-specific certificates of analysis (COA). Depending on the specific assays requested, one or more of the following Calibrators will be provided (Table 4). If combining calibrators, please refer to the Specificity section for more information.

Table 4. Analytes included in the Calibrator blends available for U-PLEX Biomarker Group 1 (human) assays

Name	Storage	Catalog No.	Size	Quantity Supplied			Assays
				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 Assays designed for use on specific instrument platforms (Table 5).

Table 5. Instrument compatibility

Instrument	Assays on U-PLEX 96-well SECTOR™ Plates	Assays on U-PLEX 384-well SECTOR Plates
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	—	—

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 (1,000–1,500 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

Note: If including user-supplied antibody pairs, you will also need:

- ❑ MSD GOLD SULFO-TAG NHS-Ester (catalog No. R91AO-1) for conjugating detection reagents or SULFO-TAG conjugated antispecies antibodies for use as reporters with unconjugated detection antibodies
- ❑ Sulfo-NHS-LC-Biotin for biotinylating the capture reagents (e.g., EZ-Link Sulfo-NHS-LC-Biotin, Thermo Fisher Scientific, catalog No. 21327 or equivalent)
- ❑ Zeba Desalting Columns (Thermo Fisher Scientific, catalog numbers 87766-87773)
- ❑ Coating diluent such as 0.5% bovine serum albumin in PBS, or MSD Diluent 100 (50 mL, Catalog No. R50AA-4) for diluting the capture antibody

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(s) (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

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 reagents 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 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 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 inter-plate 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.
- Avoid cross-contamination between Linkers and antibodies by following the techniques below:
    - Pulse centrifuge the vials to get all of the contents to the bottom of the vial.
    - Open one vial at a time. Close the cap after use.

- Each Linker vial is color-coded; ensure that each cap and tube have matching colors when opening and closing vials.
- Use filtered pipette tips.
- Use a fresh pipette tip after each reagent addition.

- For long-term studies using multiple plates of the same assay, it is recommended that the same Linker be coupled with the same antibody for the duration of the study.
- For multiplex U-PLEX assays that are provided in more than one box, each box is assembled with antibody pairs and calibrators for optimal performance. Components should not be mixed between boxes except for Stop Solution, Diluents, and Read Buffer.
- 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.

Reagent Preparation

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

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

To prepare materials for optional open spots, please refer to the Appendix B.

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

Prepare U-PLEX Plate

The preparation of a U-PLEX plate involves coating the provided plate with Linker-coupled capture antibodies. U-PLEX, 4-Assay plates are shown below as an example (Figure 3). This graphic shows plates with 4 activated spots. Assign each antibody to a unique Linker and record the antibody identity next to the assigned Linker, as shown in the examples below.

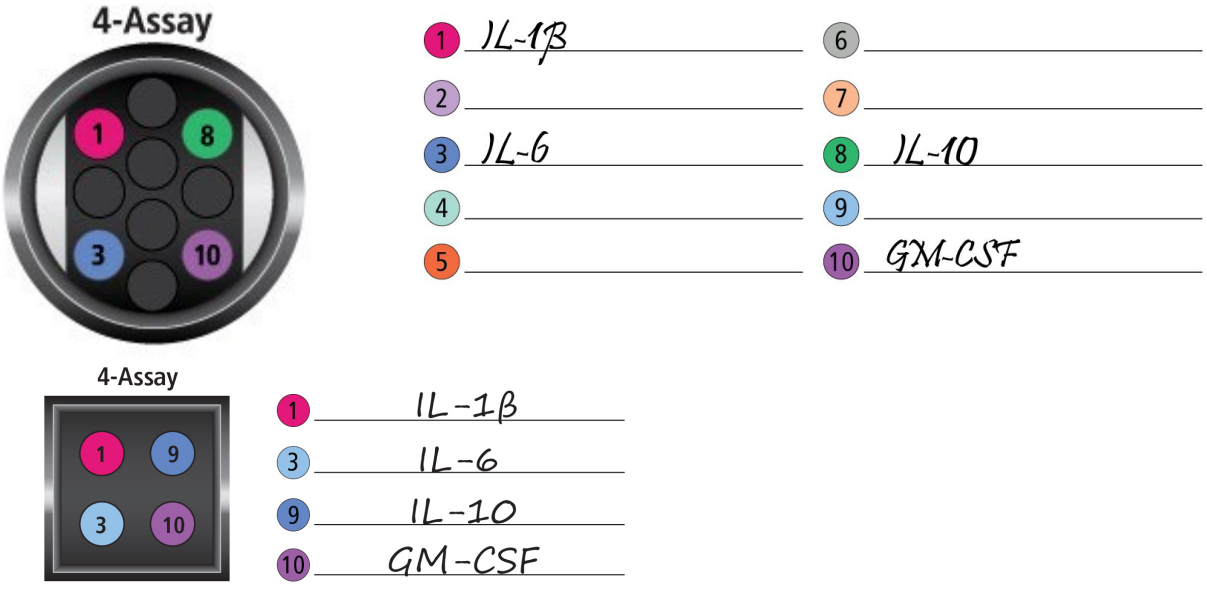


*Figure 3. U-PLEX 4-Assay Plates and assigned Linkers. **Top,** 96-well plate; **Bottom,** 384-well plate*

The protocol in this section describes the preparation of a multiplex coating solution for one plate. The volumes can be adjusted depending on the number of plates or wells, but the ratios of the reagents should remain the same (Table 6).

STEP 1: Create Individual U-PLEX Linker-Coupled Antibody Solutions

A different Linker must be used for each unique biotinylated antibody. Below are the steps to complete the coupling reactions for a 4-Assay plate.

Couple each biotinylated capture antibody to a unique Linker and record the antibody identity next to the Linker number on the Spot Map (a blank Spot Map is provided in Appendix B).

- ❑ Add 200 µL of each biotinylated antibody to 300 µL of the assigned Linker. Mix by vortexing. Incubate at room temperature for 30 minutes. Do not shake.

Notes:

- Each Linker vial has a matching colored cap and label.
- To remove liquid from the cap, briefly centrifuge the Linker vial and open the cap gently.
- Open one Linker at a time and close its cap as soon as you are done using it. Take precautions to avoid reagent contamination.
- For studies using multiple plates of the same assay, it is recommended that the same Linker be coupled with the same antibody for the duration of the study.

- ❑ Add 200 µL of Stop Solution, then mix by vortexing. Incubate at room temperature for 30 minutes.

Note: At the end of step 1, each iU-PLEX Linker-coupled antibody solution is at 10X the coating concentration and can be stored at 2–8 °C. Do not store for more than 7 days.

Adjust the volumes for multiple plates. The volumetric ratio of Linker:Antibody:Stop Solution is 3:2:2.

STEP 2a: Prepare the Multiplex Coating Solution for 96-well Plates

- ❑ Combine 600 µL of each U-PLEX Linker-coupled antibody solution (10X) into a single tube and mix by vortexing. Up to 10 U-PLEX Linker-coupled antibodies can be pooled. Do not combine U-PLEX Linker-coupled antibody solutions that share the same Linker.
- ❑ When combining fewer than 10 antibodies, bring the solution up to 6 mL with Stop Solutionto obtain a 1X concentration. Mix by vortexing.

Note: At the end of Step 2a, the U-PLEX multiplex coating solution is at 1X and can be stored at 2–8 °C. Do not store for more than 7 days.

STEP 2b: Prepare the Multiplex Coating Solution for 384-well Plates

- ❑ Combine 600 µL of each U-PLEX Linker-coupled antibody solution into a single tube and mix by vortexing. Up to 4 U-PLEX Linker-coupled antibodies can be pooled. Do not combine U-PLEX Linker-coupled antibody solutions that share the same Linker.
- ❑ Bring the solution up to 12 mL by mixing it with Stop Solution. Mix by vortexing.

Note: At the end of Step 2b, the U-PLEX multiplex coating solution is at 0.5X and can be stored at 2–8 °C. Do not store for more than 7 days.

STEP 3a: Coat U-PLEX 96-well Plates

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

STEP 3b: Coat U-PLEX 384-well Plates

- ❑ Wash the plate 3 times with 90 µL/well of 1X Wash Buffer.
- ❑ Add 25 µL of the 0.5X multiplex coating solution to each well. Seal the plate with an adhesive plate seal and shake for 4 hours at room temperature.
- ❑ Wash the plate 3 times with 90 µL/well of 1X Wash Buffer.

Note: The plate is now coated and ready for use. Plates may be stored in the original pouch with desiccant and sealed for up to 7 days at 2–8 °C.

The recommended volumes of Linker, biotinylated capture antibody, and Stop Solution for coating one or multiple U-PLEX plates are provided below in Table 6. If using a partial plate, refer to Table 11 and Table 12 in Appendix A.

Table 6. Amount of each component required for U-PLEX coating solution per plate

No. of Plate(s)	Individual Linker (µL)	Individual Biotinylated Antibody (µL)	Stop Solution (µL)
1	300	200	200
2	600	400	400
3	900	600	600
4	1,200	800	800
5	1,500	1,000	1,000
N	300 × N	200 × N	200 × N

Prepare Calibrator Standards

Depending on the assays ordered, you may receive one or more Calibrator vials with your order. If combining calibrators, please refer to the Specificity section (page 20) for more information. Guidance for using non-MSD calibrators is provided in Appendix A.

Bring the Calibrator vial(s) to room temperature. Reconstitute each vial of Calibrator by adding 250 µL of Assay Diluent to the vial. This will result in a 10X concentrated stock of each 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). A 2-fold dilution in the assay plate completes the 10-fold dilution. 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 dilutions at room temperature.

Note: A maximum of 5 Calibrator blends can be mixed in the Calibrator Standard 1 preparation step. It is recommended to mix the Calibrators for the assays that you are developing to prepare a single blend for ease in dilution. If you are using your own Calibrator, prepare 250 µL of a 10X concentrated blend of the calibrators in Assay Diluent. Use this concentrated stock to generate the Calibrator Standard 1 (Table 7). The following instructions will enable you to prepare seven Calibrator Standard solutions and a zero Calibrator Standard for up to six replicates.

Note: We recommend that reconstituted calibrators be used immediately. If storage is necessary, divide into 60 µL aliquots and store immediately at ≤–70 °C. For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the product. You can also find a copy of the COA at www.mesoscale.com.

Depending on the number of calibrator blends being used, prepare Calibrator Standard 1 (top of the curve) in a clean polypropylene tube by mixing and diluting the reconstituted or thawed calibrator. Mix by vortexing.

Table 7. Combining Calibrators to generate the Calibrator Standard 1 (top of the curve) level

No. of Calibrator Blends Provided	Volume of Reconstituted Calibrator (µL)	Assay Diluent (µL)	Total volume (µL)
1	50	200	250
2	50 each	150	250
3	50 each	100	250
4	50 each	50	250
5	50 each	0	250

Prepare the subsequent 6 dilutions for the curve (4-fold serial dilutions) in Assay Diluent (Figure 4). Use Assay Diluent for the Calibrator Standard 8 (zero Calibrator/blank). Mix by vortexing the tubes between each serial dilution.

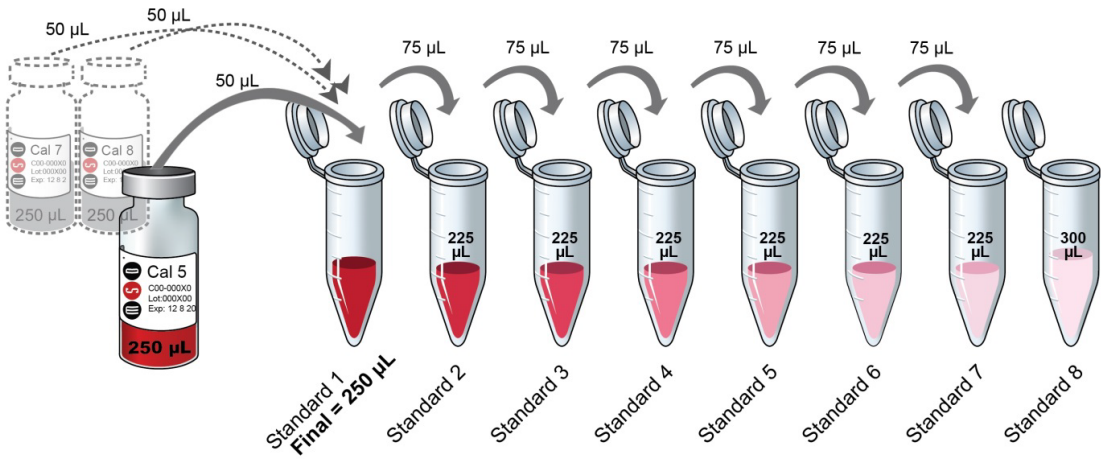


Figure 4. Dilution schema for preparation of Calibrator Standards for U-PLEX Biomarker Group 1 (human) Assays.

Table 8. 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 vials	See Table 7		
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

Alternate Calibrator handling procedures

If an assay needs more than 5 Calibrators blended together, reconstitute each Calibrator with 125 µL of Assay Diluent. This will result in a 20X concentrated stock of the Calibrator. Take extra care that all of the lyophilized material is reconstituted. Follow the instructions (Figure 4; Table 7), but blend 25 µL of each Calibrator (rather than 50 µL) and add enough Assay Diluent to get a final volume of 250 µL.

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, in-house data indicate that a large sample dilution (100-fold) is required to generate optimal results. Refer to the product-specific datasheets for additional information.

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X for 96-well assays and 0.5X for 384-well assays. Prepare the detection antibody solution immediately before use. Adjust the volumes proportionally for partial plates.

For one plate, combine:

- ❑ 60 µL of each 100X detection antibody
- ❑ Diluent 3 to bring the final volume to 6 mL (12 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 to use. Do not dilute.

Assay Protocols

Note: Follow Reagent Preparation before beginning this assay protocol.

96-well Plate Assays

STEP 1: Add Sample or Calibrator Standard

- ❑ 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 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 4 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, Coincubation:** Coincubating samples and detection antibody solution may improve the sensitivity for some assays. Note that the use of the coincubation 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, 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.
- ❑ **Alternate Protocol 3, Shortened Incubations**: Some assays in 384-well plates may achieve acceptable performance with shorter incubations. Consider incubating samples in the plate for 2 hours.

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 plates. The data were generated during the development of the assay and do not represent the product specifications. Under your experimental conditions and with your specific multiplex, the assay may perform differently than the representative data shown.

Specificity

To assess specificity, the Antibody Set for each analyte was tested individually against a larger panel of recombinant human analytes for nonspecific binding (CTACK, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, EPO, FLT3L, Fractalkine, G-CSF, GM-CSF, GRO-α, I-309, IFN-α2a, IFN-β, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-2Rα, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-17E/IL-25, IL-17F, IL-18, IL-21, IL-22, IL-23, IL-27, IL-29/IFN-λ1, IL-31, IL-33, IP-10, I-TAC, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1α, MIP-1β, MIP-3α, MIP-3β, MIP-5, SDF-1α, TARC, TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, and YKL-40).

Nonspecific binding was less than 0.5% for all assays in U-PLEX Biomarker Group 1 (human) using the following calculation.

$$\%\ nonspecificity = \frac{nonspecific\ signal}{specific\ signal} \times 100$$

Exceptions are noted below:

- The IL-12p70 and IL-23 analytes contain the p40 subunit. These analytes will cross-react with the IL-12/IL-23p40 assay as expected. We do not recommend multiplexing IL-12p70 or IL-23 assays on the same plate with the IL-12/IL-23p40 assay. If measuring IL-12/IL-23p40 assay, Calibrator 3 should not be combined with either Calibrator 1 or 6.
- The IL-17A analyte cross-reacts with the IL-17A/F assay. The IL-17A/F analyte cross-reacts with the IL-17A assay. We do not recommend multiplexing these assays on the same plate. If measuring IL-17A or IL-17A/F assays, Calibrator 6 should not be combined with Calibrator 1.
- The MCP-2 and MCP-3 detection antibodies nonspecifically bind the Eotaxin Calibrator (2.8% and 3.7%, respectively). We do not recommend multiplexing the MCP-2 or MCP-3 assay and the Eotaxin assay on the same plate.
- The TPO capture antibody nonspecifically binds the FLT3L Calibrator/detection antibody pair (4%). We do not recommend multiplexing the TPO assay and the FLT3L assay on the same plate.

Appendix A

U-PLEX Combos

U-PLEX Combos (Table 9) are preconfigured multiplex assays that include U-PLEX Plates, Linkers, Antibody Sets, Calibrators, Stop Solution, Diluents, and Read Buffer.

Table 9. Catalog numbers of U-PLEX Biomarker Group 1 (human) Combos

Product	Analytes	Catalog Numbers (-1/-5/-25 Plate Size)
U-PLEX TH1/TH2 Combo (human) SECTOR	IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNFα	K15010K-1/-2/-4
U-PLEX TH17 Combo 1 (human) SECTOR	IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, IL-23, IL-27, IL-31, IL-33	K15075K-1/-2/-4
U-PLEX TH17 Combo 2 (human) SECTOR	IFN-γ, IL-1β, IL-6, IL-10, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, TNF-α	K15076K-1/-2/-4
U-PLEX Proinflam Combo 1 (human) SECTOR	IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α	K15049K-1/-2/-4
U-PLEX Proinflam Combo 2 (human) SECTOR	GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70	K15066K-1/-2/-4
U-PLEX Proinflam Combo 3 (human) SECTOR	IFN-γ, IL-1β, IL-6, TNF-α	K15025K-1/-2/-4
U-PLEX Proinflam Combo 4 (human) SECTOR	IL-1β, IL-6, IL-8, TNF-α	K15072K-1/-2/-4
U-PLEX Cytokine Combo 1 (human) SECTOR	GM-CSF, IL-1α, IL-5, IL-7, IL-12/IL-23p40, IL-15, IL-16, IL-17A, TNF-β, VEGF-A	K15045K-1/-2/-4
U-PLEX Chemokine Combo 1 (human) SECTOR	Eotaxin, Eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC	K15047K-1/-2/-4
U-PLEX Chemokine Combo 2 (human) Sector	CTACK, ENA-78, Fractalkine, ITAC, MIP-3α, MIP-3β, SDF-1α	K15046K-1/-2/-4
U-PLEX T-Cell Combo (human) SECTOR	GM-CSF, IFN-γ, IL-2, IL-4, IL-9, IL-10, IL-13, IL-17A, IL-17E/IL-25, IL-17F, IL-21, IL-22, MIP-3α, TNF-α	K15093K-1/-2/-4
U-PLEX Interferon Combo (human) SECTOR	IFN-α2a, IFN-β, IFN-γ, IL-29/IFN-λ1	K15094K-1/-2/-4
U-PLEX Viral Combo 1 (human) SECTOR	G-CSF, GM-CSF, IFN-α2a, IFN-β, IFN-γ, IL-1β, IL-1RA, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IP-10, MCP-1, MIP-1α, VEGF-A, TNF-α	K15343K-1/-2/-4

U-PLEX Antibody Sets

Antibody Sets (Table 10) include a biotinylated capture antibody and SULFO-TAG conjugated detection antibody.

Table 10. Catalog numbers of Antibody Sets available for the 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-γ 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

Working with Partial Plates

A portion of a plate may be used. Volumes should be adjusted proportionally when preparing reagents for partial plates (Table 11; Table 12).

For convenience, the recommended volumes of Linker, biotinylated capture antibody, and Stop Solution for coating partial plates are provided below.

Table 11. Amount of each component required for U-PLEX coating solution (partial 96-well plate)

No. of wells	Individual Linker (µL)	Individual Biotinylated Antibody (µL)	Stop Solution per Reaction (µL)	Vol. to Pull from Each Reaction (µL)
16	60	40	40	100
32	120	80	80	200
48	150	100	100	300
64	210	140	140	400
80	240	160	160	500
96	300	200	200	600

Table 12. Amount of each component required for U-PLEX coating solution (partial 384-well plate)

No. of Wells	Individual Linker (µL)	Individual Biotinylated Antibody (µL)	Stop Solution per Reaction (µL)	Vol. to Pull from Each Reaction (µL)	Add Stop Solution and bring Vol to (µL)
64	60	40	40	100	2,000
128	120	80	80	200	4,000
192	150	100	100	300	6,000
256	210	140	140	400	8,000
320	240	160	160	500	10,000

When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. The uncoated wells of a partially used plate may be sealed and stored for up to 30 days at 2–8 °C in the original foil pouch with desiccant.

Multiplate Assays

Multiplex U-PLEX assays can occupy more than one plate, depending on the number and compatibility of the selected assays.

An example of a multiplate U-PLEX assay is the U-PLEX Cytokine Combo 1 (human) (catalog number K15045K; Table 13). The assay is supplied in two separate U-PLEX boxes. Each box includes one 10-Spot, U-PLEX 96-well plate (with the appropriate number of activated spots), Linkers, antibody pairs, and Calibrators that run optimally together. Components should not be mixed between boxes, except for Stop Solution, Diluents, and Read Buffer.

To perform any multiplate multiplex assay, we recommend that you position the boxes in numerical order. When multiple Calibrators are in one box, they should be blended as instructed in this product insert. Do not combine with any other Calibrators from another box. There will be a unique Calibrator curve for each box.

Table 13. U-PLEX Cytokine Combo 1 (human) layout

Box 1 4-Activated Spots Plate (Calibrator 1)	Box 2 6-Activated Spots Plate (Calibrator 3)
GM-CSF	IL-1α
IL-5	IL-7
IL-17A	IL-12/IL-23p40
VEGF-A	IL-15
–	IL-16
	TNF-β

Dash (—) = not applicable

Appendix B

Open Spots

Prepare Conjugated Capture and Detection Antibodies

The U-PLEX platform uses a biotinylated capture antibody and a SULFO-TAG conjugated detection antibody. Therefore, for assays that are being developed with your own antibody pairs, the capture antibodies (or other suitable capture reagents) must be biotinylated before starting the U-PLEX protocol. Similarly, the detection antibody must be conjugated with SULFO-TAG; however, you may choose to use a SULFO-TAG conjugated secondary detection antibody that is raised against the host of the detection antibody. In such cases, the detection antibody should be raised in different host species than the capture antibodies in the U-PLEX assay to avoid cross-reactivity. For example, if the capture antibody is raised in a rabbit, choose a detection antibody raised in a different host species than rabbit (e.g., mouse).

Note: Since the capture antibody is always biotinylated, do not use a biotinylated detection antibody or SULFO-TAG streptavidin as a method for detection. SULFO-TAG streptavidin will cause high backgrounds, as it will bind to the biotin on the capture antibody.

Prepare Biotinylated Capture Antibody

The working concentration of biotinylated capture antibody needed to prepare the multiplex coating solution for the U-PLEX Plate is 10 µg/mL. Prepare a stock solution of the biotinylated capture antibody by following the manufacturer's guidelines for the conjugation of an antibody to Sulfo-NHS-LC-Biotin (such as EZ-Link Sulfo-NHS-LC-Biotin (Thermo Fisher Scientific) or an equivalent product. At least one biotin must be present on the capture antibody for it to be coupled to the U-PLEX Linker. We recommend starting with a biotin challenge ratio of 10 biotins to 1 capture antibody. This challenge ratio typically leads to the conjugation of an average of 2–4 biotins per antibody.

Note: Free biotin will interfere with the U-PLEX assay signal. Therefore, after conjugation, it is recommended to purify the biotinylated antibody from the free biotin reagent by using Zeba Desalting Columns.

For long-term storage, it is recommended that you perform a buffer exchange to store the final biotinylated antibody in the Conjugate Storage buffer.

Prepare SULFO-TAG Conjugated Detection Antibody

The optimal concentration of the SULFO-TAG conjugated detection antibody for use in the U-PLEX assay is typically within the range of 0.5–1 µg/mL. Prepare a concentrated stock solution of 100X for each SULFO-TAG conjugated detection antibody by following the guidelines for SULFO-TAG conjugation available at www.mesoscale.com. We recommend using a 20:1 challenge ratio for SULFO-TAG conjugation of antibodies. This challenge ratio leads to a typical conjugation ratio of 10 SULFO-TAG labels per antibody molecule. Optimization of the SULFO-TAG challenge ratio may be necessary to reduce background and increase assay signal. To find out more details on optimizing the SULFO-TAG conjugation of the detection antibody, please refer to the MSD GOLD SULFO-TAG Conjugation Quick Guide or the MSD GOLD SULFO-TAG NHS-Ester technical note available at www.mesoscale.com.

For long-term storage, purify the SULFO-TAG conjugated antibody to remove the unconjugated SULFO-TAG NHS-ESTER. Antibody conjugates are typically stable for at least 1 year in conjugation storage buffer at 2–8 °C. Protect from direct exposure to light.

Prepare non-MSD Calibrator

For assays that are being developed with your antibody pairs, a recombinant protein that is representative of the native protein can be used for the calibration curve. A good starting concentration is 10 ng/mL for the high calibrator and 0.001 ng/mL for the low calibrator. We recommend testing an 8-point titration curve and optimizing the calibrator diluent if required. Additional guidance on using recombinant protein calibrators can be found in the Development Pack Product Insert at www.mesoscale.com/U-PLEX-documents.

Summary Protocols

Prepare U-PLEX 96-well Plates

Step 1 Created Individual U-PLEX Linker-Coupled Antibody Solutions

Couple an individual biotinylated antibody to a unique Linker, and record the antibody identity next to the Linker number on the Spot Map (Figure 5).

- ❑ Add 200 µL of each biotinylated antibody to 300 µL of the assigned Linker. Refer to the U-PLEX plate Spot Map to determine which Linkers can be combined. A different Linker must be used for each unique biotinylated antibody. Mix by vortexing. Incubate at room temperature for 30 minutes.
- ❑ Add 200 µL of Stop Solution. Mix by vortexing. Incubate at room temperature for 30 minutes.

STEP 2: Prepare the Multiplex Coating Solution for a 96-well Plate

- ❑ Combine 600 µL of each U-PLEX Linker-coupled antibody solution into a single tube and mix by vortexing. Up to 10 U-PLEX Linker-coupled antibodies can be pooled. Do not combine U-PLEX Linker-coupled antibody solutions that share the same Linker.
- ❑ When combining fewer than 10 antibodies, bring the solution up to 6 mL by mixing with Stop Solution to result in a final 1X concentration. Mix by vortexing.

STEP 3: Coat a U-PLEX 96-well Plate

- ❑ Add 50 µL of the 1X multiplex coating solution to each well. 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 Wash Buffer. The plate is now coated and ready for use.

96-Well Assay Protocol

STEP 1: Add Samples or Calibrator Standards

- ❑ 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 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 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.

Prepare U-PLEX 384-well Plates

STEP 1: Create Individual U-PLEX Linker-Coupled Antibody Solutions

Couple an individual biotinylated antibody to a unique Linker, and record the antibody identity next to the Linker number on the Spot Map (Figure 5).

- ❑ Add 200 µL of each biotinylated antibody to 300 µL of the assigned Linker. Refer to the U-PLEX plate Spot Map to determine which Linkers can be combined. A different Linker must be used for each unique biotinylated antibody. Mix by vortexing. Incubate at room temperature for 30 minutes.
- ❑ Add 200 µL of Stop Solution. Mix by vortexing. Incubate at room temperature for 30 minutes.

STEP 2: Prepare the Multiplex Coating Solution for a 384-well Plate

- ❑ Combine 600 µL of each U-PLEX Linker-coupled antibody solution into a single tube and mix by vortexing. Up to 4 U-PLEX Linker-coupled antibodies can be pooled. Do not combine U-PLEX Linker-coupled antibody solutions that share the same Linker.
- ❑ Bring the solution up to 12 mL by mixing with Stop Solution to result in a final 0.5X concentration. Mix by vortexing.

STEP 3: Coat a U-PLEX 384-well Plate

- ❑ Wash the plate 3 times with 90 µL of 1X Wash Buffer.
- ❑ Add 25 µL of the 0.5X multiplex coating solution to each well. Seal the plate with an adhesive plate seal and shake for 4 hours at room temperature.
- ❑ Wash the plate 3 times with 90 µL/well of 1X Wash Buffer. The plate is now coated and ready for use.

384-well Assay Protocol

STEP 1: Add Sample or Calibrator Standards

- ❑ 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 4 hours.

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. 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 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.

Spot Maps

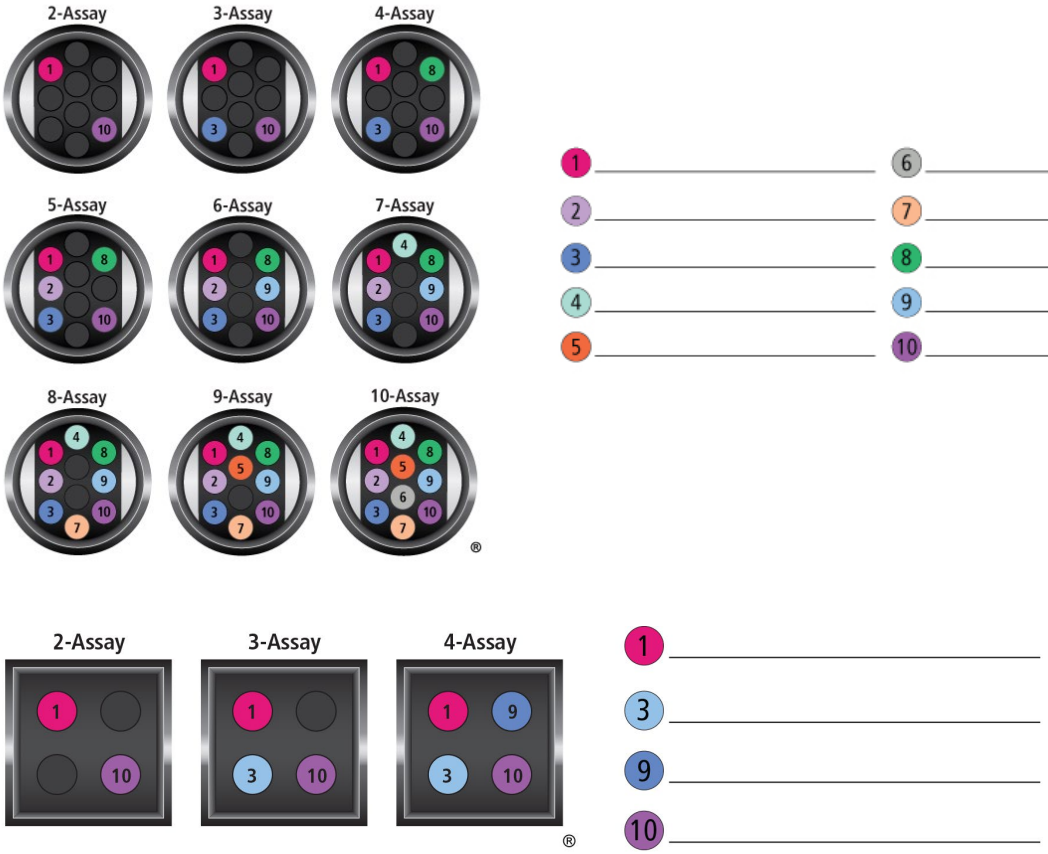


Figure 5. Spot maps: (***top***) 96-well plate, (***bottom***) 384-well plate.

Plate Diagrams

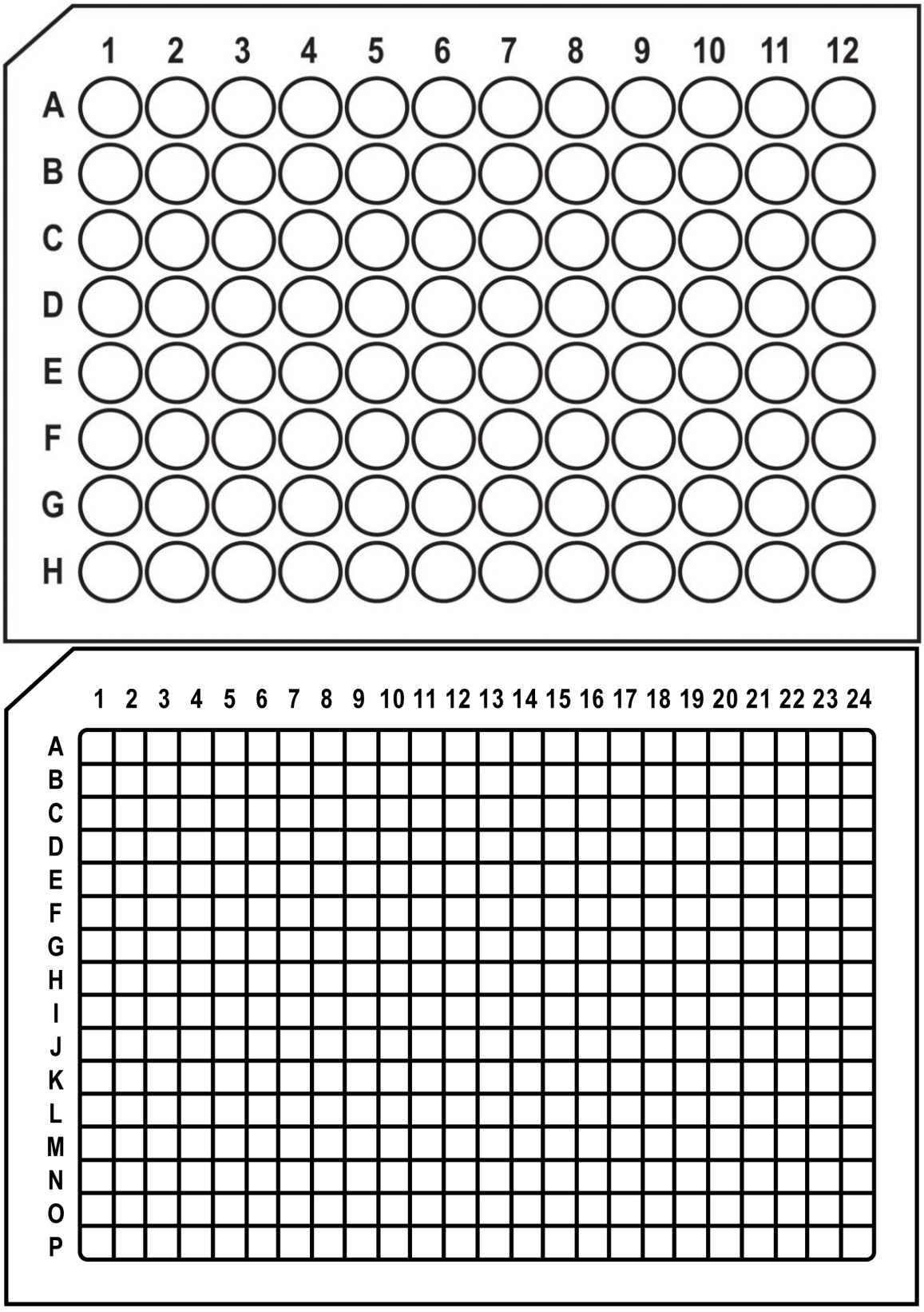


Figure 6. Plate diagrams; similar plate layouts can be created in Excel and in the DISCOVERY WORKBENCH® software.