U-PLEX® Biomarker Group 1 (mouse)

Singleplex Assay

MSD U-PLEX Platform

U-PLEX Biomarker Group 1 (mouse) Singleplex Assays

Tested on serum, EDTA plasma, and cell culture supernatants.

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

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

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 (mouse) contains 50 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 (mouse) is presented in the product-specific datasheets available at www.mesoscale.com/support/datasheets.

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

Assays				
6CKine/CCL21	IL-1β	IL-15	IL-27p28/IL-30	MIP-2 (CXCL2)
BAFF	IL-2	IL-16	IL-31	MIP-3α (CCL20)
BCA-1/BLC	IL-4	IL-17A	IL-33	MMP-9 (total)
CD40/TNFRSF5	IL-5	IL-17A/F	IP-10 (CXCL10)	NGAL/LCN2
Eotaxin (CCL11)	IL-6	IL-17C	KC/GRO	RANTES (CCL5)
EPO	IL-9	IL-17E	MCP-1 (CCL2)	SDF-1α (CXCL12)
GM-CSF (CSF3)	IL-10	IL-17F	MCP-5 (CCL12)	TARC (CCL17)
IFN-α	IL-12/IL-23p40	IL-21	MDC (CCL22)	TNF-α
IFN-β	IL-12p70	IL-22	MIP-1α (CCL3)	TNF-R1
IFN-γ	IL-13	IL-23	MIP-1β (CCL4)	VEGF-A

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.

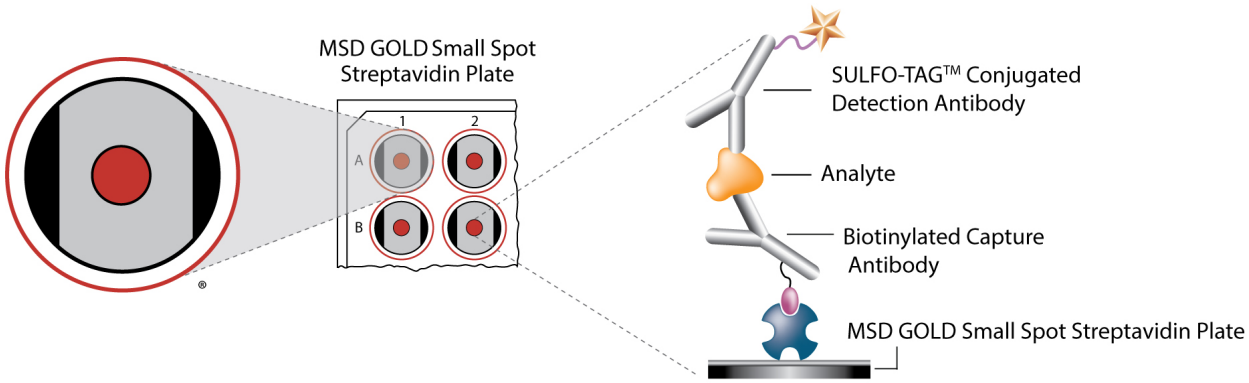

Figure 1. U-PLEX Singleplex Assay on an MSD GOLD 96-well Small Spot Streptavidin Plate. The 384-well assay is similar.

Components

Tables 2, 3, and 4 list the components provided with U-PLEX Biomarker Group 1 (mouse) Singleplex Assays. U-PLEX Singleplex Assays are available with either SECTOR™ or QuickPlex® 96-well plates or SECTOR 384-well plates (Table 6).

Table 2. Reagents that are supplied with all U-PLEX Biomarker Group 1 (mouse) 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-spot	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 41	≤–10 °C	R50AH-1	10 mL	1 bottle	—	—	Diluent for samples and calibrators
		R50AH-2	50 mL	—	1 bottle	5 bottles	
Diluent 45	≤–10 °C	R50AI-3	8 mL	1 bottle	—	—	Diluent for detection antibody
		R50AI-4	40 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

Table 3. Reagents that are supplied with all U-PLEX Metabolic Group 1 (mouse) 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 41	≤–10 °C	R50AH-2	50 mL	2 bottles	10 bottles	Diluent for samples and calibrators
Diluent 45	≤–10 °C	R50AI-4	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

Dash (—) = not applicable
RT = room temperature

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 (mouse) and their respective catalog numbers is 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 Mouse 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 one or more analytes and may be either lyophilized or frozen 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 (mouse)

Name	Storage	Catalog No.	Size	Quantity Supplied			Analytes
				1 Plate	5 Plates	25 Plates	
Calibrator 5	2–8 °C	C0065-2	1 vial	1 vial	5 vials	25 vials	EPO, GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, KC/GRO, TNF-α, VEGF-A
Calibrator 7	2–8 °C	C0073-2	1 vial	1 vial	5 vials	25 vials	IL-16, IL-17A, IL-17C, IL-17E/IL-25, IL-21, IL-22, IL-23
Calibrator 8	2–8 °C	C0074-2	1 vial	1 vial	5 vials	25 vials	IL-15, IL-17F, IL-31, IL-33, IL-27p28/IL-30
Calibrator 12	2–8 °C	C0092-2	1 vial	1 vial	5 vials	25 vials	IL-9, IL-17A/F, IP-10, MCP-1, MIP-1α, MIP-1β, MIP-2, MIP-3α
Calibrator 16	≤–70 °C	C0295-2	1 vial	1 vial	5 vials	25 vials	6CKine/Ccl21, BAFF, BCA-1/BLC, CD40, IFN-β, MCP-5/Ccl12, MDC
Calibrator 17	2–8 °C	C0296-2	1 vial	1 vial	5 vials	25 vials	Eotaxin, MMP-9 (total), NGAL/LCN2, RANTES, SDF-1α, TARC, TNF-RI
Mouse IFN-α Calibrator	2–8 °C	C02W1-2	1 vial	1 vial	5 vials	25 vials	IFN-α

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 Plates	Assays on 96-well QuickPlex Plates	Assays on 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	—	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 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

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 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 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.
- 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 reagents to room temperature and refer to the Best Practices section (page 9) before beginning this 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.

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. They may be sealed and stored overnight at 4 °C.

Prepare Calibrator Standards

The following instructions will enable you to prepare 7 Calibrator Standards plus a zero Calibrator standard for up to four replicates.

For Lyophilized Calibrators:

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 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.

For Liquid Calibrators:

Thaw the stock calibrator(s) and keep it on ice. The thawed calibrator is added as is to Calibrator Standard 1. Once thawed, the calibrator is ready to use. Keep dilution(s) at room temperature. A 2-fold dilution in the assay plate completes the dilution.

Note: We recommend that reconstituted or thawed calibrators be used immediately. If storage is necessary, divide calibrators into suitably sized aliquots (60 µL aliquots are recommended) 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.

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

- ❑ Prepare Calibrator Standard 1 by adding 50 µL of the reconstituted or thawed 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/blank).

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.

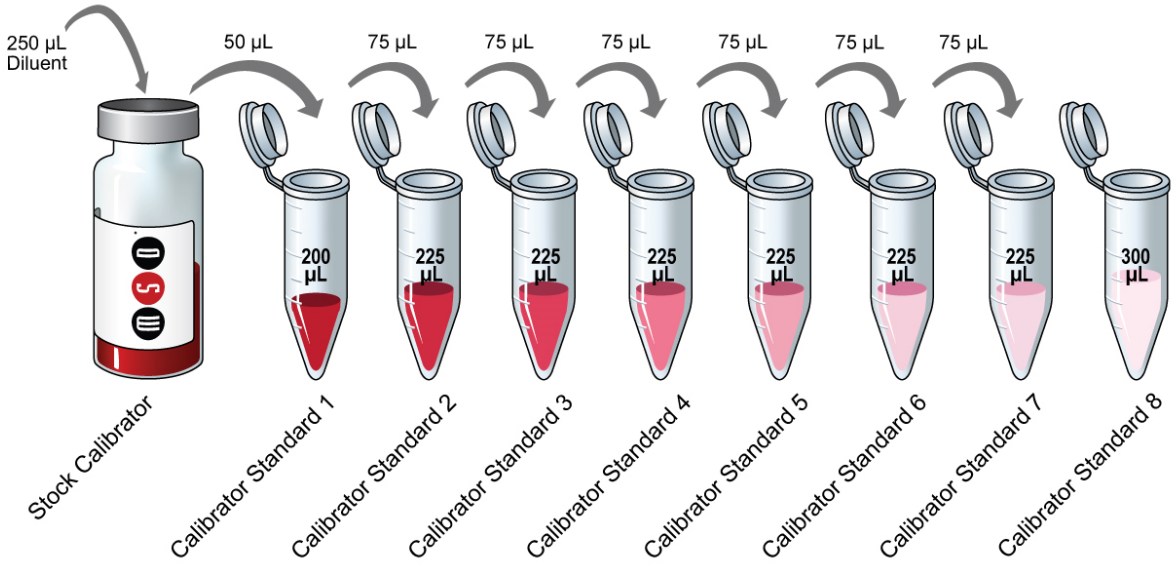

Figure 2. Dilution schema for Calibrator Standards for U-PLEX Biomarker Group 1 (mouse) Singleplex Assays.

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, 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: The concentrations of 6CKine/CCL21, BAFF, and NGAL/LCN2 in normal serum and EDTA plasma may exceed the standard working range of the assays. Preassay 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 for 96-well plates is 1X and 0.5X for 384-well plates. 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 45 (11.94 mL for 384-well assays)

Read Buffer

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

Prepare 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.

Assay Protocol

96-well Plate Assays

Note: Follow Reagent Preparation before beginning this assay protocol.

STEP 1: Add Samples and Calibrators

- ❑ Wash the plate 3 times with 90µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of Diluent 41 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

- ❑ Wash the plate 3 times with 90µL/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 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, Shortened Incubation:** Some 384-well 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 2, Extended Incubation:** Incubating samples overnight at 2–8 °C may improve sensitivity for some assays.
- ❑ **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/U-PLEX-documents. 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, the assay may perform differently than the representative data shown.

Appendix

U-PLEX Singleplex Assays

U-PLEX Singleplex 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 (mouse) Singleplex Assays

Product	96-Well SECTOR Assays (1/5/25 Plates)	96-Well QuickPlex Assays (1/5/25 Plates)	384-Well SECTOR Assays (5/25 plates)
U-PLEX Mouse 6CKine/CCL21 Assay	K152C0K-1/-2/-4	K152C0K-21/-22/-24	K252C0K-2/-4
U-PLEX Mouse BAFF Assay	K1523QK-1/-2/-4	K1523QK-21/-22/-24	K2523QK-2/-4
U-PLEX Mouse BCA-1/BLC Assay	K152F0K-1/-2/-4	K152F0K-21/-22/-24	K252F0K-2/-4
U-PLEX Mouse CD40/TNFRSF5 Assay	K152D0K-1/-2/-4	K152D0K-21/-22/-24	K252D0K-2/-4
U-PLEX Mouse Eotaxin Assay	K152UDK-1/-2/-4	K152UDK-21/-22/-24	K252UDK-2/-4
U-PLEX Mouse EPO Assay	K152VXK-1/-2/-4	K152VXK-21/-22/-24	K252VXK-2/-4
U-PLEX Mouse GM-CSF Assay	K152UMK-1/-2/-4	K152UMK-21/-22/-24	K252UMK-2/-4
U-PLEX Mouse IFN-α Assay	K152W1K-1/-2/-4	K152W1K-21/-22/-24	K252W1K-2/-4
U-PLEX Mouse IFN-β Assay	K152G0K-1/-2/-4	K152G0K-21/-22/-24	K252G0K-2/-4
U-PLEX Mouse IFN-γ Assay	K152TTK-1/-2/-4	K152TTK-21/-22/-24	K252TTK-2/-4
U-PLEX Mouse IL-1β Assay	K152TUK-1/-2/-4	K152TUK-21/-22/-24	K252TUK-2/-4
U-PLEX Mouse IL-2 Assay	K152TVK-1/-2/-4	K152TVK-21/-22/-24	K252TVK-2/-4
U-PLEX Mouse IL-4 Assay	K152TWK-1/-2/-4	K152TWK-21/-22/-24	K252TWK-2/-4
U-PLEX Mouse IL-5 Assay	K152UOK-1/-2/-4	K152UOK-21/-22/-24	K252UOK-2/-4
U-PLEX Mouse IL-6 Assay	K152TXK-1/-2/-4	K152TXK-21/-22/-24	K252TXK-2/-4
U-PLEX Mouse IL-9 Assay	K152XKK-1/-2/-4	K152XKK-21/-22/-24	K252XKK-2/-4
U-PLEX Mouse IL-10 Assay	K152TZK-1/-2/-4	K152TZK-21/-22/-24	K252TZK-2/-4
U-PLEX Mouse IL-12/IL-23p40 Assay	K152UQK-1/-2/-4	K152UQK-21/-22/-24	K252UQK-2/-4
U-PLEX Mouse IL-12p70 Assay	K152UAK-1/-2/-4	K152UAK-21/-22/-24	K252UAK-2/-4
U-PLEX Mouse IL-13 Assay	K152UBK-1/-2/-4	K152UBK-21/-22/-24	K252UBK-2/-4
U-PLEX Mouse IL-15 Assay	K152URK-1/-2/-4	K152URK-21/-22/-24	K252URK-2/-4
U-PLEX Mouse IL-16 Assay	K152USK-1/-2/-4	K152USK-21/-22/-24	K252USK-2/-4
U-PLEX Mouse IL-17A Assay	K152UTK-1/-2/-4	K152UTK-21/-22/-24	K252UTK-2/-4
U-PLEX Mouse IL-17A/F Assay	K152VYK-1/-2/-4	K152VYK-21/-22/-24	K252VYK-2/-4
U-PLEX Mouse IL-17C Assay	K152WJK-1/-2/-4	K152WJK-21/-22/-24	K252WJK-2/-4
U-PLEX Mouse IL-17E/IL-25 Assay	K152VZK-1/-2/-4	K152VZK-21/-22/-24	K252VZK-2/-4
U-PLEX Mouse IL-17F Assay	K152WAK-1/-2/-4	K152WAK-21/-22/-24	K252WAK-2/-4
U-PLEX Mouse IL-21 Assay	K152WBK-1/-2/-4	K152WBK-21/-22/-24	K252WBK-2/-4
U-PLEX Mouse IL-22 Assay	K152WIK-1/-2/-4	K152WIK-21/-22/-24	K252WIK-2/-4
U-PLEX Mouse IL-23 Assay	K152WGK-1/-2/-4	K152WGK-21/-22/-24	K252WGK-2/-4
U-PLEX Mouse IL-27p28/IL-30 Assay	K152WCK-1/-2/-4	K152WCK-21/-22/-24	K252WCK-2/-4
U-PLEX Mouse IL-31 Assay	K152WEK-1/-2/-4	K152WEK-21/-22/-24	K252WEK-2/-4
U-PLEX Mouse IL-33 Assay	K152WFK-1/-2/-4	K152WFK-21/-22/-24	K252WFK-2/-4
U-PLEX Mouse IP-10 Assay	K152UFK-1/-2/-4	K152UFK-21/-22/-24	K252UFK-2/-4
U-PLEX Mouse KC/GRO Assay	K152VWK-1/-2/-4	K152VWK-21/-22/-24	K252VWK-2/-4
U-PLEX Mouse MCP-1 Assay	K152UGK-1/-2/-4	K152UGK-21/-22/-24	K252UGK-2/-4

Product	96-Well SECTOR Assays (1/5/25 Plates)	96-Well QuickPlex Assays (1/5/25 Plates)	384-Well SECTOR Assays (5/25 plates)
U-PLEX Mouse MCP-5/CCL12 Assay	K152Y1K-1/-2/-4	K152Y1K-21/-22/-24	K252Y1K-2/-4
U-PLEX Mouse MDC Assay	K152X1K-1/-2/-4	K152X1K-21/-22/-24	K252X1K-2/-4
U-PLEX Mouse MIP-1α Assay	K152UJK-1/-2/-4	K152UJK-21/-22/-24	K252UJK-2/-4
U-PLEX Mouse MIP-1β Assay	K152UKK-1/-2/-4	K152UKK-21/-22/-24	K252UKK-2/-4
U-PLEX Mouse MIP-2 Assay	K152XLK-1/-2/-4	K152XLK-21/-22/-24	K252XLK-2/-4
U-PLEX Mouse MIP-3α Assay	K152UZK-1/-2/-4	K152UZK-21/-22/-24	K252UZK-2/-4
U-PLEX Mouse MMP-9 (total) Assay	K152ZGK-1/-2/-4	K152ZGK-21/-22/-24	K252ZGK-2/-4
U-PLEX Mouse NGAL/LNC2 Assay	K152Z1K-1/-2/-4	K152Z1K-21/-22/-24	K252Z1K-2/-4
U-PLEX Mouse RANTES Assay	K152A2K-1/-2/-4	K152A2K-21/-22/-24	K252A2K-2/-4
U-PLEX Mouse SDF-1α Assay	K152VBK-1/-2/-4	K152VBK-21/-22/-24	K252VBK-2/-4
U-PLEX Mouse TARC Assay	K152B0K-1/-2/-4	K152B0K-21/-22/-24	K252B0K-2/-4
U-PLEX Mouse TNF-α Assay	K152UCK-1/-2/-4	K152UCK-21/-22/-24	K252UCK-2/-4
U-PLEX Mouse TNF-RI Assay	K1520VK-1/-2/-4	K1520VK-21/-22/-24	K2520VK-2/-4
U-PLEX Mouse VEGF-A Assay	K152UVK-1/-2/-4	K152UVK-21/-22/-24	K252UVK-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 (mouse)

Product	Catalog Numbers (-1/-5 Plate Size)
U-PLEX Mouse 6CKine/CCL21 Antibody Set	B22C0-2/-3
U-PLEX Mouse BAFF Antibody Set	B223Q-2/-3
U-PLEX Mouse BCA-1/BLC Antibody Set	B22F0-2/-3
U-PLEX Mouse CD40/TNFRSF5 Antibody Set	B22D0-2/-3
U-PLEX Mouse Eotaxin Antibody Set	B22UD-2/-3
U-PLEX Mouse EPO Antibody Set	B22VX-2/-3
U-PLEX Mouse GM-CSF Antibody Set	B22UM-2/-3
U-PLEX Mouse IFN-α Antibody Set	B22W1-2/-3
U-PLEX Mouse IFN-β Antibody Set	B22G0-2/-3
U-PLEX Mouse IFN-γ Antibody Set	B22TT-2/-3
U-PLEX Mouse IL-1β Antibody Set	B22TU-2/-3
U-PLEX Mouse IL-2 Antibody Set	B22TV-2/-3
U-PLEX Mouse IL-4 Antibody Set	B22TW-2/-3
U-PLEX Mouse IL-5 Antibody Set	B22U0-2/-3
U-PLEX Mouse IL-6 Antibody Set	B22TX-2/-3
U-PLEX Mouse IL-9 Antibody Set	B22XK-2/-3
U-PLEX Mouse IL-10 Antibody Set	B22TZ-2/-3
U-PLEX Mouse IL-12/IL-23p40 Antibody Set	B22UQ-2/-3
U-PLEX Mouse IL-12p70 Antibody Set	B22UA-2/-3
U-PLEX Mouse IL-13 Antibody Set	B22UB-2/-3
U-PLEX Mouse IL-15 Antibody Set	B22UR-2/-3
U-PLEX Mouse IL-16 Antibody Set	B22US-2/-3
U-PLEX Mouse IL-17A Antibody Set	B22UT-2/-3
U-PLEX Mouse IL-17A/F Antibody Set	B22VY-2/-3
U-PLEX Mouse IL-17C Antibody Set	B22WJ-2/-3

Product	Catalog Numbers (-1/-5 Plate Size)
U-PLEX Mouse IL-17E/IL-25 Antibody Set	B22VZ-2/-3
U-PLEX Mouse IL-17F Antibody Set	B22WA-2/-3
U-PLEX Mouse IL-21 Antibody Set	B22WB-2/-3
U-PLEX Mouse IL-22 Antibody Set	B22WI-2/-3
U-PLEX Mouse IL-23 Antibody Set	B22WG-2/-3
U-PLEX Mouse IL-27p28/IL-30 Antibody Set	B22WC-2/-3
U-PLEX Mouse IL-31 Antibody Set	B22WE-2/-3
U-PLEX Mouse IL-33 Antibody Set	B22WF-2/-3
U-PLEX Mouse IP-10 Antibody Set	B22UF-2/-3
U-PLEX Mouse KC/GRO Antibody Set	B22VW-2/-3
U-PLEX Mouse MCP-1 Antibody Set	B22UG-2/-3
U-PLEX Mouse MCP-5/CCL12 Antibody Set	B22Y1-2/-3
U-PLEX Mouse MDC Antibody Set	B22X1-2/-3
U-PLEX Mouse MIP-1α Antibody Set	B22UJ-2/-3
U-PLEX Mouse MIP-1β Antibody Set	B22UK-2/-3
U-PLEX Mouse MIP-2 Antibody Set	B22XL-2/-3
U-PLEX Mouse MIP-3α Antibody Set	B22UZ-2/-3
U-PLEX Mouse MMP-9 (total) Antibody Set	B22ZG-2/-3
U-PLEX Mouse NGAL/LNC2 Antibody Set	B22Z1-2/-3
U-PLEX Mouse RANTES Antibody Set	B22A2-2/-3
U-PLEX Mouse SDF-1α Antibody Set	B22VB-2/-3
U-PLEX Mouse TARC Antibody Set	B22B0-2/-3
U-PLEX Mouse TNF-α Antibody Set	B22UC-2/-3
U-PLEX Mouse TNF-RI Antibody Set	B200V-2/-3
U-PLEX Mouse VEGF-A Antibody Set	B22UV-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 Diluent 41 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 MSD 384-well Streptavidin 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

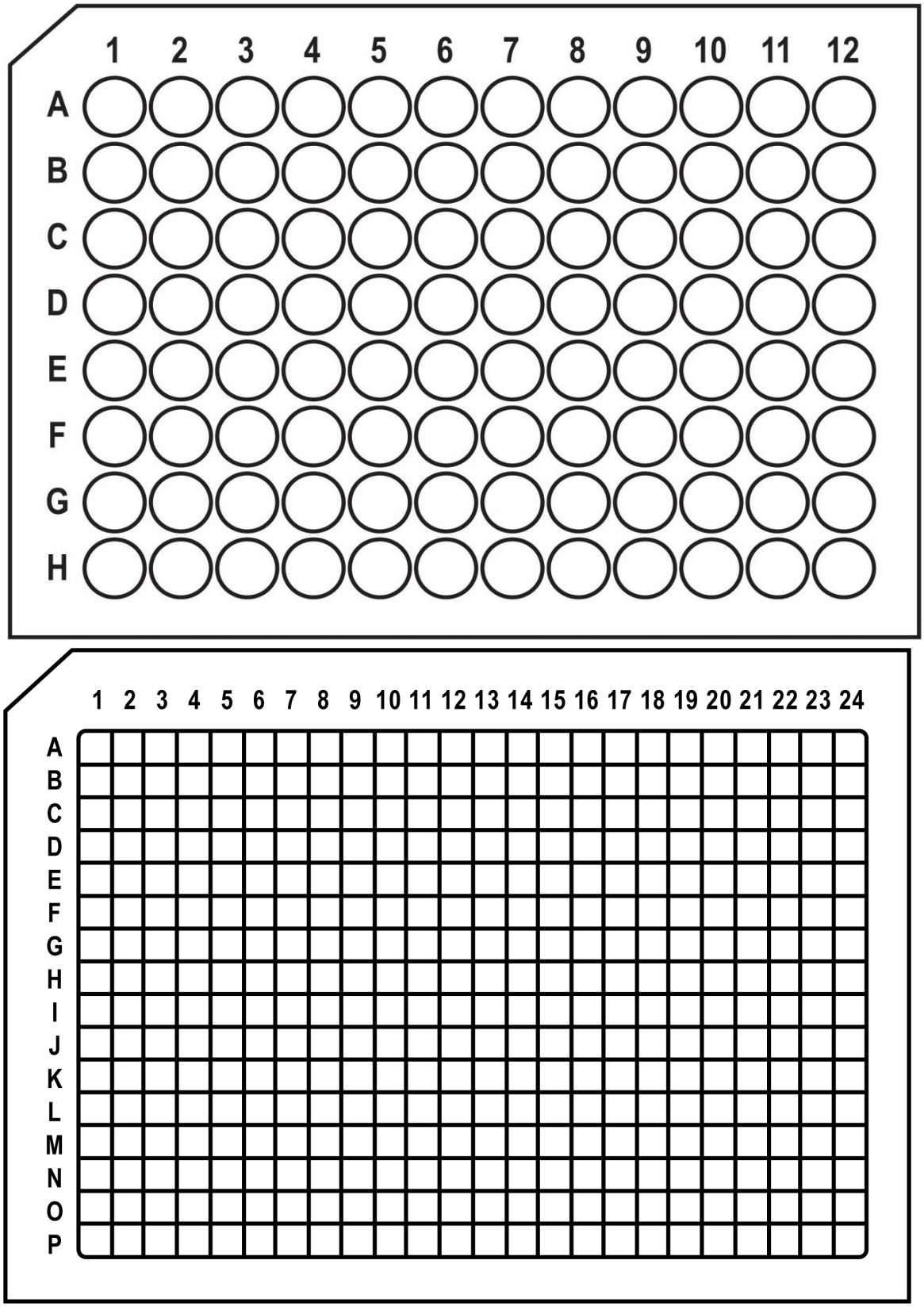

Figure 3. *Plate diagrams. Similar layouts can be created in Excel and in the DISCOVERY WORKBENCH® software.*