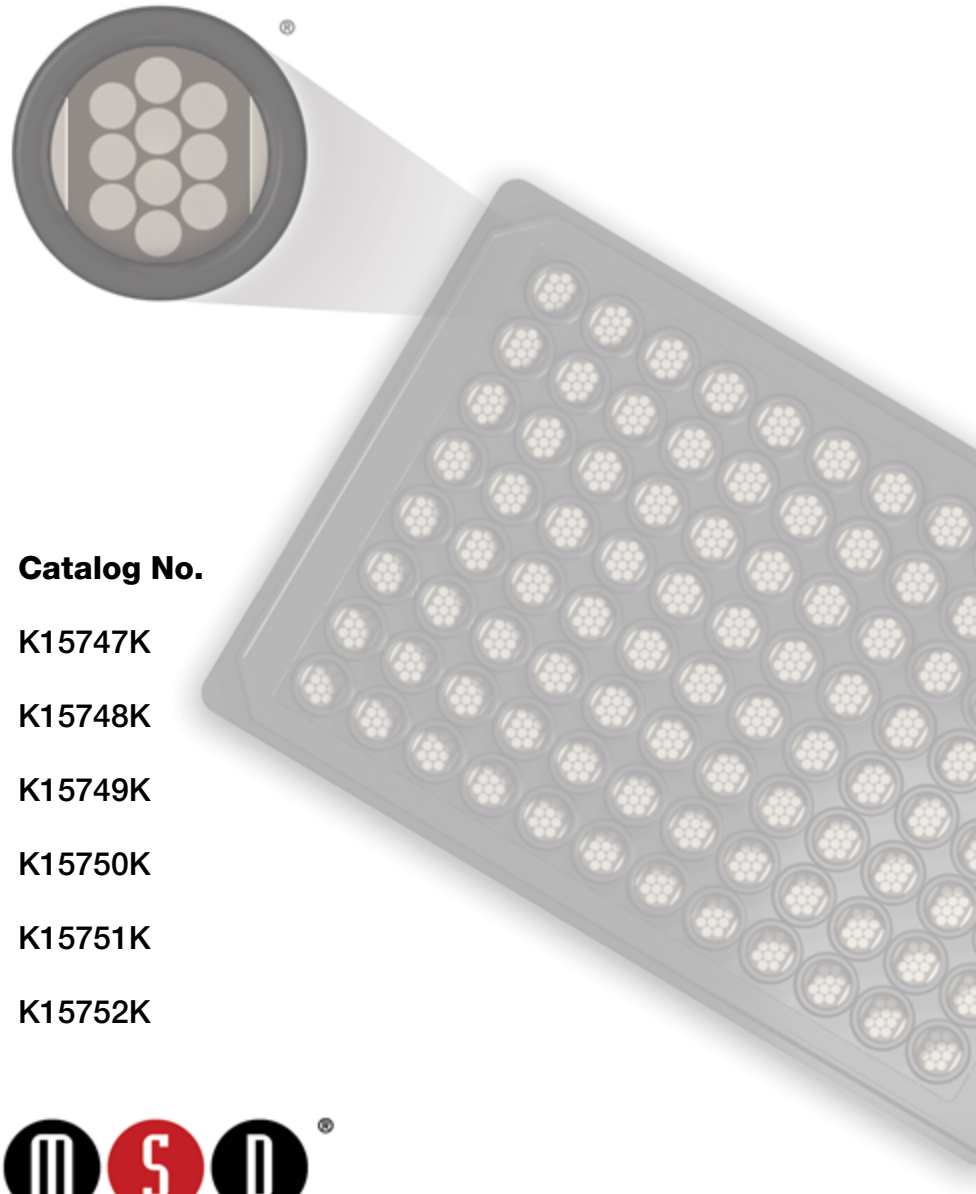
U-PLEX® Preblended Panels



	Catalog No.
Inflammation Panel 1	K15747K
Immune Signaling Panel 1	K15748K
Chemokine Panel 1	K15749K
T-cell Differentiation Panel 1	K15750K
High Dilution Panel 1	K15751K
50-Plex Discovery Panel	K15752K

MSD U-PLEX Platform

U-PLEX Preblended Panels

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

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

MESO SCALE DISCOVERY® U-PLEX Preblended Panels offer the convenience of ready-to-dilute blends of capture antibodies coupled to U-PLEX linkers. Detection antibodies are also provided as a blend for each panel. Preblended Panels feature short assay protocols with minimal hands-on time and reduced storage space requirements.

Analytes Detected by U-PLEX Preblended Panels

The analytes detected by the U-PLEX Preblended Panels are listed in Table 1. The panels can be ordered individually or combined as the 50-Plex Discovery Panel; see *Catalog Numbers* on page 17 for ordering information.

Table 1. *U-PLEX Preblended Panels Analytes*

Inflammation Panel 1	Immune Signaling Panel 1	Chemokine Panel 1	T-cell Differentiation Panel 1	High Dilution Panel 1
IFN-γ	GM-CSF	Eotaxin	IL-12/IL-23p40	BCMA/TNFRSF17
IL-10	IFN-α2a	Eotaxin-3	IL-13	HAVCR2/TIM-3
IL-17A	IFN-β	GRO-α	IL-15	MMP-2
IL-1β	IL-12p70	IL-23	IL-17A/F	MMP-9 (total)
IL-2	IL-18	I-TAC	IL-1α	RANTES
IL-6	IL-1RA	MDC	IL-21	S100A12
IL-8	IL-29/IFN-λ1	MIP-1α	IL-22	Tie-2
IP-10	IL-2Rα	MIP-1β	IL-4	TNF-RI
MCP-1	IL-7	MIP-3α	IL-5	TNF-RII
TNF-α	TSLP	TARC	VEGF-A	YKL-40

Representative data for each U-PLEX Preblended Panel assay is presented in the product-specific datasheets available at the www.mesoscale.com website.

Principle of the Assay

Linker-coupled capture antibodies 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.

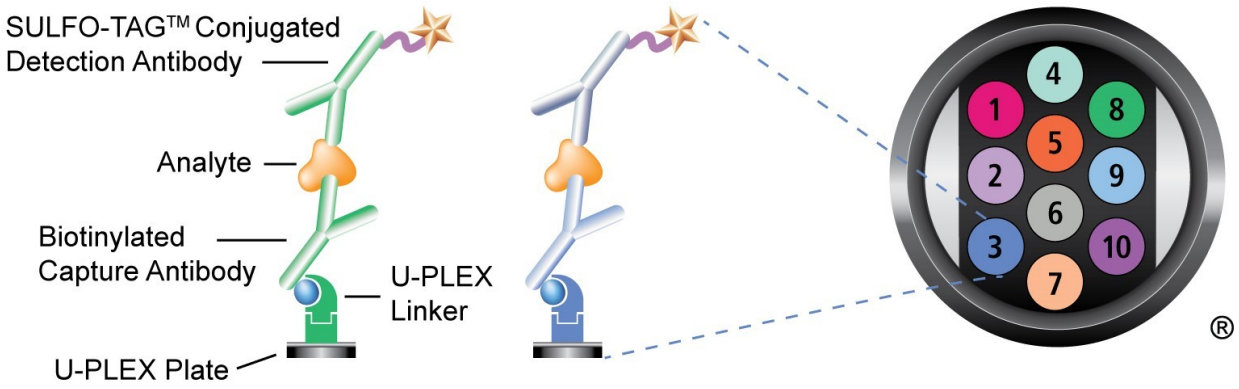

Figure 1. U-PLEX multiplex immunoassay on a U-PLEX 96-well 10-Assay Plate.

Standard Workflow for U-PLEX Preblended Panels

An overview of the steps in the U-PLEX Preblended Panels workflow with incubation durations is outlined below. This is an overview for planning purposes, for the full workflow see *Protocol* on page 10.

Step	Substep	Incubation
Prepare Plate	Coat U-PLEX plate	1 hr
Assay Protocol	Add sample and Calibrator	2 hr
	Add Detection Antibody	1 hr
	Add Read Buffer	No incubation
	Read plate	Read time is dependent on instrument model

Materials and Equipment

This section lists the components provided with the various U-PLEX Preblended Panels. Analyte-specific components are distinct for each panel.

U-PLEX Preblended Panels Assay-Specific Reagents

Table 2. *U-PLEX Preblended Panels assay spot map*

Spot #	Inflammation Panel 1	Immune Signaling Panel 1	Chemokine Panel 1	T-cell Differentiation Panel 1	High Dilution Panel 1
1	IL-2	IFN-β	I-TAC	IL-17A/F	MMP-2
2	IL-6	IL-7	Eotaxin-3	IL-15	BCMA/TNFRSF17
3	TNF-α	IL-12p70	MDC	IL-13	Tie-2
4	IL-8	IL-18	GRO-α	IL-5	YKL-40
5	IL-1β	IL-29/IFN-λ1	MIP-1α	IL-21	HAVCR2/TIM-3
6	IL-17A	IL-2Rα	Eotaxin	IL-1α	MMP-9 (total)
7	IFN-γ	IL-1RA	MIP-3α	IL-22	RANTES
8	IL-10	TSLP	MIP-1β	IL-12/IL-23p40	S100A12
9	IP-10	IFN-α2a	IL-23	VEGF-A	TNF-RI
10	MCP-1	GM-CSF	TARC	IL-4	TNF-RII

The panels can be ordered individually or combined as the 50-Plex Discovery Panel; see *Catalog Numbers* on page 17 for ordering information.

Table 3. *U-PLEX Preblended Panels Detection and Capture Antibody Blends*

Reagents	Storage	Size	Quantity Supplied			Description
			1 Plate	5 Plates	25 Plates	
Capture Antibody Blend	≤–70 °C	1 Plate	1	—	—	20X Capture Antibody Blend for each panel
		5 Plates	—	1	5	
Detection Antibody Blend	2–8 °C	1 Plate	1	—	—	20X Detection Antibody Blend for each panel
		5 Plates	—	1	5	

U-PLEX Plates

U-PLEX plates are provided in a sealed foil pouch with desiccant. The spots correspond to unique U-PLEX Linkers.

U-PLEX Calibrators

Calibrators are lyophilized. Individual analyte concentrations are provided in lot-specific certificates of analysis (COA). Depending on the specific assays requested, one or more of the following Calibrators is provided (Table 4).

Table 4. *Analytes included in the Calibrators available for U-PLEX Preblended Panels.*

Name	Storage	Catalog No.	Analytes
Calibrator 1	2–8 °C	C0060-2	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	Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC
Calibrator 3	2–8 °C	C0062-2	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	CTACK, ENA-78, Fractalkine, I-TAC, MIP-3α, MIP-3β, SDF-1α
Calibrator 6	2–8 °C	C0072-2	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	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	Eotaxin-2, GRO-α, I-309, MCP-2, MCP-3 M-CSF, MIF, MIP-5, TRAIL, YKL-40
Calibrator 22	2–8 °C	C0091-2	BCMA, gp130 (soluble), HAVCR2/TIM-3, Tie-2
Calibrator 29	2–8 °C	C0091-2	ICOS-L/B7-H2, MMP-2, proMMP-9[#], P-Selectin, RANTES, S100A12, TNF-RI, TNF-RII

[#]Analytes used for two assays.

The Calibrators for the U-PLEX Preblended Panels are outlined in Table 5.

Table 5. *U-PLEX Preblended Panels Assay Calibrators*

Reagent	Inflammation Panel 1	Immune Signaling Panel 1	Chemokine Panel 1	T-cell Differentiation Panel 1	High Dilution Panel 1
Calibrators	1, 2	1, 3, 6, 9	2, 4, 6, 10	1, 3, 6	10, 22, 29

Diluents and Buffers

Table 6. Common diluents and buffers that are supplied with U-PLEX Preblended Panels, depending on the specific assay requirements.

Diluents and Buffers	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 58	≤–10 °C	R50CA-1	10 mL	1 bottle	—	—	Diluent for samples and calibrators
		R50CA-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	
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Diluent for samples and calibrators
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

The assay-specific diluents for the U-PLEX Preblended Panels are outlined in Table 7.

Table 7. U-PLEX Preblended Panels Assay-specific Diluents

Reagent	Inflammation Panel 1	Immune Signaling Panel 1	Chemokine Panel 1	T-cell Differentiation Panel 1	High Dilution Panel 1
Assay Diluent	Diluent 57	Diluent 57	Diluent 57	Diluent 57	Diluent 58
Antibody Diluent	Diluent 3	Diluent 3	Diluent 3	Diluent 3	Diluent 3
Sample Diluent	—	—	—	—	Diluent 100

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.
- The standard protocol uses a minimum of 130 mL of 1X Wash Buffer for a 96-well plate. Automated plate washers need higher volumes. Additional MSD Wash Buffer (20X, 100 mL) can be ordered using catalog number R61AA-1.
- Adhesive plate seals.
- Deionized water.
- Vortex mixer.

Instrument Compatibility

MSD offers U-PLEX Assays designed for use on specific instrument platforms (Table 8).

Table 8. Instrument compatibility and read time

Instrument	Assays on U-PLEX 96-well SECTOR™ Plate	Plate Read Time	Assays on U-PLEX 96-well QuickPlex Ultra™ Plates	Plate Read Time
MESO® QuickPlex SQ 120	Y	90 sec	N/A	N/A
MESO QuickPlex® SQ 120MM	Y	90 sec	N/A	N/A
MESO SECTOR® S 600	Y	70 sec	N/A	N/A
MESO SECTOR S 600MM	Y	70 sec	N/A	N/A
MESO QuickPlex Q 60MM	N/A	N/A	Y	83 sec

N/A = not applicable

Safety

Use safe laboratory practices. Wear appropriate personal protective equipment to include 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.

Protocol

This protocol is for U-PLEX 96-well plates.

Best Practices

Read this product insert in its entirety before using this product, and follow the best practices:

Reagent Preparation

Do Not Mix Lots	Do not mix components between boxes of multiplex U-PLEX panels. However, Stop Solution and Read Buffer are not panel- or lot-specific, and can be used interchangeably between different kits.
Thaw Reagents	Equilibrate all assay components to room temperature before use. Mix well before use. Bring plates to room temperature before opening the packet.

Reagent Handling

Avoid Bubbles During Pipetting	Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is very important when adding Read Buffer (just prior to reading the plate).

Plate Handling

Dispensing Fluids	Dispense reagents and wash fluids at the side of the well towards the bottom corner away from the coated spots.
Plate Shaking Guidelines	Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm for 96-well plates. Keep the shaking speed and shaker model consistent for long-term studies.
Use New Plate Seals	Use a new adhesive plate seal for all incubation steps. Avoid re-using plate seals to prevent contamination.
Washing Fluid Removal	Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
Do Not Dry After Washing	Avoid excessive drying of the plate after 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.

Plate Reading

Remove Plate Seal	Remove the plate seal before reading the plate in the instrument.
Do Not Shake Plate	Do not shake the plate after adding Read Buffer.
Timing of Plate Reads	Keep time intervals consistent between the addition of Read Buffer and reading the plate to improve interplate precision. Prepare an MSD instrument before adding Read Buffer.
Plate Barcode	Do not obscure or damage the plate barcode. The barcode is required for the MSD instrument.

Working with Multiple Preblended Panels

Each panel contains components that are intended to be run together. Each panel has a unique Calibrator curve.

Working with Partial Plates

Volumes should be adjusted proportionally when preparing reagents for partial plates.

When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. Partially used plates not coated with capture blends may be sealed and stored for up to 30 days at 2–8 °C in the original foil pouch with desiccant. Ensure that only the sectors in use are selected when reading the plate.

Reagent Preparation

IMPORTANT

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

Coat U-PLEX Plate

The preparation of a U-PLEX plate involves coating the provided plate with Linker-coupled capture antibodies.

IMPORTANT

Thaw and dilute linker-coupled capture antibodies immediately before use. Minimize the time linker-coupled capture antibodies are unfrozen.

- ❑ 1. Dilute the 1X Capture Antibody Solution by adding 300 µL of 20X Capture Antibody Blend to 5.7 mL of Stop Solution.
- ❑ 2. Add 50 µL of the 1X Capture Antibody Solution to each well.
  Seal the plate with an adhesive plate seal.
  Incubate at room temperature while shaking for 1 hour.
- ❑ 3. Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.

SAFE STOPPING POINT

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

Prepare Calibrator Standards

This section describes the preparation of the Calibrator Standards.

If you are performing plate preparation for more than one plate, adjust the volumes accordingly.

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

Use reconstituted or thawed Calibrators immediately. If storage is necessary, divide Calibrators into suitably sized aliquots (100 µL aliquots are recommended) and store immediately at ≤–70 °C.

❑ 2. Prepare Calibrator Standard 1 (top of the standard curve) in a polypropylene tube by mixing and diluting the reconstituted Calibrator as indicated in Table 9.

Mix by vortexing.

Table 9. *Combining Calibrators to generate the Calibrator Standard 1*

No. of Plates	No. of Calibrator Blends Provided	Volume of Each Reconstituted Calibrator (µL)	Assay Diluent (µL)	Total volume (µL)
1	N	25 each	250-(25 x N)	250
5	N	75 each	750-(75 x N)	750

❑ 3. Prepare the subsequent 6 dilutions for the curve (4-fold serial dilutions) in Assay Diluent:

- For 1 plate, see Table 10 and Figure 2.
- For 5 plates, see Table 11 and Figure 3.

☐ Mix by vortexing the tubes between each serial dilution.

☐ Use Assay Diluent for the Calibrator Standard 8 (zero Calibrator/blank).

Table 10. *Serial dilution to generate the standard curve for 1 plate*

Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Reconstituted Calibrator (µL)	Assay Diluent (µL)	Total volume (µL)
1	1	Calibrator Standard 1 (top of curve)	See Table 9		
2	2	From tube 1	50	150	200
3	3	From tube 2	50	150	200
4	4	From tube 3	50	150	200
5	5	From tube 4	50	150	200
6	6	From tube 5	50	150	200
7	7	From tube 6	50	150	200
8 (zero Calibrator)	8	—	0	200	200

Dash (—) = not applicable

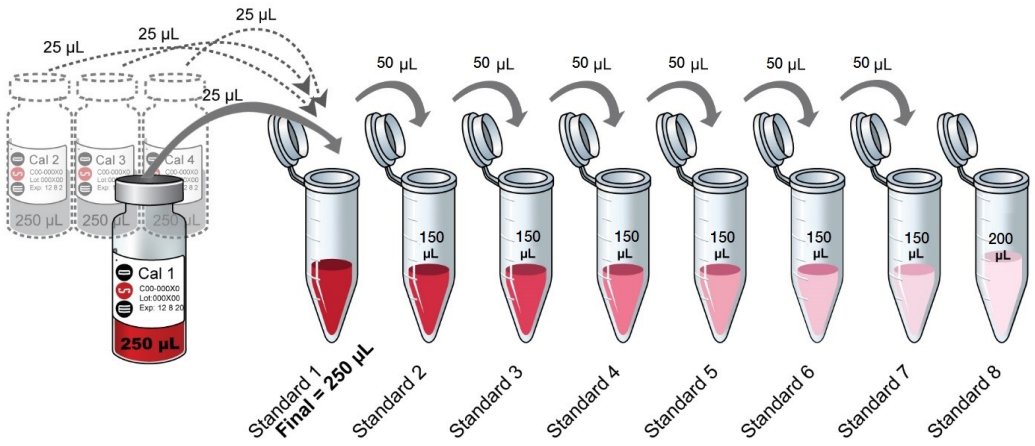

Figure 2. *Dilution schema for preparation of Calibrator Standards for a single U-PLEX assay plate.*

Table 11. *Serial dilution to generate the standard curve for 5 plates*

Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Reconstituted Calibrator (µL)	Assay Diluent (µL)	Total volume (µL)
1	1	Calibrator Standard 1 (top of curve)	See Table 9		
2	2	From tube 1	200	600	800
3	3	From tube 2	200	600	800
4	4	From tube 3	200	600	800
5	5	From tube 4	200	600	800
6	6	From tube 5	200	600	800
7	7	From tube 6	200	600	800
8 (zero Calibrator)	8	—	0	800	800

Dash (—) = not applicable

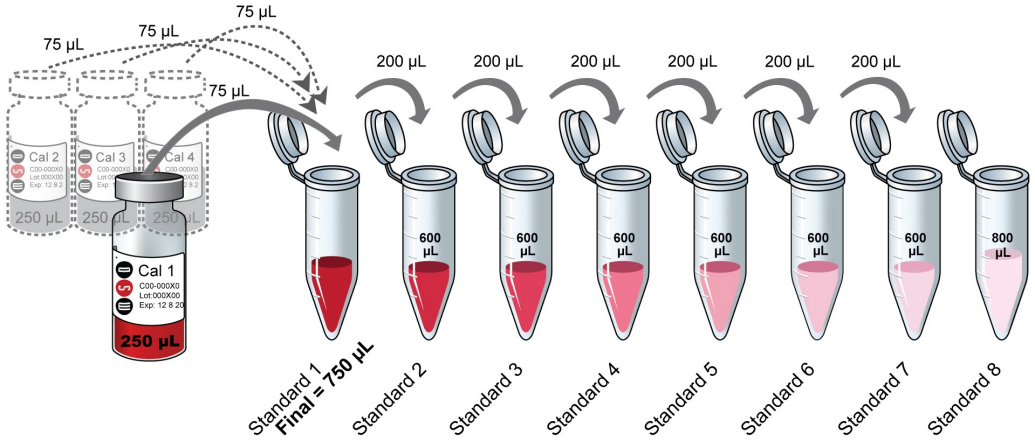

Figure 3. *Dilution schema for preparation of Calibrator Standards for 5 U-PLEX assay plates.*

Dilute Samples

Dilute samples for most panels two-fold using panel-specific Assay Diluent (see Table 7 on page 8). It is recommended that samples to be run in the High Dilution Panel 1 be diluted 100-fold in Diluent 100. The dilution factor for each sample type may need to be optimized. Consult MSD Technical Support if assistance or additional information is required.

Prepare Detection Antibody Solution

The Detection Antibody Blend is provided as a 20X stock solution. The working solution for 96-well plates is 1X. Prepare the 1X Detection Antibody Solution immediately before use.

For one plate, combine:

- 300 µL of 20X Detection Antibody Solution
- 5.7 mL of Diluent 3 to bring the final volume to 6 mL

> **i** If you are preparing for more than one plate or a partial plate, adjust the volumes accordingly.

Prepare Wash Buffer

Prepare a 1X working solution by diluting the 20X stock with deionized water.

Read Buffer

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

Assay Protocol

This section describes the assay protocol. Perform the *Reagent Preparation* on page 11 before beginning this protocol.

STEP 1: Add Sample and Calibrator

❑ 1. Add 50 µL of the prepared Calibrator 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

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

❑ 3. Add 50 µL of 1X 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

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

❑ 5. Add 150 µL of MSD GOLD Read Buffer B to each well.
Read the plate on an MSD instrument. Incubation in Read Buffer is not required before reading the plate.

Alternate Protocols

The following alternative protocols may be considered.

IMPORTANT

The use of an alternate protocol may result in sample concentrations that vary from concentrations obtained with the standard protocol. MSD recommends using the same protocol for the entirety of a research project. Note that alternate protocols should be tested with representative samples before using for the entirety of the study.

- **Alternate Protocol 1, Co-incubation**: Adding samples and 1X Detection Antibody Solution without a wash before addition of the Detection Antibody Solution may improve the sensitivity for some assays.
- **Alternate Protocol 2, Reduced Wash**: For cell culture supernatants, Alternate Protocol 1 can be further simplified by reducing the number of washes at each wash step from 3 to 2.
- **Alternate Protocol 3, Overnight Incubation**: Samples can be incubated overnight at 4- 8° C if required. First shake the plate for 15 minutes at room temperature if not shaking during the overnight incubation period. Bring the plate to room temperature before proceeding with the next steps.
- **Alternate Protocol 4, Limited Sample Volume**: Sample volume can be decreased to 30 µL if the available sample volume is limited. Use the same volume of sample and Calibrator.

Additional Information

Plate Diagram

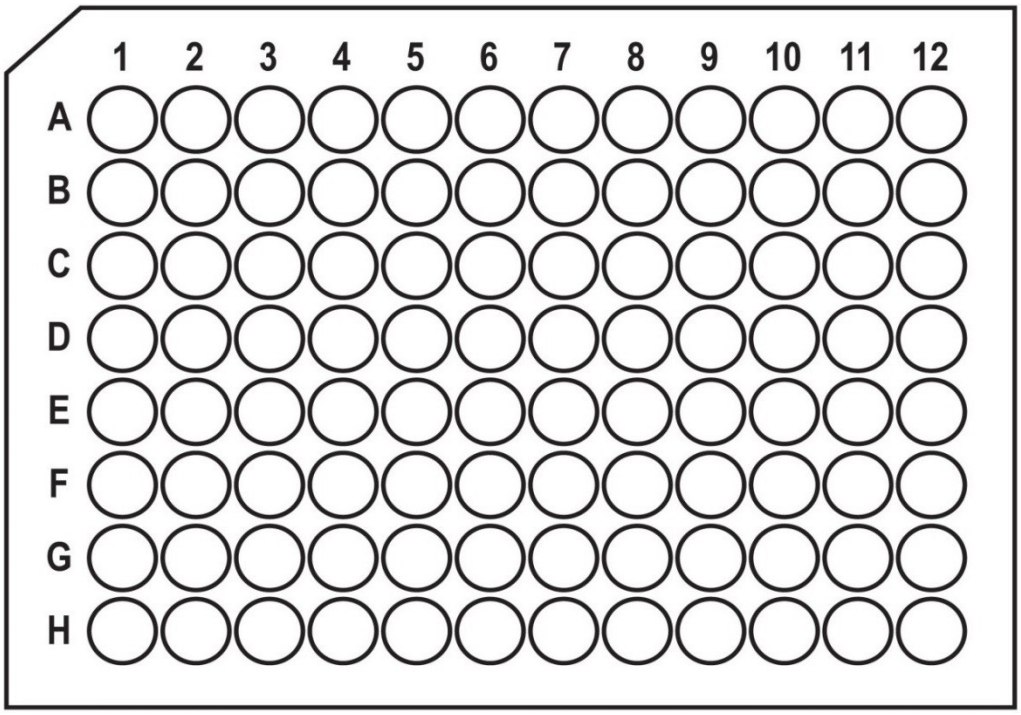

Figure 4. Plate diagram.

Recommended Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL-01		Sample-01		Sample-09		Sample-17		Sample-25		Sample-33	
B	CAL-02		Sample-02		Sample-10		Sample-18		Sample-26		Sample-34	
C	CAL-03		Sample-03		Sample-11		Sample-19		Sample-27		Sample-35	
D	CAL-04		Sample-04		Sample-12		Sample-20		Sample-28		Sample-36	
E	CAL-05		Sample-05		Sample-13		Sample-21		Sample-29		Sample-37	
F	CAL-06		Sample-06		Sample-14		Sample-22		Sample-30		Sample-38	
G	CAL-07		Sample-07		Sample-15		Sample-23		Sample-31		Sample-39	
H	CAL-08		Sample-08		Sample-16		Sample-24		Sample-32		Sample-40	

Figure 5. Recommended plate layout for the assay. Each sample and calibrator is measured in duplicate in side-by-side wells.

Catalog Numbers

Table 12. Catalog numbers associated with the U-PLEX Preblended Panels

Kit Name	SECTOR Plate			QuickPlex Ultra Plate		
	1-Plate Kit	5-Plate Kit	25-Plate Kit	1-Plate Kit	5-Plate Kit	25-Plate Kit
Inflammation Panel 1	K15747K-1	K15747K-2	K15747K-4	K15747K-21	K15747K-22	K15747K-24
Immune Signaling Panel 1	K15748K-1	K15748K-2	K15748K-4	K15748K-21	K15748K-22	K15748K-24
Chemokine Panel 1	K15749K-1	K15749K-2	K15749K-4	K15749K-21	K15749K-22	K15749K-24
T-cell Differentiation Panel 1	K15750K-1	K15750K-2	K15750K-4	K15750K-21	K15750K-22	K15750K-24
High Dilution Panel 1	K15751K-1	K15751K-2	K15751K-4	K15751K-21	K15751K-22	K15751K-24
50-Plex Discovery Panel	K15752K-1	K15752K-2	K15752K-4	K15752K-21	K15752K-22	K15752K-24

Table 13. Instrument compatibility for each plate type

Plate Type	Instrument Compatibility
SECTOR™ Plate	MESO SECTOR S 600, MESO SECTOR S 600MM, MESO QuickPlex SQ 120, MESO QuickPlex SQ 120MM
QuickPlex Ultra Plate	MESO QuickPlex Q 60MM