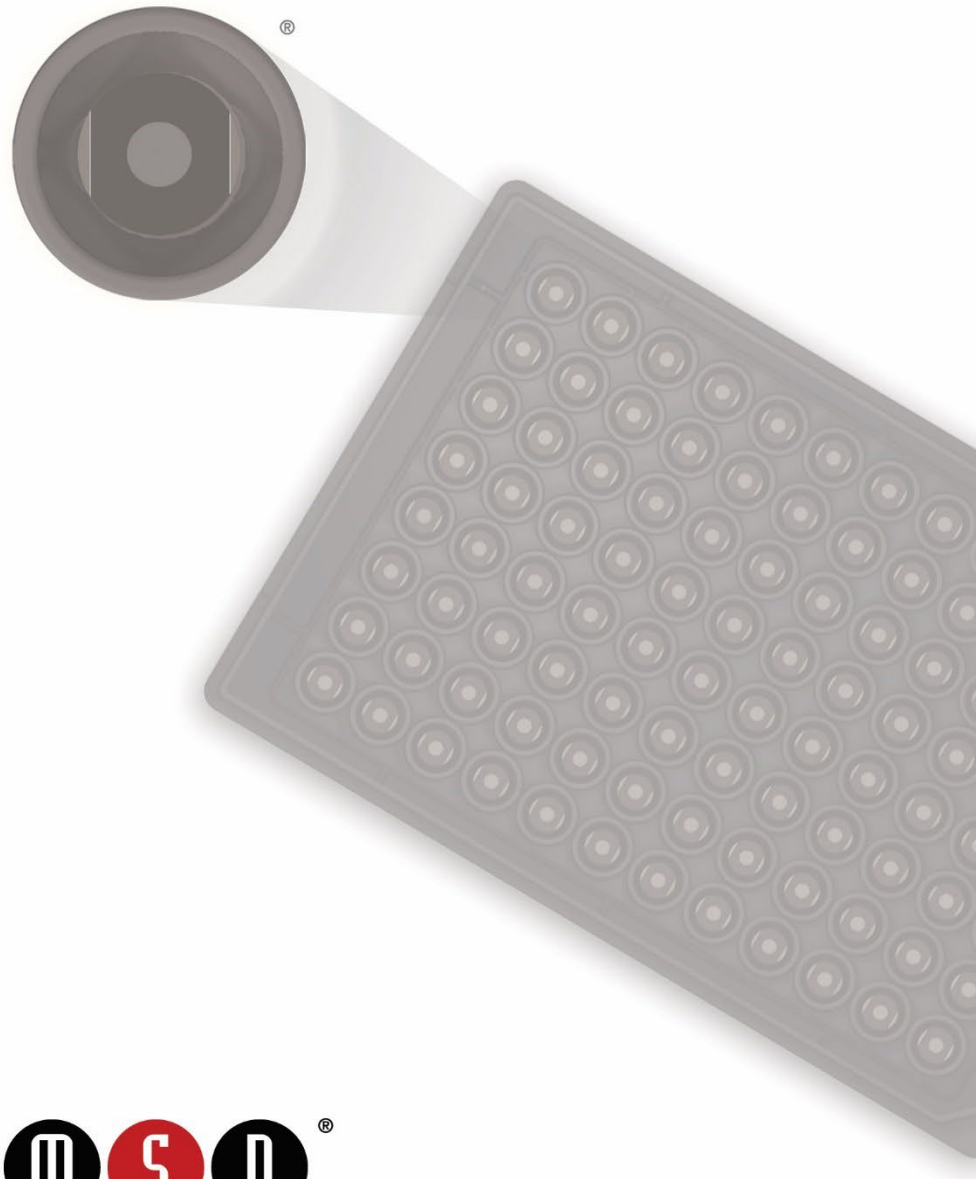
U-PLEX® Biomarker Group 3 (Human)

Singleplex Assays



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MSD U-PLEX Platform

U-PLEX Biomarker Group 3 (Human) Singleplex Assays

For use with EDTA plasma and serum.

Catalog numbers of U-PLEX Biomarker Group 3 (human) Singleplex Assays are provided in Table 12 on page 17.

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 3 (human) contains 21 analytes (Table 1) that are important in many biological processes.

A representative data set for each of the assays in U-PLEX Biomarker Group 3 (human) is presented in the product-specific datasheets available at www.mesoscale.com/datasheets.

Table 1. Assays in U-PLEX Biomarker Group 3 (human) should use this singleplex product insert

Assays		
A2M	Complement factor D	RBP4
Adiponectin	CRP	SAA
ApoA1	Cystatin C	Serpin A1
ApoC3	DPPIV	SHBG
CA1	Factor VII	sTfR-1
Clusterin	ICAM-1	VCAM-1
Complement C9	NGAL/LCN2	vWF

Principle of the Assay

Singleplex assays are supplied on MSD GOLD™ Small Spot Streptavidin 96-well or MSD Streptavidin 384-well plates.

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 labeled with electrochemiluminescent labels (MSD GOLD SULFO-TAG™) bind to the analytes to complete the sandwich immunoassay (Figure 1). Once the 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.

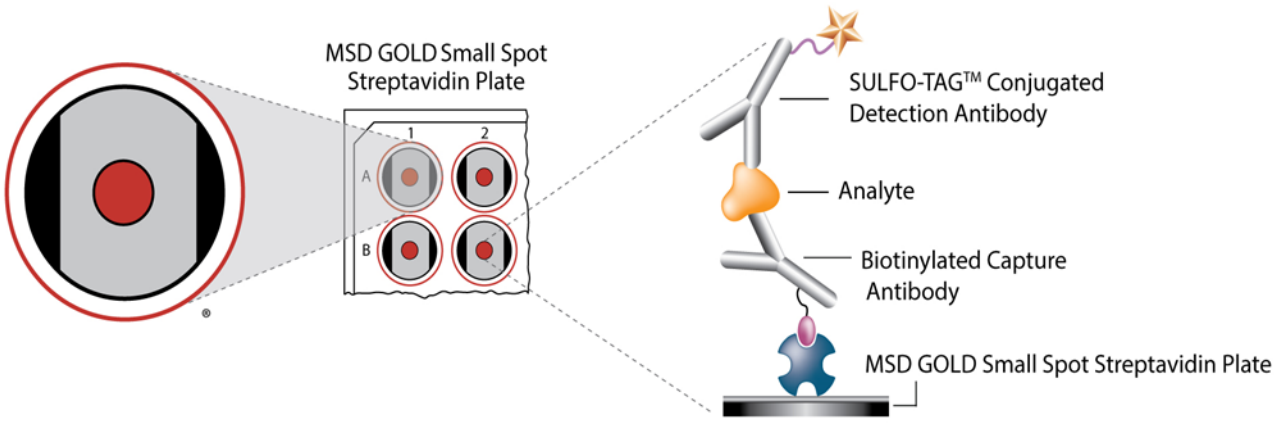

Figure 1. U-PLEX singleplex assay on an MSD GOLD 96-well Small Spot Streptavidin Plate. The 384-well assay is similar.

Components

Table 2 and Table 3 list the components provided with U-PLEX Biomarker Group 3 (human) Singleplex Assays.

Reagents Supplied with U-PLEX Singleplex Assays

Table 2. Reagents that are supplied with all U-PLEX Biomarker Group 3 (human) Singleplex Assays

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 plate	5 plates	25 plates	
MSD GOLD 96-Well Small Spot Streptavidin SECTOR Plate	2–8 °C	L45SA-1	—	1 plate	5 plates	25 plates	96-well plate, foil sealed, with desiccant
MSD GOLD 96-Well Small Spot Streptavidin QuickPlex Plate		L4BSA-1					
Diluent 100	2–8 °C	R50AA-2	200 mL	1 bottle	3 bottles	3 bottles	Diluent for capture antibody and samples
		R50AA-3	1,000 mL	—	—	2 bottles	
Diluent 12	≤–10 °C	R50JA-3	50 mL	1 bottle	3 bottles	—	Diluent for samples and Calibrator
		R50JA-2	200 mL	—	—	3 bottles	
Diluent 11	≤–10 °C	R55BA-5	10 mL	1 bottle	—	—	Diluent for detection antibody
		R55BA-3	50 mL	—	1 bottle	5 bottles	
MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent reaction
		R60AM-2	90 mL	—	1 bottle	5 bottles	

Dash (—) = not applicable
RT = room temperature

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

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

Dash (—) = not applicable
RT = room temperature

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 (Table 4). A complete list of all Antibody Sets available for U-PLEX Biomarker Group 3 (human) and their respective catalog numbers is provided in the Appendix (Table 13).

Table 4. Contents of U-PLEX Antibody Sets

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

Dash (—) = not applicable

Calibrators

Calibrators (Table 5) 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). Depending on the specific assays requested, one or more of the following Calibrators will be provided.

Table 5. Analytes included in the Calibrator blends available for U-PLEX Biomarker Group 3 (human)

Name	Storage	Catalog No.	Size	Quantity Supplied			Analytes
				1 Plate	5 Plates	25 Plates	
Calibrator 24	2–8 °C	C0351-2	1 vial	1 vial	5 vials	25 vials	DPPIV, ICAM-1, SAA, SHBG, VCAM-1
Calibrator 25	2–8 °C	C0352-2	1 vial	1 vial	5 vials	25 vials	CA1, Complement factor D, CRP, Cystatin C, Factor VII, NGAL/LCN2, sTfR-1
Calibrator 26	2–8 °C	C0353-2	1 vial	1 vial	5 vials	25 vials	A2M, Adiponectin, ApoA1, ApoC3, Complement C9, RBP4, Serpin A1
Human Clusterin Calibrator	≤–70 °C	C01B9-2	1 vial	1 vial	5 vials	25 vials	Clusterin
Human vWF Calibrator	2–8 °C	C01C9-2	1 vial	1 vial	5 vials	25 vials	vWF

Instrument Compatibility

MSD offers U-PLEX 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 or 384-well microtiter plate
- ❑ Plate-washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm (1,500 rpm for 384-well plates)
- ❑ MSD® Wash Buffer (20X, 100 mL, catalog number R61AA-1) for plate washing.
    - The standard protocol uses a minimum of 415 mL of 1X Wash Buffer for a 384-well plate and 130 mL for a 96-well plate. Automated plate washers may need overage added to these volumes.
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer
- ❑ Diluent 100 (catalog number R50AA) may be needed to dilute samples.

Safety

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

Additional product-specific safety information is available in the applicable safety data sheet(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, prewet 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 it from airflow and add solutions to the plate as soon as possible.
- Use a new adhesive plate seal for all incubation steps. Avoid reusing 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.
- Aliquot and freeze Diluent 100 to prevent contamination after opening.

Reagent Preparation

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

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

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

Coat 96-well Plate

Coat the plate accordingly:

- ❑ 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 the wells 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 or at 2–8 °C overnight.
- ❑ 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. Plates 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 (Figure 2; Table 7).

For Lyophilized Calibrators

Bring the Calibrator vial that is provided to room temperature. Reconstitute each vial of Calibrator by adding 250 µL of Assay Diluent Working Solution to the glass vial. This will result in a 10X concentrated stock of each Calibrator, which will need to be diluted 10-fold (Figure 2; Table 7) to generate the highest point in the standard curve (i.e., Calibrator Standard 1). Invert the reconstituted Calibrator at least 3 times. Do not vortex. Let the reconstituted solution equilibrate at room temperature for 15–30 minutes and then vortex briefly. The Calibrator is now ready for use. Keep the dilutions at room temperature.

For Liquid Calibrators:

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

Note: We recommend that reconstituted 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 (see 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 25 µL of the reconstituted or thawed Calibrator to 225 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.

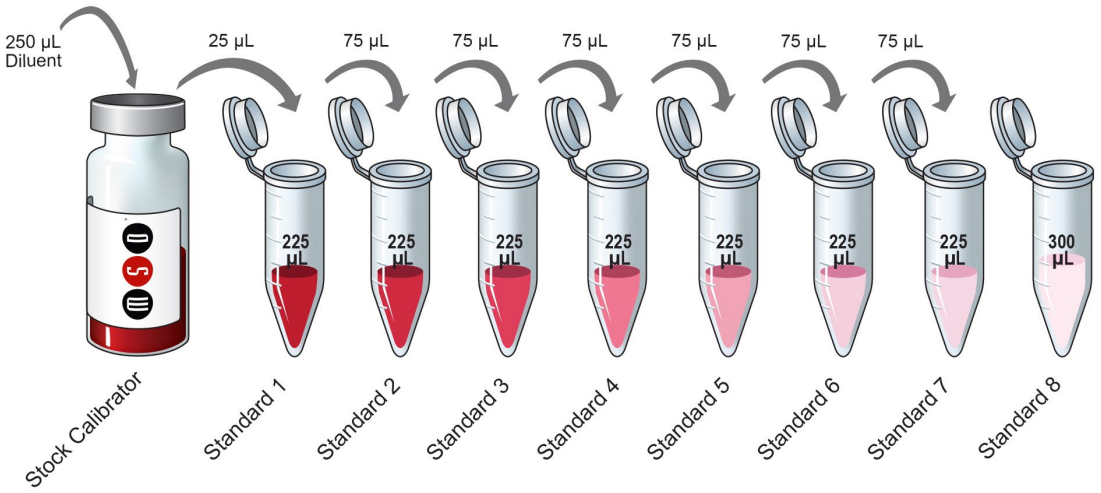

Figure 2. Dilution schema for Calibrator standards for U-PLEX Biomarker Group 3 (human) Singleplex Assays.

Table 7. Serial dilution to generate the standard curve

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

Dash (—) = not applicable

Sample Dilution, 4,000-fold

Based on in-house testing of normal samples, a 4,000-fold dilution is recommended for CA1, Clusterin, Complement factor D, CRP, Cystatin C, DPPIV, Factor VII, ICAM-1, NGAL/LCN2, SAA, SHBG, sTfR-1, VCAM-1 and vWF before loading onto the plate (Table 8). See Table 5 for recommended Calibrators.

Table 8. Dilute samples 4,000-fold

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

Dash (—) = not applicable

A two-step dilution procedure is encouraged. First, dilute the sample 100-fold by adding 10 µL of samples to 990 µL of Diluent 100. Dilute the sample one more time 40-fold by adding 10 µL of diluted sample to 390 µL of Diluent 12. The sample is now diluted 4,000-fold (Figure 3; Table 9).

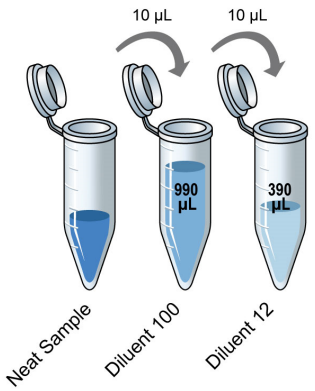

Figure 3. Dilution schema for preparation of samples diluted 4,000-fold.

Table 9. Dilution for optimal sample analysis

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

Sample Dilution, 200,000-fold

Based on in-house testing of normal samples, a 200,000-fold dilution is recommended for A2M, Adiponectin, Apo1, ApoC3, Complement 9, RBP4, and SerpinA1 before loading onto the plate (Table 10). See Table 5 for recommended Calibrators.

Table 10. Dilute samples 200,000-fold

Analytes			
A2M	Adiponectin	Apo1	ApoC3
Complement C9	RBP4	Serpin A1	—

Dash (—) = not applicable

A three-step dilution procedure is encouraged. First, dilute the sample 100-fold by adding 10 µL of sample to 990 µL of Diluent 100. Dilute the sample again 100-fold by adding 10 µL of diluted sample to 990 µL of Diluent 100. Dilute the sample one more time 20-fold by adding 10 µL of diluted sample to 190 µL. The sample is now diluted 200,000-fold (Figure 4; Table 11).

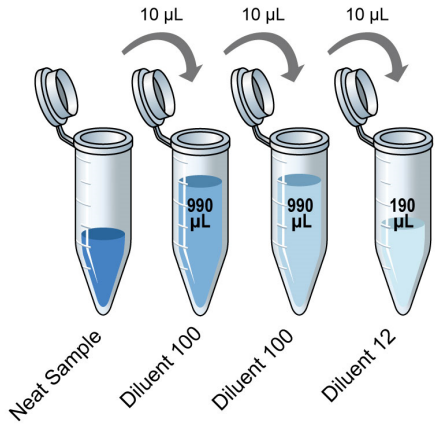

Figure 4. Dilution schema for preparation of samples diluted 200,000-fold.

Table 11. Dilution for optimal sample analysis

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

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X for 96-well plates 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 11 (11.94 mL for 384-well assays)

Wash Buffer

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

Read Buffer

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

Assay Protocols

Note: Follow Reagent Preparation before beginning this assay protocol.

96-well Plate Assays

STEP 1: Add Samples and Calibrators

- ❑ Add 50 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 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 90 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of the prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 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 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, Co-incubation:** Co-incubating samples with detection antibody solution may improve the sensitivity for some assays. Note that the use of the co-incubation protocol may result in sample concentrations that vary from concentrations obtained with the standard protocol. If this protocol is followed, we recommend that this protocol be used for the entirety of the research project.
- ❑ **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. These 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 Biomarker Group 3 (human) Singleplex Assays

Assays (Table 12) include Antibody Sets, plates, diluents, Calibrators, and MSD GOLD Read Buffer B.

Table 12. Catalog numbers of U-PLEX Biomarker Group 3 (human) 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 Human A2M Assay	K151Q9K-1/-2/-4	K151Q9K-21/-22/-24	K251Q9K-2/-4
U-PLEX Human Adiponectin Assay	K151R9K-1/-2/-4	K151R9K-21/-22/-24	K251R9K-2/-4
U-PLEX Human ApoA1 Assay	K151S9K-1/-2/-4	K151S9K-21/-22/-24	K251S9K-2/-4
U-PLEX Human ApoC3 Assay	K151T9K-1/-2/-4	K151T9K-21/-22/-24	K251T9K-2/-4
U-PLEX Human CA1 Assay	K151J9K-1/-2/-4	K151J9K-21/-22/-24	K251J9K-2/-4
U-PLEX Human Clusterin Assay	K151B9K-1/-2/-4	K151B9K-21/-22/-24	K251B9K-2/-4
U-PLEX Human Complement C9 Assay	K151U9K-1/-2/-4	K151U9K-21/-22/-24	K251U9K-2/-4
U-PLEX Human Complement Factor	K151K9K-1/-2/-4	K151K9K-21/-22/-24	K251K9K-2/-4
U-PLEX Human CRP Assay	K151L9K-1/-2/-4	K151L9K-21/-22/-24	K251L9K-2/-4
U-PLEX Human Cystatin C Assay	K151M9K-1/-2/-4	K151M9K-21/-22/-24	K251M9K-2/-4
U-PLEX Human DPPIV Assay	K151D9K-1/-2/-4	K151D9K-21/-22/-24	K251D9K-2/-4
U-PLEX Human Factor VII Assay	K151N9K-1/-2/-4	K151N9K-21/-22/-24	K251N9K-2/-4
U-PLEX Human ICAM-1 Assay	K151E9K-1/-2/-4	K151E9K-21/-22/-24	K251E9K-2/-4
U-PLEX Human NGAL/LCN2 Assay	K151Z1K-1/-2/-4	K151Z1K-21/-22/-24	K251Z1K-2/-4
U-PLEX Human RBP4 Assay	K151V9K-1/-2/-4	K151V9K-21/-22/-24	K251V9K-2/-4
U-PLEX Human SAA Assay	K151F9K-1/-2/-4	K151F9K-21/-22/-24	K251F9K-2/-4
U-PLEX Human Serpin A1 Assay	K151W9K-1/-2/-4	K151W9K-21/-22/-24	K251W9K-2/-4
U-PLEX Human SHBG Assay	K151G9K-1/-2/-4	K151G9K-21/-22/-24	K251G9K-2/-4
U-PLEX Human sTfR-1 Assay	K151P9K-1/-2/-4	K151P9K-21/-22/-24	K251P9K-2/-4
U-PLEX Human VCAM-1 Assay	K151H9K-1/-2/-4	K151H9K-21/-22/-24	K251H9K-2/-4
U-PLEX Human vWF Assay	K151C9K-1/-2/-4	K151C9K-21/-22/-24	K251C9K-2/-4

U-PLEX Biomarker Group 3 (human) Antibody Sets

Antibody Sets (Table 13) include a biotinylated capture antibody and a SULFO-TAG conjugated detection antibody.

Table 13. Catalog numbers of Antibody Sets available for U-PLEX Biomarker Group 3 (human)

Product	Catalog Number (1/5 Plate Size)
U-PLEX Human A2M Antibody Set	B21Q9-2/-3
U-PLEX Human Adiponectin Antibody Set	B21R9-2/-3
U-PLEX Human ApoA1 Antibody Set	B21S9-2/-3
U-PLEX Human ApoC3 Antibody Set	B21T9-2/-3
U-PLEX Human CA1 Antibody Set	B21J9-2/-3
U-PLEX Human Clusterin Antibody Set	B21B9-2/-3
U-PLEX Human Complement C9 Antibody Set	B21U9-2/-3
U-PLEX Human Complement factor D Antibody Set	B21K9-2/-3
U-PLEX Human CRP Antibody Set	B21L9-2/-3
U-PLEX Human Cystatin C Antibody Set	B21M9-2/-3
U-PLEX Human DPPIV Antibody Set	B21D9-2/-3
U-PLEX Human Factor VII Antibody Set	B21N9-2/-3
U-PLEX Human ICAM-1 Antibody Set	B21E9-2/-3
U-PLEX Human NGAL/LCN2 Antibody Set	B21Z1-2/-3
U-PLEX Human RBP4 Antibody Set	B21V9-2/-3
U-PLEX Human SAA Antibody Set	B21F9-2/-3
U-PLEX Human Serpin A1 Antibody Set	B21W9-2/-3
U-PLEX Human SHBG Antibody Set	B21G9-2/-3
U-PLEX Human sTfR-1 Antibody Set	B21P9-2/-3
U-PLEX Human VCAM-1 Antibody Set	B21H9-2/-3
U-PLEX Human vWF Antibody Set	B21C9-2/-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. Alternatively, you can also shake the plate overnight while incubating it at 2–8 °C.
- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use.

96-well Assay Protocol

STEP 1: Add Samples and Calibrators

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

STEP 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

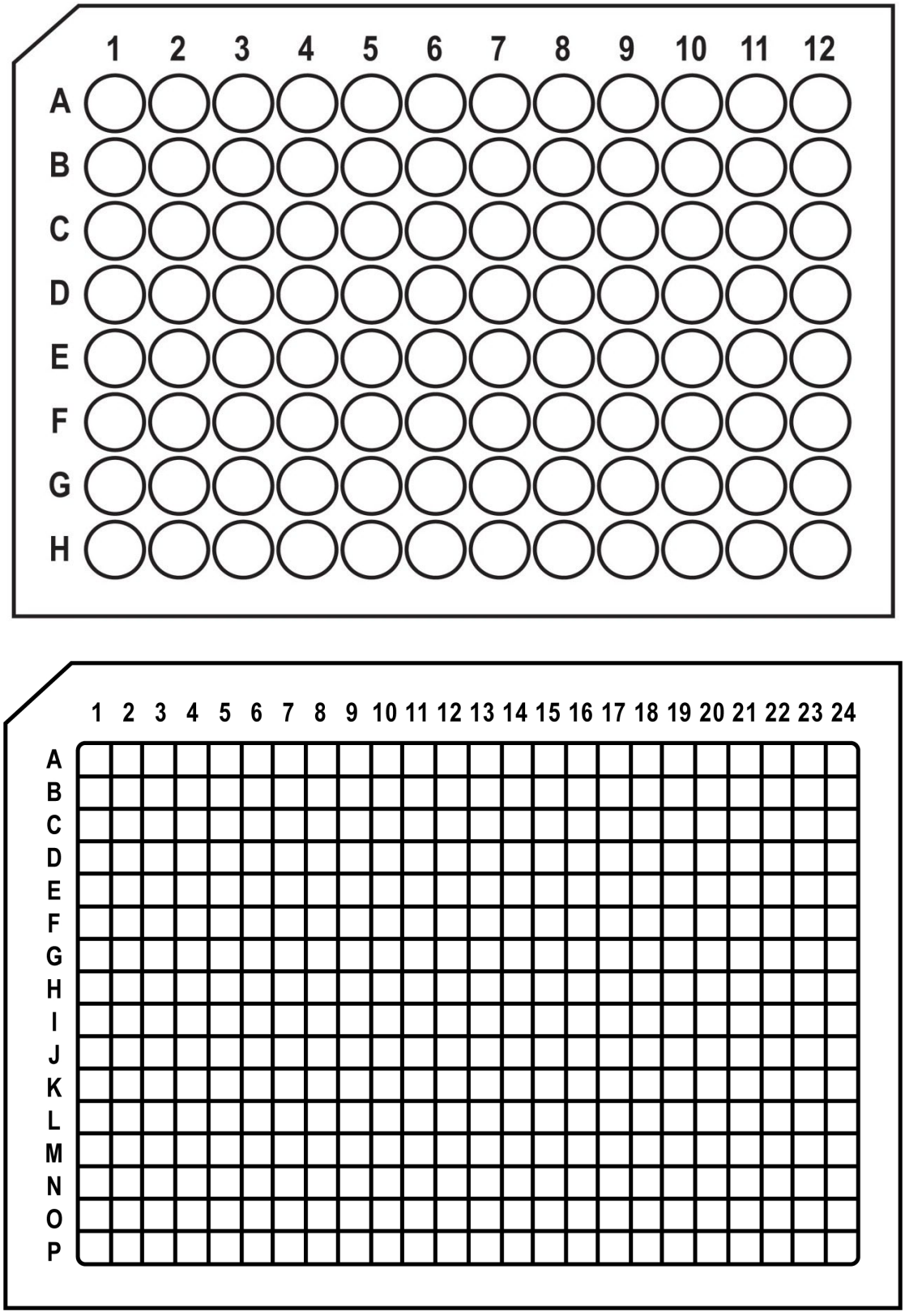

Figure 5. Plate diagrams. Similar plate layouts can be created in Excel and easily imported into DISCOVERY WORKBENCH® software.