R-PLEX® Antibody Sets
Singleplex Assays

MSD R-PLEX Platform

R-PLEX Antibody Sets
Singleplex Assays

Use for the development of R-PLEX singleplex assays.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Table of Contents

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Introduction

The MESO SCALE DISCOVERY® R-PLEX platform provides an expanded menu of electrochemiluminescence assays for biomarker discovery and development.

R-PLEX Antibody Sets include a matched biotinylated capture and SULFO-TAG™ conjugated detection antibody pair and a calibrator for the quick and easy development of highly sensitive immunoassays on MSD instruments. Diluents, plates, and MSD Read Buffers are available separately.

For greater ordering convenience, R-PLEX Assays include an Antibody Set, its primary assay and antibody diluents, MSD GOLD™ Read Buffer B, and either SECTOR™ or QuickPlex Ultra™ plates. Collectively, they provide a complete set of components required to develop an MSD immunoassay.

R-PLEX enables the development of singleplex immunoassays on:

- MSD GOLD 96-well Small Spot Streptavidin SECTOR Plates for use on MESO® SECTOR S 600, MESO SECTOR® S 600MM, MESO QuickPlex® SQ 120, and MESO QuickPlex SQ 120MM instruments.
- 96-well Small Spot Streptavidin QuickPlex Ultra plates for use on the MESO QuickPlex Q 60MM instrument.

This product insert provides the details for developing a singleplex assay on the MSD platform. R-PLEX Antibody Sets can also be used to develop multiplex immunoassays. See www.mesoscale.com/en/products_and_services/assay_kits/r-plex for more details.

During development, MSD observed improved performance for certain groups of assays when following a modified procedure. Therefore, four different assay protocols are provided in this product insert. Refer to Table 1 to determine the optimal assay protocol for your R-PLEX assay.

- Assay Protocol 1—R-PLEX standard procedure (used for most assays)
- Assay Protocol 2—requires coincubation of Calibrator or samples and detection antibody (or tracer) for 2 hours on capture-coated plate
- Assay Protocol 3—uses 50 µL/well of Calibrator or sample and requires 1X MSD Tris Wash Buffer to wash the plate
- Assay Protocol 4—some R-PLEX assays come on U-PLEX® spot plates and use a different plate preparation protocol

Table 1. Recommended Assay Protocols for R-PLEX Antibody Sets and Assays

Protocol Guidance	R-PLEX Antibody Set/Assay
Assay Protocol 1	Recommended for most R-PLEX Antibody Sets/Assays. If your antibody set is not listed below, follow Assay Protocol 1.
Assay Protocol 2	This is a homogenous assay; it is recommended for Human Aβ (all 6E10) (order Diluent 35 separately), CKBB, and Rat Albumin Sets/Assays.
Assay Protocol 3	Recommended for these Human Antibody Sets/Assays: Aiolos, Bcl-2, BIM/Bcl2-L-11, Hemoglobin alpha, Hemopexin, Ikaros, IRS-1, Lactotransferrin, LRRK2, LRRK2 (pS935), Mcl-1/Bcl2-L-3, Mcl-1/BAK Complex, Mcl-1/BIM Complex, and Serpin F2.
Assay Protocol 4	Recommended for these R-PLEX Antibody Sets/Assays: ADAM12, Human Amphiregulin, Arginase-1, Complement C3b, Human Complement C5a, DKK-1, Human Galectin-3, HB-EGF, IGFBP-3, Lymphotactin, MIS/AMH, Neurotropin-3, NPY, OX40L, Oncostatin-M, PDGF-A/B, Human Perisotin/OSF-2, PRAS40 (total), and Thryoglobulin.

The complete list of R-PLEX products is available at www.mesoscale.com/R-PLEX. Representative data is available in the product-specific datasheets available at https://www.mesoscale.com/R-PLEX-datasheets.

We recommend that sample dilution and other assay parameters be optimized before running samples.

Principle of the Assay

Singleplex assays can be easily developed on MSD Small Spot Streptavidin plates. These plates provide high sensitivity, consistent performance, and excellent inter- and intralot uniformity. The typical R-PLEX Antibody Set includes a biotinylated capture antibody that binds to streptavidin on the plate surface. Analyte in the sample binds to the capture reagent; a detection antibody conjugated with an electrochemiluminescent label (MSD GOLD SULFO-TAG label) binds to the analyte to complete the sandwich immunoassay (Figure 1). 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 the analyte in the sample.

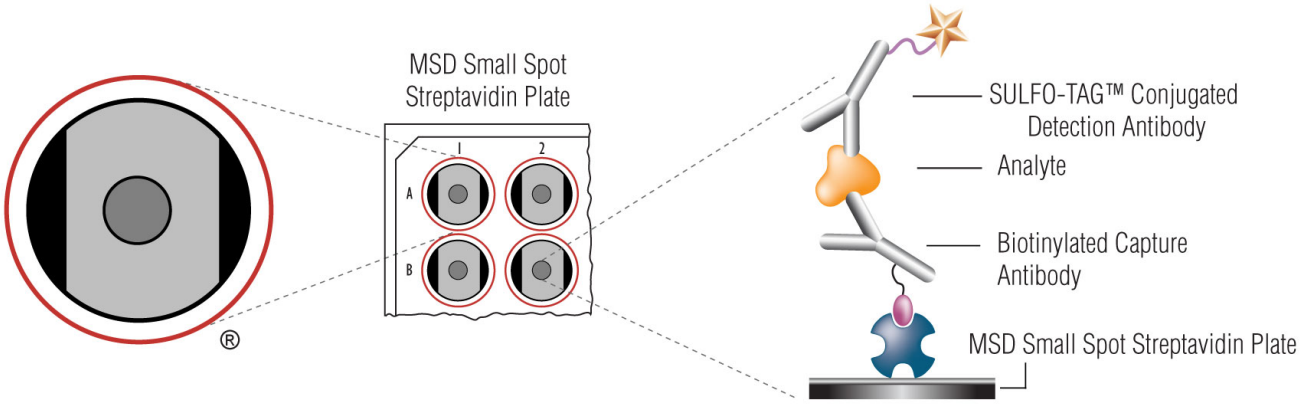

Figure 1. *R-PLEX singleplex assay on an MSD Small Spot Streptavidin Plate.*

Components

R-PLEX Assays

For R-PLEX assay instrument compatibility, please refer to the Instrument Compatibility section on page 9.

R-PLEX Assays contain the components listed in Table 2 along with the R-PLEX Antibody Set components (Table 3).

Table 2. R-PLEX Assay Components

Reagent	Storage	Catalog #	Size	Quantity Supplied	Description
MSD GOLD 96-Well Small Spot Streptavidin SECTOR Plate	2–8 °C	L45SA-1	1 spot	5 plates	96-well plate—foil sealed, with desiccant
96-Well Small Spot Streptavidin QuickPlex Ultra Plate		L4BLA-1			
Diluent 100	2–8 °C	R50AA-4	50 mL	1 bottle	Diluent for biotinylated capture antibody
Assay Diluent	≤–10 °C	The Assay Diluent provided is noted on the assay datasheet.			Diluent for samples and Calibrator, typically contains proteins, blockers, and preservatives
Antibody (or Tracer) Diluent	≤–10 °C	The Antibody Diluent provided is noted on the assay datasheet.			Diluent for detection antibody or tracer, typically contains protein, blockers, and preservatives
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1 bottle	Buffer to catalyze the electrochemiluminescence reaction

RT = room temperature

R-PLEX assays are provided with sufficient diluent to support up to a 10-fold dilution. If additional sample dilution is required for your study, Assay Diluent or Diluent 100 is recommended.

See www.mesoscale.com/en/products_and_services/assay_kits/r-plex for more information on specific assays and Diluent 100. For information on diluent volume requirements, see the R-PLEX Assay Diluent Volume Calculation handout. For information on diluents for R-PLEX, see the R-PLEX Assay/Antibody Diluent Combinations handout. Both are available at www.mesoscale.com/technical_literature/handouts.

R-PLEX Antibody Sets

R-PLEX Antibody Sets contain a biotinylated capture antibody, a SULFO-TAG conjugated detection antibody (or tracer), and a frozen calibrator (Table 3). The calibrator is provided at a 20-fold higher concentration than the suggested Calibrator Standard 1 (top of curve) concentration. The representative Calibrator Standard 1 concentration for each assay is shown in the product-specific datasheet.

Table 3. Contents of R-PLEX Antibody Sets

Name	Storage	Size	Quantity Supplied	Description
Biotin Capture Antibody (analyte-specific)	2–8 °C	5 Plates	1 vial	Biotinylated capture antibody. Provided as one vial per five plates.
SULFO-TAG Detection Antibody (or Tracer) (analyte-specific)	2–8 °C	5 Plates	1 vial	SULFO-TAG conjugated detection antibody (or tracer) (100X). Provided as one vial per five plates.
Calibrator (analyte specific)	≤-70 °C	5 Plates	5 vials	Calibrator (analyte-specific)

Plates and Reagents for Separate Purchase

MSD offers a range of assays, plates (Table 4), and reagents (Table 5 and Table 6) to enable assay development using R-PLEX Antibody Sets. Plates and reagents are also available for individual purchase and in different pack sizes. For a complete listing of all available assay development plates and reagents, visit our website at www.mesoscale.com.

Plates

Table 4. Singleplex Plates (included in assays)

Name	1 Plate	5 Plates	30 Plates	120 Plates	510 Plates
MSD GOLD 96-well Small Spot Streptavidin SECTOR Plates	L45SA-1	L45SA-2	L45SA-5	L45SA-6	L45SA-7
96-well Small Spot Streptavidin QuickPlex Ultra Plates	L4BLA-1	L4BLA-2	L4BLA-5	L4BLA-6	L4BLA-7

Read Buffers

MSD GOLD Read Buffer B is recommended. It is included in R-PLEX Assays and is provided at the working concentration. Other popular Read Buffers are included in Table 5.

Table 5. Read Buffers

Name	Catalog #	Size	Storage	Description
MSD GOLD Read Buffer B	R60AM-1	18 mL	15–30 °C	Buffer to catalyze the electrochemiluminescence reaction Provided at the working concentration of the assay
	R60AM-2	90 mL		
	R60AM-3	200 mL		
	R60AM-4	1000 mL		
MSD GOLD Read Buffer A	R92TG-3	18 mL	15–30 °C	Buffer to catalyze the electrochemiluminescence reaction Provided at the working concentration of the assay
	R92TG-4	90mL		
	R92TG-1	200 mL		
	R92TG-2	1000 mL		
Read Buffer T (4X)	R92TC-3	50 mL	15–30 °C	Buffer to catalyze the electrochemiluminescence reaction Dilute 1:1 with deionized water
	R92TC-2	200 mL		
	R92TC-1	1000 mL		

Diluents

The catalog numbers for commonly used diluents are provided in Table 6, but a range of diluents are available for purchase.

Table 6. *Common diluents used in R-PLEX singleplex assays*

Name	Catalog #	Size
Diluent 1	R50CK-4	50 mL
	R50CK-2	200 mL
Diluent 2	R51BB-4	8 mL
	R51BB-3	40 mL
	R51BB-2	200 mL
Diluent 3	R50AP-1	8 mL
	R50AP-2	40 mL
Diluent 5	R52BA-5	25 mL
	R52BA-6	125 mL
Diluent 6		
	R53BB-3	40 mL
Diluent 7	R54BB-4	5 mL
	R54BB-3	50 mL
Diluent 8		
	R54BA-3	50 mL
Diluent 10	R55BB-3	50 mL
Diluent 11	R55BA-5	10 mL
	R55BA-3	50 mL
Diluent 12	R50JA-4	10 mL
	R50JA-3	50 mL
Diluent 13	R56BB-4	10 mL
	R56BB-3	50 mL
Diluent 17	R50KA-4	6 mL
	R50KA-3	30 mL
Diluent 22	R50BB-8	8 mL
	R50BB-4	40 mL
Diluent 27	R500A-3	30 mL

Name	Catalog #	Size
Diluent 29	R50HA-4	15 mL
	R50HA-3	40 mL
Diluent 30	R50AB-4	25 mL
Diluent 35	R50AE-3	30 mL
	R50AE-2	150 mL
Diluent 37	R50AF-3	25 mL
	R50AF-6	125 mL
Diluent 39	R5ABB-2	50 mL
Diluent 40	R50AJ-1	5 mL
	R50AJ-2	25 mL
Diluent 41	R50AH-1	10 mL
	R50AH-2	50 mL
Diluent 42	R50AK-1	10 mL
	R50AK-2	50 mL
Diluent 43	R50AG-1	10 mL
	R50AG-2	50 mL
Diluent 45	R50AI-3	8 mL
	R50AI-4	40 mL
Diluent 57	R50BZ-1	10 mL
	R50BZ-2	50 mL
Diluent 58	R50CA-2	50 mL
Dilunet 65	R50CJ-1	10 mL
	R50CJ-2	50 mL
Diluent 100*	R50AA-4	50 mL
	R50AA-2	200 mL
	R50AA-3	1000 mL
Diluent 101	R51AD-3	50 mL
	R51AD-5	150 mL
MSD Tris Lysis Buffer	R60TX-2	200 mL
	R60TX-3	50 mL
MSD Tris Wash Buffer (10X)	R61TX-1	1000 mL
	R61TX-2	200 mL

*Diluent 100 is recommended as a coating diluent.

Note: To run five plates, 50 mL of assay diluent and 40 mL of antibody diluent are required when assaying samples that are diluted up to 10-fold (40 samples per plate, run in duplicate). Additional assay diluent is necessary for samples that are diluted greater than 10-fold. Diluent 100 may be used in place of assay diluent for samples that require high dilution. Testing different diluents can help optimize assays for specific experimental conditions.

R-PLEX assays may have specific diluents for sample and calibrator dilution as well as for the preparation of the detection antibody (or tracer) solutions. Refer to the R-PLEX product-specific datasheet supplied with the product for the diluents tested in the assay. The datasheet is also available at www.mesoscale.com/support/data_sheets.

Wash Buffer

Table 7. Catalog number of Wash Buffer

Name	Storage	Catalog #	Size	Description
MSD Wash Buffer (20X)	RT	R61AA-1*	100 mL	Phosphate-buffered saline (PBS) plus surfactant
MSD Tris Wash Buffer (10X)	2–8 °C	R61TX-2*	200 mL	Tris-buffered solution with surfactant (required for Assay Protocol 3)
		R61TX-1	1000 mL	

*This size of Wash Buffer (Table 7) is sufficient for washing four plates manually or for washing two plates with an automated plate washer.

- ❑ Prepare a 1X working solution with MSD Wash Buffer or MSD Tris Wash Buffer.
    i. MSD Wash Buffer: for one plate, combine 15 mL of MSD Wash Buffer (20X) with 285 mL of deionized water.
    ii. MSD Tris Wash Buffer: for one plate, combine 30 mL of MSD Tris Wash Buffer (10X) with 270 mL of deionized water.

Instrument Compatibility

Depending on which MSD instrument you are using, you will need to select the R-PLEX Singleplex Assay that includes the appropriate plate. R-PLEX Singleplex Assays being run on SECTOR plates should be used with either MESO SECTOR S 600 instruments or MESO QuickPlex SQ 120 instruments. The MESO QuickPlex Q 60MM only reads R-PLEX Assays on QuickPlex Ultra plates. Be sure you have selected the correct plate type for your MSD instrument (Table 8).

Table 8. R-PLEX Assay and Instrument Compatibility

Instrument	SECTOR plate	QuickPlex Ultra plate
MESO QuickPlex SQ 120	Y	—
MESO QuickPlex SQ 120MM	Y	—
MESO SECTOR S 600	Y	—
MESO SECTOR S 600MM	Y	—
MESO QuickPlex Q 60MM	—	Y

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
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex mixer
- ❑ Mini-centrifuge

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 all components to room temperature; a 22–25 °C water bath can be used for frozen diluents.
- Prepare Calibrator Standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by briefly vortexing after each dilution.
- Avoid prolonged exposure of the detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light (except for direct sunlight).
- Avoid bubbles in wells during all pipetting steps because they may lead to variable results. Bubbles introduced when adding MSD Read Buffers may interfere with signal detection. Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm, optimally at 700 rpm or above.
- Avoid letting the plate surface dry completely between steps. If a step needs to be delayed, leave the antibody coating solution, sample, or detection antibody (or tracer) solution in the plate until you are ready to perform the next step to keep the plate from drying out.
- Remove the plate seal before reading the plate.
- Make sure that the MSD Read Buffer is at room temperature when added to a plate.
- Do not shake the plate after adding MSD Read Buffer.
- To improve interplate precision, keep time intervals consistent between adding MSD Read Buffer and reading the plate. Read the plate as soon as possible after adding MSD Read Buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading and follow guidelines on how to read partial plates provided in the instrumental manual. Partially used plates may be stored up to 30 days at 2–8 °C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature and refer to the **Best Practices** section before beginning the protocol. Determine which assay protocol is optimal based on the R-PLEX analytes (see Table 1). Reagent preparation may vary based on the assay protocol.

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

To prepare supplemental reagents such as MSD Wash Buffer, please refer to the Components section.

Coat Plate

Note: If you are following Assay Protocl 4, skip this step and go directly to assay protocol 4 (page 17).

- ❑ Add 200 µL of biotinylated capture antibody to 3.3 mL of coating diluent. Mix by vortexing.

Note: Coating diluents can be simple diluents containing 0.5% BSA in PBS, or use 0.5% MSD Blocker A or MSD Diluent 100.

- ❑ Add 25 µL of the above solution to each well of the MSD Small Spot Streptavidin plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate at room temperature for 1 hour. Shake the plate during incubation (required).
- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer. If following Assay Protocol 3, wash the plates with at least 150 µL/well 1X of MSD Tris Wash Buffer. The plate is now coated with capture antibody.

Prepare Calibrator Standards

MSD supplies calibrator for R-PLEX Antibody Sets at a concentration that is 20-fold higher than the recommended highest standard. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep it on ice, then add to assay diluent at room temperature to make the calibration curve solutions.

To prepare seven calibrator solutions plus a zero calibrator for up to 4 replicates, see Figure 2.

- ❑ Prepare the most concentrated calibrator solution (Calibrator Standard 1) by adding 15 µL of the stock calibrator to 285 µL of assay diluent. Mix well.
- ❑ Prepare the next calibrator solution by transferring 100 µL of the most concentrated calibrator to 300 µL of assay diluent. Mix well. Repeat 4-fold serial dilution 5 times to generate 7 calibrator solutions.
- ❑ Use assay diluent as the blank.

Discard any unused, diluted calibrator solutions.

Notes:

- Assay Protocol 3 requires twice as much Calibrator Standard (50 µL/well) as Assay Protocols 1 and 2 (25 µL/well).
- Dilution volumes can be adjusted for fewer replicates.
- For the recommended Calibrator Standard 1 (top of curve) concentration, refer to the product-specific datasheet supplied with the R-PLEX Antibody Set. The datasheet is also available at: https://www.mesoscale.com/en/support/product_information/search_data_sheets.

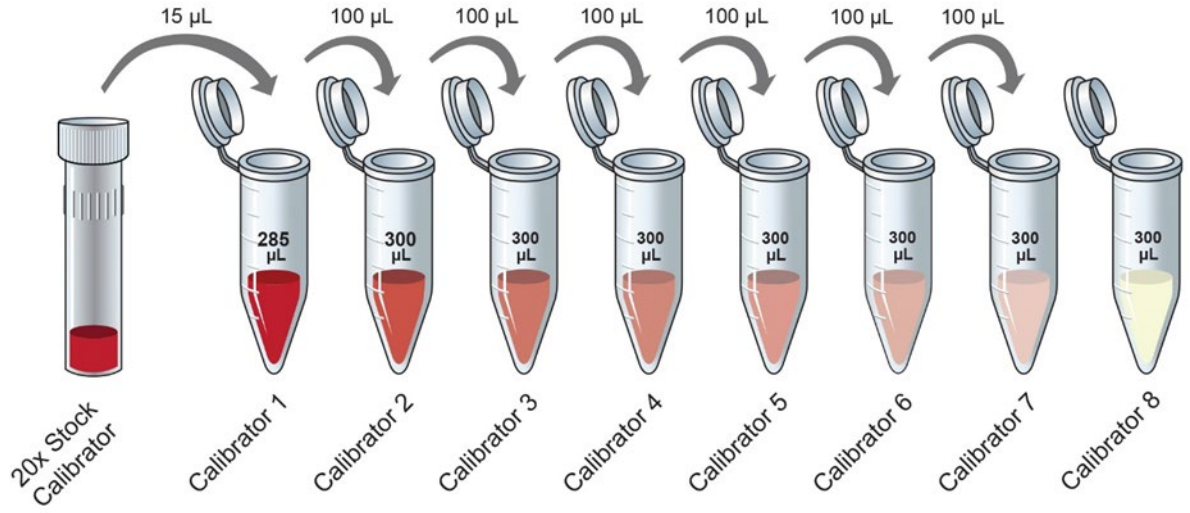

Figure 2. Dilution schema for Calibrator Standards for R-PLEX Singleplex Assays

Prepare Detection Antibody (or Tracer) Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X. Prepare the detection antibody (or tracer) solution immediately before use.

For one plate, combine:

- ❑ 60 µL of the supplied 100X detection antibody (or tracer)
- ❑ 5,940 µL of antibody diluent

Note: Assay Protocol 2 uses 25 µL/well of 1X detection antibody (or tracer) solution. Prepare 3,000 µL of 1X working solution for one plate.

Dilute Samples

Depending on the sample set under investigation, a dilution may be necessary. Suggested dilution factors are provided in the R-PLEX product-specific datasheet. Assay diluent may be used for sample dilution. The dilution factor for a given sample type should be optimized.

Notes:

- The sample preparation for biomarkers for Assay Protocol 3 may not be compatible with secreted biomarkers. Refer to the R-PLEX product-specific datasheet for information on biomarker diluent/sample preparation.
- Additional assay diluent may be necessary for samples that are diluted greater than 10-fold. Diluent 100 may be used in place of assay diluent for samples that require high dilutions.

Prepare MSD Read Buffer

MSD GOLD Read Buffer B and MSD GOLD Read Buffer A are provided at the working concentrations. Equilibrate the read buffer to room temperature before use. To avoid bubbles, do not vortex.

If using MSD Read Buffer T (4X), dilute the buffer two-fold in deionized water to make a 2X working solution. Equilibrate the read buffer to room temperature before use. To avoid bubbles, do not vortex.

Assay Protocol 1

Refer to the **Introduction** (Table 1) for the optimal assay protocol for your R-PLEX assay.

Note: Before beginning STEP 1, coat the plate as described on page 12.

STEP 1: Add Samples and Calibrators

- ❑ Add 25 µL of assay diluent to each well. Tap the plate gently on all sides.
- ❑ Add 25 µL of the prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1 hour.

STEP 2: Wash and Add Detection Antibody Solution

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

STEP 3: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of read buffer to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Assay Protocol 2

Refer to the **Introduction** (Table 1) for the optimal assay protocol for your R-PLEX assay.

Note: Before beginning STEP 1, prepare the plate as described on page 12.

STEP 1: Add Blocker and Wash (This step is NOT required for Human CKBB or Rat Albumin).

- ❑ Add 150 µL of Diluent 35 as a blocking solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1 hour.
- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Wash Buffer.

STEP 2: Add Samples, Calibrators, and Detection Antibody Solution (or Tracer)

- ❑ Add 25 µL of detection antibody (or tracer) solution to each well.
- ❑ 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 3: Wash and Read

- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of read buffer to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Assay Protocol 3

Refer to the **Introduction** (Table 1) for the optimal assay protocol for your R-PLEX assay.

Note: Before beginning STEP 1, prepare the plate as described on page 12.

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 1 hour.

***Note:** For Human Aiolos, Ikaros, and IRS-1, add 25 µL of assay diluent and 25 µL of the prepared Calibrator Standard or sample to each well per the standard R-PLEX protocol.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Tris 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 three times with at least 150 µL/well of 1X MSD Tris Wash Buffer.
- ❑ Add 150 µL of MSD Read Buffer to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Alternate Wash Steps

Alternate wash steps may be employed for the following protocols: Assay Protocol 1, Assay Protocol 2, and Assay Protocol 3. These alternates may be used to simplify the protocol or improve the assay performance.

- ❑ **Alternate 1**: For simple sample matrices such as cell culture supernatants, the protocol may be simplified by eliminating one of the wash steps. After incubating the Calibrator Standard or sample, add detection antibody solution (or tracer) to the plate without decanting or washing the plate.
- ❑ **Alternate 2**: For assays with complex matrices such as serum and plasma, washing with a larger volume (up to 400 µL) may improve the performance of some assays.

Assay Protocol 4

Refer to the **Introduction** (Table 1) for the optimal assay protocol for your R-PLEX assay.

The preparation of a U-PLEX plate involves coating the provided plate with Linker-coupled capture antibodies. The protocol in this section describes the preparation of a singleplex coating solution for one 96-well plate. The volumes can be adjusted depending on the number of plates or wells, but the ratios of the reagents should remain the same.

STEP 1: Create Individual Linker-Coupled Antibody Solutions

- ❑ Add 200 µL of the biotinylated antibody to 300 µL of Linker 1. Mix by vortexing. Incubate at room temperature for 30 minutes. Do not shake.

  Notes:

  - To remove liquid from the cap, briefly centrifuge the Linker vial and open the cap gently.
  - Close the Linker cap as soon as you are done using it. Take precautions to avoid reagent contamination.
- ❑ Add 200 µL of Stop Solution. Mix by vortexing. Incubate at room temperature for 30 minutes.

  Note: At the end of Step 1, the individual 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 2: Prepare the Singleplex Coating Solution

- ❑ Take 600 µL of the Linker-coupled antibody solution into a single tube and add 5,400 uL of stop solution and vortex.

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

STEP 3: Coat the U-PLEX Plate

- ❑ Add 50 µL of singleplex coating solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature for 1 hour or overnight at 2–8 °C. Shaking the plate during incubation is required.
- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer

The plate is now coated and ready for use. Plates may be stored in the original pouch with desiccant and sealed, for up to 7 days at 2–8 °C. Please refer to **Assay Protocol 1** to continue the assay.

The recommended volumes of Linker, biotinylated capture antibody, and Stop Solution for coating one or multiple U-PLEX plates are provided below (Table 10)

Table 10. Amount of each component needed for U-PLEX Coating Solution (partial plate)

No. of Wells	Individual Linker (µL)	Individual Biotinylated Capture 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

Assay Performance

A representative data set for each assay is presented in the product-specific datasheet shipped with the product; also available at www.mesoscale.com/R-PLEX-documents. The data represent the performance of the assay tested in singleplex 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.

Summary Protocols for R-PLEX Singleplex Assays

Refer to the **Introduction** (Table 1) for the optimal assay protocol for your R-PLEX Antibody Set or Singleplex Assay.

Gather Required Assay Components

- ❑ R-PLEX Antibody Set with Calibrator
- ❑ MSD Small Spot Streptavidin plate
- ❑ Coating diluent, assay diluent, and antibody diluent
- ❑ MSD GOLD Read Buffer B
- ❑ MSD Wash Buffers*

*Not included in R-PLEX Assays

Coat Plate

Note: If you are following Assay Protocl 4, skip this step and go directly to the summary of assay protocol 4 (page 20).

- ❑ Add 200 µL of biotinylated capture antibody to 3.3 mL of coating diluent. Mix by vortexing.
- ❑ Add 25 µL of the above solution to each well of an MSD Small Spot Streptavidin plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.
- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Wash Buffer. (If following Assay Protocol 3, wash plates with at least 150 µL/well of 1X MSD Tris Wash Buffer.) The plate is now coated and ready for use.

Summary Assay Protocol 1

STEP 1: Add Samples and Calibrators

- ❑ Add 25 µL of assay diluent to each well of the coated plate. 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 three 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 and incubate at room temperature with shaking for 1 hour.

STEP 3: Wash and Read

- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of MSD Read Buffer to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Summary Assay Protocol 2

STEP 1: Add Blocker and Wash (This step is NOT required for Human CKBB)

- ❑ Add 150 µL of Diluent 35 as a blocking solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 1 hour.
- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Wash Buffer.

STEP 2: Add Samples, Calibrators, and Detection Antibody Solution (or Tracer)

- ❑ Add 25 µL of detection antibody (or tracer for competitive formats) solution to each well.
- ❑ 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 3: Wash and Read

- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Wash Buffer.
- ❑ Add 150 µL of MSD Read Buffer to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Summary Assay Protocol 3

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 1 hour.

*For Human Aiolos, Ikaros, and IRS-1, add 25 µL of assay diluent and 25 µL of the prepared Calibrator Standard or sample to each well.

STEP 2: Wash and Add Detection Antibody Solution

- ❑ Wash the plate three times with at least 150 µL/well of 1X MSD Tris 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 three times with at least 150 µL/well of 1X MSD Tris Wash Buffer.
- ❑ Add 150 µL of MSD Read Buffer to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Summary Assay Protocol 4

STEP 1: Create Individual Linker-Coupled Antibody Solutions

- ❑ Add 200 µL of the biotinlated antibody to 300 µL of Linker 1. Mix by vortexing. Incubate at room temperature for 30 minutes. Do not shake
- ❑ Add 200 µL of Stop Solution. Mix by vortexing. Incubate at room temperature for 30 minutes.

STEP 2: Prepare the Singleplex Coating Solution

- ❑ Take 600 µL of the Linker-coupled antibody solution into a single tube and add 5,400 µL of stop solution and vortex.

STEP 3: Coat the U-PLEX Plate

- ❑ Add 50 µL of singleplex coating solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature for 1 hour or overnight at 2-8 °C. Shaking the plate during incubation is required.
- ❑ 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.

Plate Diagram