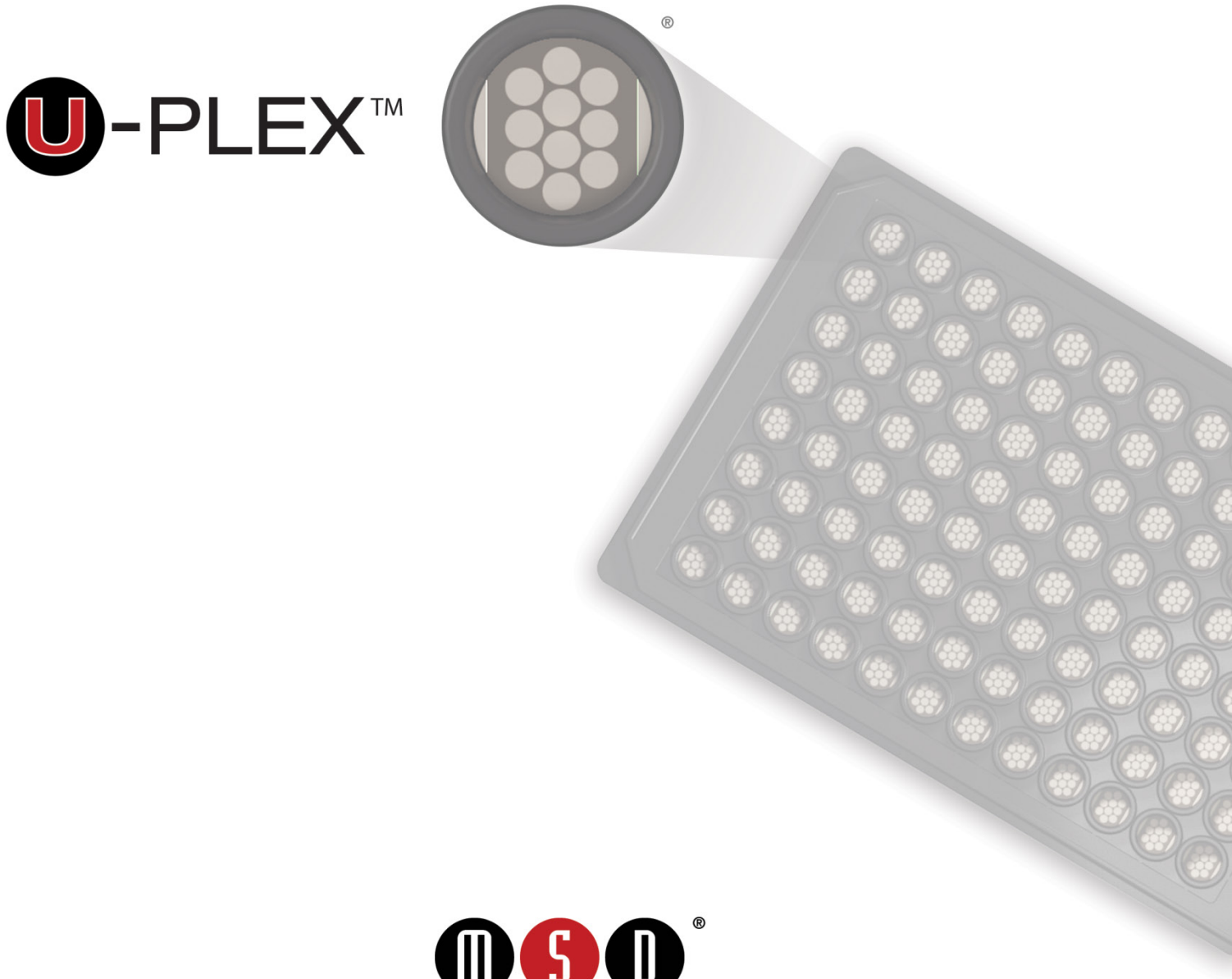
MSD® U-PLEX Assays

Proinflammatory (hu) Demonstration Kit

1-Plate Kit K15064K-1



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MSD U-PLEX® Assays

Proinflammatory (hu) Demonstration Kit

IL-1β, IL-6, IL-8, TNF-α

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

U-PLEX is a technology that allows you to create your own multiplex assays. Using two simple tools— a 10-spot U-PLEX plate and unique linkers— you can build custom multiplex panels for any combination of analytes. In U-PLEX immunoassays (Figure 1), biotinylated capture reagents (e.g., antibodies, peptides, proteins, nucleic acids) are coupled to U-PLEX linkers. These linkers 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 SULFO-TAG™) bind to the analytes to complete the immunoassay sandwich. 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, to provide a quantitative measure of each analyte in the sample.

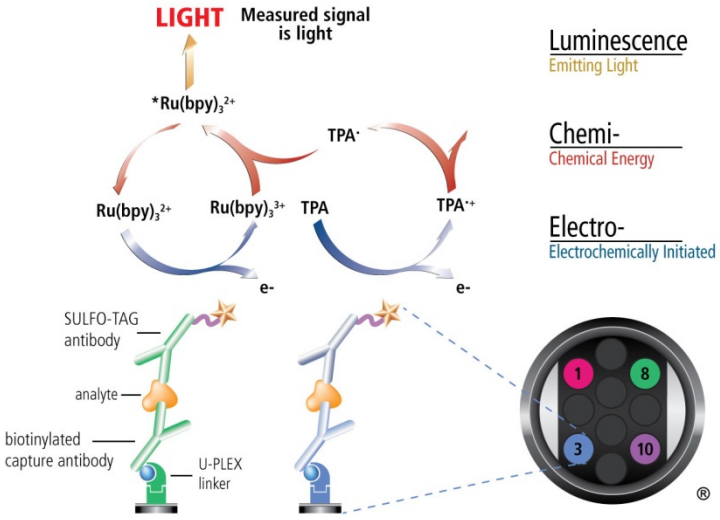


Figure 1. U-PLEX immunoassay on a 4-Assay, 10-Spot plate depicting the electrochemiluminescence reaction.

Proinflammatory (hu) Demonstration Kit

MSD offers preconfigured multiplex kits for the measurement of specific biomarkers on the U-PLEX platform. The U-PLEX Proinflammatory (hu) Demonstration Kit provides assay components that have been optimized for detection and quantification of human IL-1β, IL-6, IL-8, and TNF-α in cell culture supernatants, serum, and plasma.

Kit Components

The following tables list the components provided with the U-PLEX Proinflammatory (hu) Demonstration Kit.

Reagents Supplied

U-PLEX Platform Reagents	Storage	Catalog #	Size	Quantity	Description
4-Assay, 96-Well SECTOR® Plate	2–8°C	N05229A-1	--	1 plate	10-spot plate with activated spots at positions 1, 3, 8, and 10.
Linker 1	2–8°C	E2226-2	300 µL	1 vial	To be coupled with the assigned biotinylated antibody.
Linker 3	2–8°C	E2228-2	300 µL	1 vial	To be coupled with the assigned biotinylated antibody.
Linker 8	2–8°C	E2233-2	300 µL	1 vial	To be coupled with the assigned biotinylated antibody.
Linker 10	2–8°C	E2235-2	300 µL	1 vial	To be coupled with the assigned biotinylated antibody.
Stop Solution	2–8°C	R50AO-1	40 mL	1 bottle	Biotin-containing buffer to stop linker-antibody coupling reaction.
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	Buffer to catalyze the electrochemiluminescence reaction.

Assay Reagents	Storage	Catalog #	Size	Quantity	Description
Human IL-1β Antibody Set	2–8°C	B21TU-2	--	1 box	Set containing biotin-conjugated capture antibody and SULFO-TAG-conjugated detection antibody.
Human IL-6 Antibody Set	2–8°C	B21TX-2	--	1 box	Set containing biotin-conjugated capture antibody and SULFO-TAG-conjugated detection antibody.
Human IL-8 Antibody Set	2–8°C	B21TY-2	--	1 box	Set containing biotin-conjugated capture antibody and SULFO-TAG-conjugated detection antibody.
Human TNF-α Antibody Set	2–8°C	B21UC-2	--	1 box	Set containing biotin-conjugated capture antibody and SULFO-TAG-conjugated detection antibody.
Calibrator 1	2–8°C	C0060-2	--	1 vial	Recombinant human proteins lyophilized in buffered diluent. Individual analyte concentration is provided in the lot-specific certificate of analysis (COA).
Diluent 43	≤-10°C	R50AG-1	10 mL	1 bottle	Diluent for samples and calibrator; contains serum, blockers, and preservatives.
Diluent 3	≤-10°C	R50AP-1	8 mL	1 bottle	Diluent for detection antibody; contains protein, blockers, and preservatives.

U-PLEX Antibodies	Storage	Catalog #	Size	Quantity	Description
SULFO-TAG Anti-hu IL-1β	2–8°C	D21TU-2	75 µL	1 vial	100X SULFO-TAG-conjugated detection antibody
Biotin Anti-hu IL-1β	2–8°C	C21TU-2	250 µL	1 vial	1X Biotin-conjugated capture antibody
SULFO-TAG Anti-hu IL-6	2–8°C	D21TX-2	75 µL	1 vial	100X SULFO-TAG-conjugated detection antibody
Biotin Anti-hu IL-6	2–8°C	C21TX-2	250 µL	1 vial	1X Biotin-conjugated capture antibody
SULFO-TAG Anti-hu IL-8	2–8°C	D21TY-2	75 µL	1 vial	100X SULFO-TAG-conjugated detection antibody
Biotin Anti-hu IL-8	2–8°C	C21TY-2	250 µL	1 vial	1X Biotin-conjugated capture antibody
SULFO-TAG Anti-hu TNF-α	2–8°C	D21UC-2	75 µL	1 vial	100X SULFO-TAG-conjugated detection antibody
Biotin Anti-hu TNF-α	2–8°C	C21UC-2	250 µL	1 vial	1X Biotin-conjugated capture antibody

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of 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
- ❑ Phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T) for plate washing or MSD Wash Buffer (Catalog # R61AA-1).

  Follow the instructions below if MSD Wash Buffer is purchased separately. MSD provides 100 mL of wash buffer as a 20X stock solution. The working solution is 1X. For 1 plate, combine:

  - 15 mL of MSD Wash Buffer (20X)
  - 285 mL of deionized water
- ❑ Adhesive plate seals
- ❑ Deionized water
- ❑ Vortex

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit 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 safety data sheet (SDS), which can be obtained from MSD Customer Service.

Reagent Preparation

Bring all reagents to room temperature.

Important: Upon first thaw, separate Diluent 43 and Diluent 3 into suitably sized aliquots before refreezing.

Prepare U-PLEX plate

Preparation of a U-PLEX plate involves coating the provided plate with linker-coupled capture antibodies. This kit includes a plate with 4 activated spots at locations 1, 3, 8, and 10. Couple each biotinylated antibody to a unique linker and record the antibody identity next to the assigned linker as shown in the example below.

1 IL-1β
2
3 IL-6
4
5
6
7
8 IL-8
9
10 TNF-α

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

A separate linker must be used for each assay. Below are the steps to complete the coupled reactions for the above example.

- ❑ Add 200 µL of individual biotin-conjugated capture antibody solution directly into the 300 µL of the assigned linker.

  Note: Each linker vial is provided with an accurately pre-measured 300 µL of linker solution; there is no need to remove the liquid from the vial.

  a) Add 200 µL of Biotin Anti-hu IL-1β into 300 µL of Linker 1 (pink cap)

  b) Add 200 µL of Biotin Anti-hu IL-6 into 300 µL of Linker 3 (blue cap)

  c) Add 200 µL of Biotin Anti-hu IL-8 into 300 µL of Linker 8 (green cap)

  d) Add 200 µL of Biotin Anti-hu TNF-α into 300 µL of Linker 10 (purple cap)
- ❑ Close each colored cap of coupling reaction vial and mix well by vortexing.
- ❑ Tap or spin briefly to bring down any solution that might be trapped in the lid.
- ❑ Incubate at room temperature without shaking for 30 minutes.
- ❑ Add 200 µL of Stop Solution to each coupling reaction vial. Vortex.
- ❑ Tap or spin briefly and incubate at room temperature without shaking for 30 minutes.

At the end of step 1, each individual U-PLEX-coupled antibody solution is at 10X the coating concentration and can be stored up to 7 days at 2-8°C.

STEP 2: Prepare Multiplex Coating Solution:

- ❑ Combine 600 µL of each U-PLEX-coupled antibody solution into a single tube.
- ❑ Add 3.6 mL of Stop Solution to bring the final volume to 6 mL.
- ❑ Vortex the multiplex coating solution before adding to the plate.

The multiplex coating solution can be stored up to 7 days at 2-8°C.

STEP 3: Coat U-PLEX plate:

- ❑ Add 50 µL solution from STEP 2 above to each well of the U-PLEX plate provided.
- ❑ Seal the plate with an adhesive plate seal.
- ❑ Incubate with shaking at room temperature for 1 hour or shaking at 2-8°C overnight.
- ❑ Wash plate 3 times with at least 150 µL/well of PBS-T or MSD Wash Buffer.

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

Prepare Calibrator Dilutions

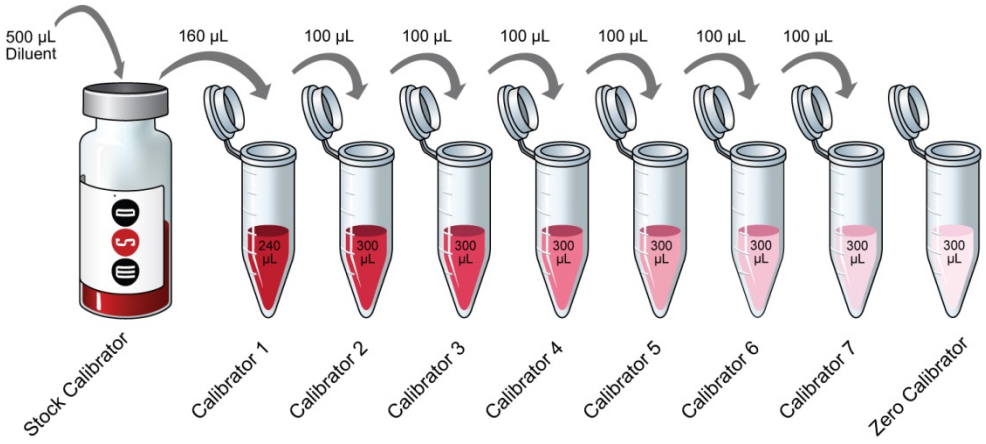
MSD supplies a multi-analyte lyophilized calibrator at 5-fold higher than the recommended highest calibrator concentration upon initial reconstitution.*

Note: Reconstituted calibrator is not stable when stored at 2–8°C; however, it may be stored frozen at ≤-70°C and is stable through 3 freeze-thaw cycles.

To prepare 7 calibrator solutions plus a zero calibrator for up to 4 replicates:

1) Reconstitute the lyophilized stock calibrator with 500 µL of Diluent 43 and incubate at room temperature without mixing for 10-15 minutes. After the incubation period, vortex stock calibrator before use. Keep all calibrator solutions on wet ice until use.
2) Prepare the first calibrator by transferring 160 µL of the calibrator stock solution into 240 µL of Diluent 43. Prepare the next calibrator by transferring 100 µL of the first calibrator into 300 µL of Diluent 43. Mix well by vortexing. Repeat 4-fold serial dilutions 5 additional times to generate 7 calibrators.
3) Use Diluent 43 as the zero calibrator.

Note: For the lot-specific concentration of each calibrator in the blend, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.



*The calibration curve can be extended by creating a more concentrated highest calibrator. This can be done by reducing the volumes of reconstituted calibrators (no less than 100 µL is recommended) when making the highest calibrator solution or by using a lower volume of Diluent 43 when reconstituting the lyophilized calibrator.

Dilute Samples

Depending on the sample set under investigation, a dilution fold may be necessary. Tissue culture supernatants may also require additional dilution based on stimulation and analyte concentration in the sample.

Prepare Detection Antibody Solution

MSD provides each detection antibody separately as a 100X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use.

For 1 plate, combine the following detection antibodies and add to 5.76 mL of Diluent 3:

- ❑ 60 µL of SULFO-TAG Anti-hu IL-1β
- ❑ 60 µL of SULFO-TAG Anti-hu IL-6
- ❑ 60 µL of SULFO-TAG Anti-hu IL-8
- ❑ 60 µL of SULFO-TAG Anti-hu TNF-α

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Protocol

NOTE: Before beginning STEP 1, prepare the plate as described in the Reagent Preparation section (pp. 7-8).

STEP 1: Add Sample or Calibrator

- ❑ Add 25 µL of Diluent 43 to each well.
- ❑ Add 25 µL of prepared calibrator 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

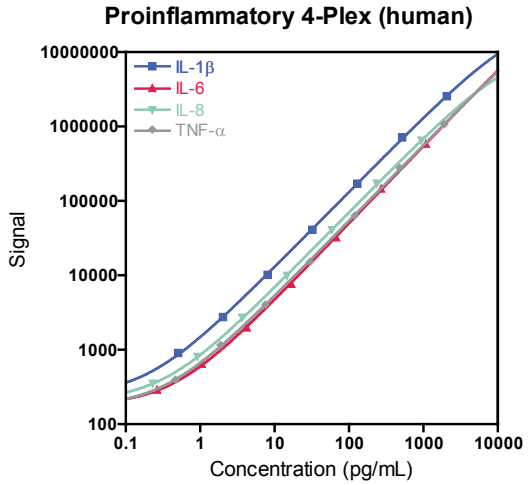
- ❑ Wash plate 3 times with at least 150 µL/well of PBS-T or MSD Wash Buffer.
- ❑ Add 50 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal.
- ❑ Incubate at room temperature with shaking for 1 hour.

STEP 3: Wash and Read

- ❑ Wash plate 3 times with at least 150 µL/well of PBS-T or MSD Wash Buffer.
- ❑ Add 150 µL of 2X Read Buffer T to each well.
- ❑ Analyze plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

Data

The following calibration curves illustrate the dynamic range of the assay. The actual signals will vary. Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of 2 replicates of calibrators.



	Median LLOD (pg/mL)	LLOD Range (pg/mL)
IL-1β	0.07	0.04–0.52
IL-6	0.23	0.10–0.91
IL-8	0.13	0.07–0.34
TNF-α	0.16	0.09–0.57

Specificity

To assess specificity, each assay antibody set in the panel is tested individually against a larger panel of recombinant human analytes for nonspecific binding (IFN-γ, IL-2, IL-4, IL-10, IL-12p70, IL-13, TNF-α, Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC, IL-1α, IL-7, IL-12p40, IL-15, IL-16, TNF-β, G-CSF, IFN-α2a, IL-18, TPO, I-TAC, CTACK, ENA-78, Fractalkine, MIP-3α, MIP-3β, SDF-1α, IFN-α, IL-5, GM-CSF, IL-17A, and VEGF). Nonspecific binding was less than 0.5% for all assays (IL-1β, IL-6, IL-8, and TNF-α) in the kit.

Best Practices

- Bring frozen diluent to room temperature in a 24°C water bath. Thaw other reagents on wet ice and use as directed without delay.
- Avoid cross-contamination between linkers by following the techniques below:
    - Open one U-PLEX linker vial at a time. Close the cap after use prior to opening another linker vial.
    - Each linker is color coded; put the cap on the vial with the matching color.
    - Use filtered pipette tips.
    - Use a fresh pipette tip after each reagent addition.
- Prepare calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of 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 at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid introduction of bubbles, and for empty wells, pipette to the bottom corner.
- Shaking should be vigorous with a rotary motion between 500 and 1,000 rpm.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- If you plan to coat U-PLEX plates for later use, keep the plate pouch and the desiccant that came with the plate. After the plates are incubated with the coating solution, wash them with PBS-T or MSD wash buffer, then return the plate to its original packaging with the desiccant and seal.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the plate.
- Remove plate seals prior to reading the plate.
- Read buffer should be at room temperature when added to the plate.
- Do not shake the plate after adding read buffer.
- Keep time intervals consistent between adding read buffer and reading the plate to improve inter-plate precision. Unless otherwise directed, read plate as soon as practical after adding read buffer.
- If assay results are above the top of the calibration curve, dilute samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. Partially used plates may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Plate Diagram