Quick Guide

U-PLEX® Biomarker Group 1 (Human) - Multiplex Assays

The MSD® U-PLEX platform provides a rapid and convenient method for creating 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. The U-PLEX assay menu is organized into groups which include a broad menu of analytes assembled together by species, abundance in serum and plasma samples, analytical compatibility, and expected use. As many as 10 U-PLEX assays from an assay group may be multiplexed on each plate for simultaneous quantification. For ultimate flexibility, custom panels can be created from a selection of MSD U-PLEX assays, your own antibodies, or a combination of both.

Read the U-PLEX Biomarker Group 1 (Human) Multiplex Assays product insert available at www.mesoscale.com/U-PLEX-documents for detailed instructions.

Components

Reagent	Storage	Description
10-spot 96-well U-PLEX Plate	2–8°C	Foil sealed, with desiccant. The appropriate number of spots is activated to match the number of assays ordered.
Linkers (1–10)	2–8°C	The appropriate number of Linkers is provided to match the number of assays ordered.
U-PLEX Antibody Sets	2–8°C	Sets containing a biotinylated capture antibody and SULFO-TAG™ conjugated detection antibody.
Calibrators	2–8°C	Multi-analyte blends, each containing multiple recombinant human proteins lyophilized in a buffered diluent. Individual analyte concentration is provided in the lot-specific certificate of analysis.
Diluent 43	≤-10°C	Diluent for samples and Calibrators; contains serum, blockers, and preservatives.
Diluent 3	≤-10°C	Diluent for detection antibody; contains protein, blockers, and preservatives.
Stop Solution	2–8°C	Biotin-containing buffer to stop Linker-antibody coupling reaction.
Read Buffer T (4X)	RT	Buffer to catalyze the electrochemiluminescence reaction. Dilute to 2X and use at room temperature.

Additional Materials

MSD Wash Buffer (20X, 100 mL, catalog # R61AA-1) or Phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T) for plate washing. For one plate, combine 15 mL of MSD Wash Buffer (20X) with 285 mL of deionized water.

Reagent Preparation

Note: Bring all reagents to room temperature.

Please consult the U-PLEX Biomarker Group 1 (Human) Multiplex Assays product insert available at www.mesoscale.com/U-PLEX-documents for detailed instructions.

Prepare the U-PLEX Plate

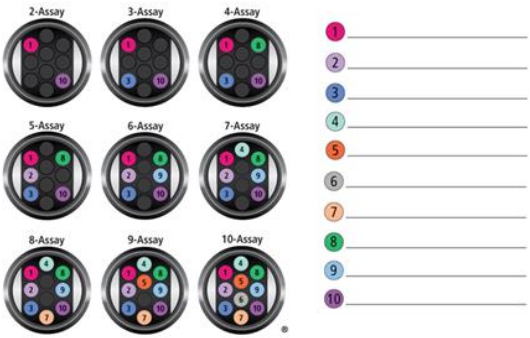
Step 1 Create the Individual U-PLEX-Coupled Antibody Solutions

The biotinylated capture antibody is provided at a ready-to-use concentration. Couple an individual biotinylated antibody to a unique Linker and record the antibody identity next to the Linker number on the Spot Map below.

- ❑ Add 200 µL of each biotinylated antibody to 300 µL of the assigned Linker. For 1-plate assay packs, the biotinylated antibody may be added directly to the Linker vial. A different Linker must be used for each biotinylated antibody. Mix by vortexing. Incubate at room temperature for 30 minutes.
- ❑ Add 200 µL of Stop Solution. Mix by vortexing. Incubate at room temperature for 30 minutes.
  Each individual U-PLEX-coupled antibody solution is at 10X the coating concentration and can be stored at 2-8°C for up to 7 days.

Step 2 Prepare the Multiplex Coating Solution

- ❑ Combine 600 µL of each U-PLEX-coupled antibody solution into a single tube and vortex. Up to 10 U-PLEX-coupled antibodies can be pooled.
  Do not combine U-PLEX-coupled antibody solutions that share the same Linker.
- ❑ When combining fewer than 10 antibodies, bring the solution up to 6 mL with Stop Solution to result in a final 1X concentration. Mix by vortexing. The 1X multiplex coating solution can be stored at 2-8°C for up to 7 days.



Reagent Preparation

Step 3 Coat the U-PLEX Plate

- ❑ Add 50 µL of 1X multiplex coating solution to each well. 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 or PBS-T (PBS plus 0.05% Tween-20). 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.

Prepare Calibrator Standards

Depending on the assays ordered, you may receive one or more multi-analyte Calibrator vials with your order. Individual analyte concentrations are provided in the lot-specific certificates of analysis (COA) that are shipped with the product.

To prepare 7 Calibrator Standard solutions plus a zero Calibrator Standard for up to 6 replicates:

- ❑ Reconstitute each vial of Calibrator by adding 250 µL of Diluent 43 to the glass vial and inverting 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.

  If you have more than one Calibrator, create a Calibrator Blend. Combine 50 µL of each reconstituted Calibrator in a clean polypropylene tube and bring the total volume up to 250 µL in Diluent 43.

  Note: We recommend that reconstituted Calibrators are used immediately. If storage is necessary, divide into 60 µL aliquots and store immediately at ≤-70°C.
- ❑ Prepare Calibrator Standard 1 by adding 50 µL of the Calibrator (or Calibrator Blend) to Diluent 43 to make a final volume of 250 µL. Mix by vortexing.
- ❑ Prepare Calibrator Standard 2 by adding 75 µL of Calibrator Standard 1 to 225 µL of Diluent 43. 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 Diluent 43 as Calibrator Standard 8 (zero Calibrator).

Dilute Samples

- ❑ Depending on the sample set under investigation, a dilution may be necessary. Diluent 43 (catalog # R50AG-1) may be used for sample dilution.

  Note: TGF-β samples require an acid treatment for activation prior to use:

  1. Add 20 µL of 1 M HCl per 100 µL of sample volume. Vortex briefly.
  2. Incubate sample for 10 min at room temperature.
  3. Neutralize the sample by adding 14 µL of 1.2M NaOH/0.5M HEPES per 100 µL of sample volume. Vortex briefly. Samples are ready to use. Use immediately.

Prepare Detection Antibody Solution

- ❑ The detection antibody is provided as a 100X stock solution. For one plate, combine 60 µL of each supplied 100X detection antibody. Add Diluent 3 to bring the final volume to 6 mL.

Reagent Preparation/Assay Protocol

Prepare 2X Read Buffer T

- ❑ For one plate, combine 10 mL of Read Buffer T (4X) and 10 mL of deionized water. You may keep excess diluted Read Buffer T in a tightly sealed container at room temperature for up to one month.

NOTE: Follow **Reagent Preparation** before beginning this assay protocol.

Step 1 Add Samples and Calibrators

- ❑ Add 25 µL of Diluent 43 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 or PBS-T.
- ❑ 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 or PBS-T.
- ❑ Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer T is not required before reading the plate.

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