 MSD® U-PLEX Development Packs provide 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.

In U-PLEX assays, 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. U-PLEX immunoassays can be run in a number of different formats.

Once the assay 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.

**Development Pack Components**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Catalog #</th>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-spot 96-well Plate</td>
<td>2–8°C</td>
<td>--</td>
<td>--</td>
<td>Foil sealed, with desiccant. An appropriate number of spots are activated to match the number of assays ordered (or included in box).</td>
</tr>
<tr>
<td>Linker (1 - 10)</td>
<td>2–8°C</td>
<td>--</td>
<td>variable</td>
<td>Number of linkers included depends on number of assays ordered.</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>2–8°C</td>
<td>R50AO-1</td>
<td>40 mL</td>
<td>Biotin-containing buffer to stop linker-antibody coupling reaction.</td>
</tr>
<tr>
<td>Read Buffer T (4X)*</td>
<td>RT</td>
<td>R92TC-3</td>
<td>50 mL</td>
<td>Buffer to catalyze the electrochemiluminescence reaction. Dilute to 1X or 2X and use at room temperature.</td>
</tr>
</tbody>
</table>

*Remove bottle from box and store it at room temperature (RT) before use.

**Optional Materials**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Catalog #</th>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash Buffer (20X)</td>
<td>RT</td>
<td>R61AA-1</td>
<td>100 mL</td>
<td>20-fold concentrated phosphate buffer solution with surfactant.</td>
</tr>
</tbody>
</table>

For Research Use Only. Not for use in diagnostic procedures.
Quick Guide

**U-PLEX Plate Preparation**

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

- Couple an individual biotinylated antibody (or other suitable capture reagent) to a unique linker and record the antibody identity next to the linker number on the Spot Map (see next page).

  - Dilute each biotinylated antibody to 10 µg/mL in coating diluent* for a final volume of ≥200 µL per plate.
  - Add 200 µL of each biotinylated antibody to 300 µL of the assigned linker. A different linker must be used for each biotinylated antibody. Vortex. Incubate at room temperature for 30 minutes. Refer to the U-PLEX plate Spot Map to determine which linkers can be combined. For example, when coating 4 assays on a 4-Assay U-PLEX plate, the antibodies should be coupled to Linkers 1, 3, 8, and 10 respectively to match the Spot Map.
  - Add 200 µL of Stop Solution. Vortex. Incubate at room temperature for 30 minutes. Each individual U-PLEX-coupled antibody solution is at 10X the coating concentration and can be stored for 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. 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. Vortex. The multiplex coating solution can be stored for up to 7 days at 2-8°C.

**Step 3  Coat U-PLEX Plate**

- Add 50 µL of multiplex coating solution to each well. Seal plate with an adhesive plate seal and incubate with shaking at room temperature for 1 hour or at 2-8°C for overnight.
- Wash plate 3 times with at least 150 µL/well of PBS-T (PBS plus 0.05% Tween-20) or MSD Wash Buffer. The plate is coated and ready for use. Plates may be stored in the original pouch with desiccant up to 7 days at 2-8°C.

*Please consult the U-PLEX Development Pack product insert for more details on plate coating procedures and guidance for assay development. The U-PLEX Development Pack product insert can be found at www.mesoscale.com/U-PLEX.

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Spot Map

Map your assay spot locations by writing the name of each analyte next to its spot number.
Typical Assay Protocol

NOTE: Follow U-PLEX Plate Preparation before beginning this assay protocol.

Step 1 Add Samples and Calibrators
- Add 50 µL of sample, calibrator, or control to each well. Seal plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

Step 2 Wash and Add Detection Antibody Solution
- Wash plate 3 times with 150 µL/well of PBS-T or MSD Wash Buffer.
- Add 50 µL of detection antibody solution to each well. Seal plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.
  User should conjugate detection antibodies using MSD GOLD SULFO-TAG NHS-Ester prior to running the experiment. Refer to MSD GOLD SULFO-TAG NHS-Ester product insert for details of detection antibody conjugation.

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 plate.

Please refer to the U-PLEX Development Pack product insert for assay optimization examples.

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