MSD S-PLEX Platform

S-PLEX Human IL-5 Kit

Tested on serum, EDTA plasma, citrate plasma, heparin plasma, and cell culture supernatants.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
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S-PLEX is MSD’s ultra-sensitive assay platform. It can dramatically improve the sensitivity of immunoassays, reducing the lower limit of detection (LLD) by 10- to 1000-fold over other assay methods. Results vary from assay to assay, but detection limits in the low femtogram/mL range are common. These low detection limits enable the measurement of analytes at lower concentrations, reduce sample volume required, and reduce the use of critical reagents.

S-PLEX uses electrochemiluminescence (ECL) technology, retaining its well-known advantages and superior analytical performance. The improved sensitivity of S-PLEX is due, in part, to the new TURBO-TAG™ and TURBO-BOOST™ reagents. When TURBO-TAG is combined with an antibody labeled with TURBO-BOOST, more signal is generated when compared to other ECL formats that use SULFO-TAG™ as the detection label.

The S-PLEX platform uses the same robust MSD instruments as other MSD assays. If you own or have access to an MSD instrument, you can run S-PLEX assays. The protocol for S-PLEX is also straightforward, similar to other MSD assay methods. It is comprised of three simple steps: (1) Assemble the immunoassay; (2) Enhance with a TURBO-TAG label; and (3) Read on an MSD instrument.

The increased sensitivity of S-PLEX assays has important implications. S-PLEX shifts the dynamic range of assays, resulting in low detection limits. S-PLEX assays provide up to 4 logs of linear dynamic range, and use minimal sample volumes. S-PLEX assays can measure analytes that were previously below the detectable range of existing assays, enabling the discovery and use of new biomarkers. As an example, the standard curve and values for native and stimulated samples for a representative cytokine assay (Human IL-17A) are shown in Figure 1. The high sensitivity of the S-PLEX assay (LLD of 52 fg/mL) allows for the detection of IL-17A in normal samples, where it is not readily detected by standard immunoassay formats (samples n = 64). Measurement of stimulated samples on the S-PLEX platform and on standard assay formats (MSD V-PLEX) confirmed concordance between platforms.

![IL-17A Calibrator Curve Comparison](a) ![IL-17A Native Samples](b) ![IL-17A Stimulated Sample Concordance](c)

**Figure 1. Standard Curves and Values for Native and Stimulated Samples for a Representative Cytokine Assay**

All assay formats shown in the figure use the same antibodies, calibrators, and diluents. (a) Calibration curves for the IL-17A assay run on three MSD assay formats. (b) IL-17A is detectable in all normal samples tested on the S-PLEX format but not with either of the other assay formats. The solid line represents the LLOD. Dashed lines show the estimated lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) for each assay format. (c) The third graph shows stimulated-sample measurement concordance between V-PLEX and S-PLEX assay formats.
Principle of the Assay

S-PLEX assays use S-PLEX 96-Well SECTOR Plates (Figure 2). These plates are coated with streptavidin and provide high sensitivity, consistent performance, and excellent inter- and intra-lot precision. S-PLEX Kits are supplied with a biotinylated capture antibody, a TURBO-BOOST conjugated detection antibody, calibrator, assay and antibody diluents, and S-PLEX-specific reagents.

![S-PLEX® 96-Well SECTOR® Plate](image)

**Figure 2.** S-PLEX Singleplex Assay on an S-PLEX 96-well SECTOR Plate

Performing an S-PLEX assay is similar to other MSD assays. The protocol is simple, robust, and uses common laboratory techniques. A graphical representation of the protocol is shown in Figure 3. The steps are outlined below:

**ASSEMBLE**

- Coat S-PLEX 96-Well SECTOR Plate using a solution of biotin-conjugated capture antibody and S-PLEX Coating Reagent C1.
- Add samples and calibrators.
- Add TURBO-BOOST detection antibody.

**ENHANCE**

- Add S-PLEX Enhance Solution.
- Add S-PLEX Detection Solution. This detection solution includes the TURBO-TAG label that is required for the electrochemiluminescent signal. During this step, TURBO-TAG binds to the enhanced TURBO-BOOST. TURBO-BOOST or TURBO-TAG alone will not generate any signal.

**READ**

- Add MSD Read Buffer and read on an MSD instrument.
Figure 3. S-PLEX Assay Format in S-PLEX 96-Well SECTOR Plate
Kit Components

S-PLEX Assay Kits are available as Singleplex assays. See below for details.

**Note:** Components will be packaged by storage conditions for ease of storage and shipping.

Table 1. Reagents and Components that are Supplied with the S-PLEX Human IL-5 Kit

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Storage</th>
<th>Catalog #</th>
<th>Size</th>
<th>Quantity Supplied</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-PLEX 96-Well SECTOR Plate</td>
<td>2–8 °C</td>
<td>L45KA-1</td>
<td>1-spot</td>
<td>1 plate</td>
<td>Plates for coating with capture antibodies</td>
</tr>
<tr>
<td>Diluent 100*</td>
<td>2–8 °C</td>
<td>R50AA-4</td>
<td>50 mL</td>
<td>1 bottle</td>
<td>Coating buffer for capture antibody and S-PLEX Coating Reagent C1</td>
</tr>
<tr>
<td>S-PLEX Coating Reagent C1 (200X)</td>
<td>≤-70 °C</td>
<td>C20H0-3</td>
<td>300 µL</td>
<td>1</td>
<td>Reagent mixed with capture antibody for plate coating. Enhances assay signals</td>
</tr>
<tr>
<td>Blocker S1 (100X)</td>
<td>≤-10 °C</td>
<td>R93AG-1</td>
<td>500 µL</td>
<td>1</td>
<td>Added to assay diluent. Reduces non-specific signals.</td>
</tr>
<tr>
<td>S-PLEX Enhance E1 (4X)</td>
<td>≤-10 °C</td>
<td>R82AA-1</td>
<td>1.7 mL</td>
<td>1</td>
<td>Reagent 1 of 3 for Enhance Step</td>
</tr>
<tr>
<td>S-PLEX Enhance E2 (4X)</td>
<td>≤-10 °C</td>
<td>R82AB-1</td>
<td>1.7 mL</td>
<td>1</td>
<td>Reagent 2 of 3 for Enhance Step</td>
</tr>
<tr>
<td>S-PLEX Enhance E3 (200X)</td>
<td>≤-70 °C</td>
<td>R82AC-1</td>
<td>50 µL</td>
<td>1</td>
<td>Reagent 3 of 3 for Enhance Step</td>
</tr>
<tr>
<td>S-PLEX Detect D1 (4X)</td>
<td>≤-70 °C</td>
<td>D20K0-2</td>
<td>1.7 mL</td>
<td>1</td>
<td>Reagent 1 of 2 for Detection Step (contains TURBO-TAG label)</td>
</tr>
<tr>
<td>S-PLEX Detect D2 (200X)</td>
<td>≤-70 °C</td>
<td>D20J0-2</td>
<td>50 µL</td>
<td>1</td>
<td>Reagent 2 of 2 for Detection Step</td>
</tr>
<tr>
<td>Biotin Human IL-5 Antibody</td>
<td>2–8 °C</td>
<td>C21J3-2</td>
<td>170 µL</td>
<td>1</td>
<td>Assay-specific biotinylated capture antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C21J3-3</td>
<td>850 µL</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>TURBO-BOOST Human IL-5 Antibody</td>
<td>2–8 °C</td>
<td>D21J3-2</td>
<td>45 µL</td>
<td>1</td>
<td>TURBO-BOOST conjugated detection antibody</td>
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<td></td>
<td></td>
<td>D21J3-3</td>
<td>225 µL</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Human IL-5 Calibrator</td>
<td>2–8 °C</td>
<td>C01J3-2</td>
<td>1vial</td>
<td>1 vial</td>
<td>Contains analyte of known concentration. Used for creating the standard curve for each assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 vials</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 vials</td>
<td></td>
</tr>
<tr>
<td>Diluent 43</td>
<td>≤-10 °C</td>
<td>R50AG-1</td>
<td>10 mL</td>
<td>1 bottle</td>
<td>Assay diluent for samples and Calibrator</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R50AG-2</td>
<td>50 mL</td>
<td>1 bottle</td>
<td></td>
</tr>
<tr>
<td>Diluent 3</td>
<td>≤-10 °C</td>
<td>R50AP-1</td>
<td>8 mL</td>
<td>1 bottle</td>
<td>Antibody diluent for diluting the TURBO-BOOST Antibody</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R50AP-2</td>
<td>40 mL</td>
<td>1 bottle</td>
<td></td>
</tr>
<tr>
<td>MSD GOLD™ Read Buffer A*</td>
<td>RT</td>
<td>R92TG-3</td>
<td>18 mL</td>
<td>1 bottle</td>
<td>Buffer to catalyze the electrochemiluminescence reaction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R92TG-4</td>
<td>90 mL</td>
<td>1 bottle</td>
<td></td>
</tr>
</tbody>
</table>

*All reagents listed above are kit-specific with the exception of Diluent 100 and MSD GOLD Read Buffer A. Lot-specific information for each assay can be found in the certificate of analysis (COA). RT = room temperature.

**Note:** For reagent handling instructions, please refer to the Best Practices section (below).
Additional Materials and Equipment

Materials

- Adhesive plate seals
- Sample diluent
- Micropipettes with filtered tips
- Tubes (polypropylene microcentrifuge tubes, conical tubes, library tubes)
- Serological pipettes and pipette controller
- Reagent reservoir
- Plastic bottles
- Wet ice and ice bucket
- Deionized water
- Molecular biology grade water
- MSD Wash Buffer (catalog no. R61AA-1) used at 1X, or phosphate-buffered saline (PBS) plus 0.05% Tween-20 (PBS-T)

Equipment

- Microtiter plate shaker capable of shaking at 500–1,000 rpm
- Microtiter plate shaker capable of shaking at 500–1,000 rpm and maintaining a controlled temperature of 27 °C (e.g., Kisker heated plate shaker)
- Plate washing equipment (automated plate washer or multichannel pipette)
- Vortex mixer
- Water bath
- Microcentrifuge

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), which can be obtained from MSD Customer Service or at www.mesoscale.com.
Best Practices

- Mixing and substituting reagents from different sources or different kit lots is not recommended. Lot information is provided in the lot-specific COA.
- Bring frozen diluents to room temperature in a 22–25 °C water bath prior to use. If a controlled water bath is not available, thaw at room temperature. Ensure that diluents are fully thawed and equilibrated to room temperature before use. Mix well after thawing and before use.
- To avoid cross-contamination between vials, open vials for one protocol step at a time (vial caps are color coded), use filtered pipette tips, and use a fresh pipette tip for each reagent addition.
- Prepare Calibrators and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid bubbles in wells during all pipetting steps as they may lead to variable results. Bubbles introduced when adding read buffer may interfere with signal detection.
- Plate shaking should be vigorous, with a rotary motion between 500 –1,000 rpm. Binding reactions may reach equilibrium sooner if shaken at the middle of this range (~700 rpm) or above.
- Use adhesive plate seal for all incubation steps.
- When using an automated plate washer, use individual wash cycles and rotate the plate 180 degrees between wash steps to improve assay precision and reduce potential assay issues due to washing.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- Avoid excessive drying of the plate during washing steps. Add solutions to the plate immediately after washing.
- Remove the plate seal prior to reading the plate.
- Make sure that the Read Buffer is at room temperature when adding to a plate.
- Do not shake the plate after adding Read Buffer.
- To improve inter-plate precision, keep time intervals consistent between adding Read Buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding Read Buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.
- If the sample requires higher dilutions, Diluent 100 may be used in place of assay diluent.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. 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.
- Avoid prolonged exposure of the S-PLEX Detect D1 reagent and detection solutions to light. Keep stocks of S-PLEX Detect D1 reagent in the dark. During the detection incubation step, plates do not need to be shielded from light except for direct sunlight.
- For washing S-PLEX assays, best results are obtained by using a low dispense flow rate and by positioning dispenser tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). This is most important after the detection solution incubation step. See Appendix A for more information on plate washing recommendations.
Reagent Preparation

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

Important: Upon first thaw, aliquot Diluent 43 and Diluent 3 into suitable volumes before refreezing.

Reagents prepared at each step are sufficient for a one-plate experiment.

STEP 1: ASSEMBLE

Prepare Coating Solution

Biotinylated capture antibody is provided as a 40X stock solution and S-PLEX Coating Reagents C1 as a 200X stock solution.

Prepare the Coating Solution immediately prior to use by combining:

- 150 µL of Biotin Human IL-5 Antibody
- 30 µL of 200X S-PLEX Coating Reagent C1
- 5.82 mL Diluent 100. Mix by vortexing.

Note: The unused S-PLEX Coating Reagent C1 should be frozen immediately after use. The reagent is stable through 5 freeze-thaw cycles.

Prepare Blocking Solution

Blocking Solution is the assay diluent supplemented with Blocker S1, and is designed to reduce non-specific binding in the sample matrix. Blocker S1 is provided as a 100X stock solution.

Prepare the Blocking Solution by combining:

- 35 µL of 100X Blocker S1
- 3,465 µL of Diluent 43. Mix by vortexing.

Note: One vial of Blocker S1 is sufficient for blocking 5 plates. If fewer than 5 plates are run, the unused Blocker S1 should be frozen immediately after use. The reagent is stable through 5 freeze-thaw cycles.

Prepare Calibrator Dilutions

MSD supplies a lyophilized calibrator that yields the recommended highest calibrator concentration when reconstituted, and then diluted as directed.

Prepare the highest calibrator concentration (Standard 1):

- Reconstitute lyophilized Human IL-5 Calibrator by adding 1,000 µL of Diluent 43 to the vial. Invert at least 3 times (do not vortex). Let the reconstituted solution equilibrate at room temperature for 15–30 minutes, and then vortex briefly using short pulses.

Note: Reconstituted calibrator is not stable when stored at 2–8 °C; however, it may be stored in aliquots at ≤-70 °C and is stable for one freeze-thaw cycle. For the lot-specific concentration of the calibrator, refer to the COA supplied with the kit. You can also find a copy of the COA at www.mesoscale.com.
This results in a **30X concentrated stock of the calibrator**, which will need to be diluted 30-fold to generate the highest point in the standard curve (Standard 1). Perform two-series dilution as below to generate Standard 1.

- Add 20 µL of the reconstituted calibrator to 180 µL of Diluent 43 to generate Intermediate 1. Mix by vortexing briefly (10-fold dilution).
- Add 100 µL of the Intermediate 1 to 200 µL of Diluent 43 to generate Standard 1. Mix by vortexing briefly (3-fold dilution).

Prepare the remaining standards plus a zero standard for up to 4 replicates (Figure 4):

- Prepare Standard 2 by adding 50 µL of Standard 1 to 150 µL of Diluent 43. Mix by vortexing.
- Repeat 4-fold serial dilutions five additional times to generate Standards 3–7. Mix by vortexing between each serial dilution.
- Use Diluent 43 as Standard 8 (zero standard).

![Figure 4](image)

**Figure 4.** Dilution Schema for Preparation of Calibrator Standards

**Sample Collection and Handling**

Below are general guidelines for sample collection, storage, and handling. If possible, use published guidelines.\(^1-5\) Evaluate sample stability under the selected method as needed.

- **Serum and plasma.** When preparing serum, allow samples to clot for 2 hours at room temperature; then centrifuge for 20 minutes at 2,000 x g prior to using or freezing. If no particulates are visible, you may not need to centrifuge.
- **Other samples.** Use immediately or freeze.

Freeze all samples in suitably-sized aliquots; they may be stored at ≤ -10 °C until needed. Repeated freeze-thaw of samples is not recommended. After thawing, centrifuge samples at 2,000 x g for 3 minutes to remove particulates prior to sample preparation. Hold on wet ice or 2–8 °C until used in the assay.
Dilute Samples
Serum and plasma samples do not require dilution for measuring IL-5. The assay requires 25 µL/well of sample. You may conserve sample by using a higher dilution. The dilution factor for other sample types will need to be optimized. Additional diluent can be purchased at www.mesoscale.com.

Prepare TURBO-BOOST Antibody Solution
Detection antibody is provided as a 200X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use.

- Add 30 µL of TURBO-BOOST Human IL-5 Antibody to 5.97 mL Diluent 3.

STEP 2: ENHANCE

Prepare Enhance Solution
Prepare the Enhance Solution up to 30 minutes prior to use. Allow 1 vial of each S-PLEX Enhance E1, E2, and E3 reagent to equilibrate to room temperature.
Label a single 15 mL tube (e.g., Enhance) and combine the following:

- 2.97 mL Molecular Biology Grade water
- 1.5 mL of 4X S-PLEX Enhance E1
- 1.5 mL of 4X S-PLEX Enhance E2
- 30 µL of 200X S-PLEX Enhance E3

Notes: S-PLEX Enhance E3 stock solution is viscous. Use slow pipetting technique to avoid bubble formation in the pipette tip and to ensure accurate pipetting volume.

Prepare TURBO-TAG Detection Solution
Prepare the TURBO-TAG Detection Solution up to 30 minutes prior to use. Allow 1 vial of each S-PLEX Detect D1 and D2 reagent to equilibrate to room temperature.
Label a single 15 mL tube (e.g., Detect) and combine the following:

- 4.47 mL Molecular Biology Grade water
- 1.5 mL of 4X S-PLEX Detect D1
- 30 µL of 200X S-PLEX Detect D2

Notes:
- Avoid prolonged exposure of the S-PLEX Detect D1 reagent and Detection Solution to light.
- S-PLEX Detect D2 solution is viscous. Use slow pipetting technique to avoid bubble formation in the tip and to ensure accurate pipetting volume.

STEP 3: READ
MSD provides MSD GOLD Read Buffer A ready for use. Do not dilute.
Assay Protocol

Note: Follow Reagent Preparation (above) before beginning this assay protocol.

STEP 1: ASSEMBLE

❑ Coat Plate with Biotin Antibody
  • Prepare coating solution containing biotinylated capture antibody and Coating Reagent C1. Wash plate. Add 50 µL of coating solution to each well. Tap the plate gently on all sides. Seal plate with an adhesive plate seal.
  • Incubate at room temperature with shaking (700 rpm) for 1 hour, or overnight without shaking at 2–8 °C.

❑ Add Samples and Calibrators
  • Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
  • Add 25 µL of blocking solution to each well. Tap the plate gently on all sides.
  • Add 25 µL of calibrator or sample to each well. Seal plate with an adhesive plate seal.
  • Incubate at room temperature with shaking (700 rpm) for 1.5 hours.

❑ Add TURBO-BOOST Antibody Solution to complete the assay
  • Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
  • Add 50 µL of TURBO-BOOST antibody solution to each well. Seal plate with an adhesive plate seal.
  • Incubate at room temperature with shaking (700 rpm) for 1 hour.

STEP 2: ENHANCE

❑ Add Enhance Solution
  • Prepare enhance solution containing Enhance E1, E2 and E3.
  • Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
  • Add 50 µL of enhance solution to each well. Seal plate with an adhesive plate seal.
  • Incubate at room temperature with shaking (700 rpm) for 30 minutes.

❑ Add TURBO-TAG Detection Solution
  • Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T.
  • Add 50 µL of detection solution to each well. Seal plate with an adhesive plate seal.
  • Incubate at 27 °C in a temperature controlled chamber with shaking for 1 hour.

STEP 3: READ

❑ Add Read Buffer
  • Wash plate 3 times with at least 300 µL/well of 1X MSD Wash Buffer or PBS-T using washer program with low dispense speed. See Appendix A for more details.
  • Add 150 µL of MSD GOLD Read Buffer A to each well. Read the plate on an MSD instrument. Incubation in MSD GOLD Read Buffer A is not required before reading the plate.
Assay Performance

A representative data set for the S-PLEX Human IL-5 assay is presented below and is also available at [www.mesoscale.com](http://www.mesoscale.com). The data represent performance of the assay tested in singleplex format. 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.

**Representative Calibrator Curve and Sensitivity**

![Calibrator Curve](image)

<table>
<thead>
<tr>
<th>Dilution from Reconstituted Calibrator to Standard 1 (top of curve)</th>
<th>15X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suggested Sample Dilution</td>
<td>Neat</td>
</tr>
<tr>
<td>LLOD (fg/mL)</td>
<td>2.02</td>
</tr>
<tr>
<td>LLOQ (fg/mL)</td>
<td>5.9</td>
</tr>
<tr>
<td>ULOQ (fg/mL)</td>
<td>19,000</td>
</tr>
</tbody>
</table>

**Figure 5. Typical Calibrator Curves for the S-PLEX Human IL-5 Kit**

The calibration curves used to calculate analyte concentrations were established by fitting the signals from the Calibrators using a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting. The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero Standard). The upper limit of quantification (ULOQ) is the highest concentration at which the CV of calculated concentration is <20% and the recovery of each analyte is within 80% to 120% of the known value. The lower limit of quantification (LLOQ) is the lowest concentration at which the CV of calculated concentration is <20% and the recovery of each analyte is within 80% to 120% of the known value. Analyte concentrations were determined from the electrochemiluminescence signals by back-fitting to the calibration curve.

**Tested Samples**

Normal serum, EDTA plasma, citrate plasma, heparin plasma, and cell culture supernatant samples were tested without dilution.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Serum (N = 26)</th>
<th>EDTA Plasma (N = 10)</th>
<th>Citrate Plasma (N = 10)</th>
<th>Heparin Plasma (N = 10)</th>
<th>Cell Culture Supernatant (N = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (fg/mL)</td>
<td>530</td>
<td>450</td>
<td>490</td>
<td>580</td>
<td>23,000</td>
</tr>
<tr>
<td>Range (fg/mL)</td>
<td>61–9,200</td>
<td>310–3,200</td>
<td>300–3,800</td>
<td>370–3,700</td>
<td>29–48,000</td>
</tr>
<tr>
<td>% Detected</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Dilution Linearity
Normal human serum, EDTA plasma, citrate plasma, and heparin plasma were tested at different dilutions. Percent recovery at each dilution level was normalized to the dilution-adjusted, neat concentration. Samples may require additional dilution with assay diluent to reduce matrix effects. NA = not applicable.

% Recovery = (measured concentration / expected concentration) X 100.

<table>
<thead>
<tr>
<th>Fold Dilution</th>
<th>Serum</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
<th>EDTA Plasma</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
<th>Citrate Plasma</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
<th>Heparin Plasma</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>100</td>
<td>NA</td>
<td></td>
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<tr>
<td>2</td>
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<td>101</td>
<td>78–123</td>
<td>4</td>
<td>100</td>
<td>84–113</td>
</tr>
<tr>
<td>8</td>
<td>102</td>
<td>83–126</td>
<td></td>
<td>8</td>
<td>93</td>
<td>79–112</td>
<td>8</td>
<td>107</td>
<td>79–140</td>
<td>8</td>
<td>101</td>
<td>89–120</td>
</tr>
</tbody>
</table>

Spike Recovery
Normal human serum, EDTA plasma, citrate plasma, and heparin plasma were spiked with calibrator at 3 levels. Spiked samples were tested neat. Samples may require additional dilution with assay diluent to reduce matrix effects.

% Recovery = (measured concentration / expected concentration) x 100.

<table>
<thead>
<tr>
<th>Spike Level</th>
<th>Serum</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
<th>EDTA Plasma</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
<th>Citrate Plasma</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
<th>Heparin Plasma</th>
<th>Average % Recovery</th>
<th>% Recovery Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>89</td>
<td>80–96</td>
<td></td>
<td>86</td>
<td>81–90</td>
<td></td>
<td>95</td>
<td>90–100</td>
<td></td>
<td>91</td>
<td>80–99</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>87</td>
<td>76–99</td>
<td></td>
<td>86</td>
<td>79–96</td>
<td></td>
<td>92</td>
<td>82–102</td>
<td></td>
<td>88</td>
<td>73–99</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>92</td>
<td>78–107</td>
<td></td>
<td>91</td>
<td>79–110</td>
<td></td>
<td>95</td>
<td>82–108</td>
<td></td>
<td>91</td>
<td>76–104</td>
<td></td>
</tr>
</tbody>
</table>

Specificity
To assess specificity, the S-PLEX Human IL-5 assay was tested individually against a larger panel of analytes for nonspecific binding (Eotaxin, Eotaxin-2, Eotaxin-3, G-CSF, GM-CSF, GRO-α, I-309, IFN-α2a, IFN-γ, IL-10, IL-12/23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17D, IL-17E/IL-25, IL-17F, IL-18, IL-1α, IL-1β, IL-1RA, IL-2, IL-21, IL-22, IL-23, IL-27, IL-29, IL-3, IL-31, IL-33, IL-4, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-2, MCP-3, MCP-4, M-CSF, MDC, MIF, MIP-1α, MIP-1β, MIP-3α, MIP-5, TARC, TNF-α, TNF-β, TPO, TRAIL, TSLP, VEGF-A, and YKL-40).
Nonspecific binding was less than 0.5%.

% Nonspecificity = (nonspecific signal/specific signal) x 100.
Assay Components

Calibrators

The assay calibrator uses the following recombinant human protein:

**Table 2. Recombinant Human Proteins Used in the Calibrator**

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Expression System</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5</td>
<td>Sf21 insect cell</td>
</tr>
</tbody>
</table>

Antibodies

**Table 3. Antibody Source Species**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Source Species</th>
<th>Assay Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSD Capture Antibody</td>
<td>MSD Detection Antibody</td>
</tr>
<tr>
<td>IL-5</td>
<td>Mouse Monoclonal</td>
<td>Mouse Monoclonal</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td></td>
</tr>
</tbody>
</table>

References

Appendix A: Recommended Plate Washer Parameters

When using an automated plate washer for S-PLEX assays, best results are obtained by using a low dispense flow rate and by positioning dispense tips at the outer edge of the well (e.g., horizontal dispense offset towards the left side of the well). This low flow rate dispense program is recommended for washing after the detection step in S-PLEX assays; all other steps can use default wash programs. However, for convenience, plates can be washed using the low dispense flow rate program for all S-PLEX assay wash steps.

We recommend creating a new program for your automated plate washer with the optimal settings before starting your S-PLEX assay. Example settings for a typical (MSD-recommended) wash program and the S-PLEX program are shown below for a common plate washer (Biotek Model 405 LS). The only different parameters are the Dispense Rate and Dispense X-Position.

We recommend creating a new program for your automated plate washer with the optimal settings before starting your S-PLEX assay. Example settings for a typical (MSD-recommended) wash program and the S-PLEX program are shown below for a common plate washer (Biotek Model 405 LS). The only different parameters are the Dispense Rate and Dispense X-Position.

**Table 4. Parameters for Customized Programs on the Biotek 405 LS Microplate Washers**

<table>
<thead>
<tr>
<th>Wash Program Parameters</th>
<th>Typical Wash Program Settings</th>
<th>Recommended S-PLEX Wash Program Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate type</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Cycles</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>ASPIRATION</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirate Type</td>
<td>TOP</td>
<td>TOP</td>
</tr>
<tr>
<td>Travel Rate</td>
<td>1 (4.1% 1.0 mm/sec)</td>
<td>1 (4.1% 1.0 mm/sec)</td>
</tr>
<tr>
<td>Aspirate Delay</td>
<td>0500 msecs</td>
<td>0500 msecs</td>
</tr>
<tr>
<td>Aspirate X-Position</td>
<td>-35 (1.600 mm)</td>
<td>-35 (1.600 mm)</td>
</tr>
<tr>
<td>Aspirate Y-Position</td>
<td>-35 (1.600 mm)</td>
<td>-35 (1.600 mm)</td>
</tr>
<tr>
<td>Asp Height</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Secondary Asp?</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>DISPENSE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispense Rate</td>
<td>05</td>
<td>02</td>
</tr>
<tr>
<td>Dispense Volume</td>
<td>0300 µL/well</td>
<td>0300 µL/well</td>
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<tr>
<td>Vacuum Delay Vol</td>
<td>0300 µL/well</td>
<td>0300 µL/well</td>
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<tr>
<td>Dispense X-Position</td>
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<td>-35 (1.600 mm)</td>
</tr>
<tr>
<td>Dispense Y-Position</td>
<td>00 (0.000 mm)</td>
<td>00 (0.000 mm)</td>
</tr>
<tr>
<td>Dispense Height</td>
<td>120 (15.245 mm)</td>
<td>120 (15.245 mm)</td>
</tr>
<tr>
<td>OPTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash Pre-dispense?</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Bottom Wash?</td>
<td>NO</td>
<td>NO</td>
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<tr>
<td>MIDCYC</td>
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<td></td>
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<tr>
<td>Wash Shake?</td>
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<td>NO</td>
</tr>
<tr>
<td>Wash Soak?</td>
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<td>NO</td>
</tr>
<tr>
<td>Home Carrier?</td>
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<td>NO</td>
</tr>
<tr>
<td>Between Cyc PreDisp?</td>
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<td>NO</td>
</tr>
<tr>
<td>POST</td>
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<td></td>
</tr>
<tr>
<td>Final Aspirate?</td>
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<td>YES</td>
</tr>
<tr>
<td>Aspirate Type</td>
<td>TOP</td>
<td>TOP</td>
</tr>
<tr>
<td>Travel Rate</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Fin Asp Delay</td>
<td>0500 msecs</td>
<td>0500 msecs</td>
</tr>
<tr>
<td>Fin Asp X-Position</td>
<td>-35 (1.600 mm)</td>
<td>-35 (1.600 mm)</td>
</tr>
<tr>
<td>Fin Asp Y-Position</td>
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<td>-35 (1.600 mm)</td>
</tr>
<tr>
<td>Fin Asp Height</td>
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<tr>
<td>Secondary Aspirate?</td>
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<td>YES</td>
</tr>
<tr>
<td>Fin Asp Sec X-Pos</td>
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<td>35 (1.600 mm)</td>
</tr>
<tr>
<td>Fin Asp Sec Y-Pos</td>
<td>35 (1.600 mm)</td>
<td>35 (1.600 mm)</td>
</tr>
<tr>
<td>Fin Asp Sec Height</td>
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<td>22</td>
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</tbody>
</table>
Figure 5. Plate Diagram