ProcartaPlex™ Multiplex Immunoassay

USER GUIDE

Using Magnetic Beads for Serum, Plasma, and Cell Culture Supernatant Samples

Instructions for Human Isotyping Assays

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product use

This user manual is for a ProcartaPlex™ Immunoassay Kit to perform quantitative, multiplexed protein measurements from serum, plasma, and cell culture supernatant samples using magnetic beads technology from Luminex™. Other biological samples might be suitable for use in the assay.

Note: ProcartaPlex™ Human Antibody Isotyping Panels cannot be combined with other ProcartaPlex™ Panels and Simplex Kits.

For the most current version of user documentation, visit our website.

How it works

ProcartaPlex™ Immunoassays incorporate magnetic microsphere technology licensed from the Luminex™ Corporation to enable the simultaneous detection and quantitation of multiple protein targets in diverse matrices. The platform allows the simultaneous detection from a single sample of up to 80 protein targets on the Luminex™ 200™ and FLEXMAP 3D™ platforms and 50 protein targets on the MAGPIX™ platform.
Materials provided and storage conditions

ProcartaPlex™ Immunoassay Kits contain the components listed below. Refer to the Certificate of Analysis for quantities and details of components supplied. Store kit at 2–8°C. Expiration date is stated on the kit. Do not use after expiration date.

<table>
<thead>
<tr>
<th>Components supplied</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen Standards, premixed</td>
</tr>
<tr>
<td>Detection Antibody, premixed (50X)[1]</td>
</tr>
<tr>
<td>Antibody Coupled Magnetic Beads, premixed (1X)[1]</td>
</tr>
<tr>
<td>Streptavidin-PE (SA-PE) (1X)[1]</td>
</tr>
<tr>
<td>Wash Buffer Concentrate (10X)[1]</td>
</tr>
<tr>
<td>Detection Antibody Diluent[1]</td>
</tr>
<tr>
<td>Universal Assay Buffer (10X)[1]</td>
</tr>
<tr>
<td>Reading Buffer[1]</td>
</tr>
<tr>
<td>8-Tube Strip</td>
</tr>
<tr>
<td>96-Well Flat Bottom Plate</td>
</tr>
<tr>
<td>Black Microplate Lid</td>
</tr>
<tr>
<td>Plate Seals</td>
</tr>
</tbody>
</table>

[1] Contains sodium azide. See WARNING.

**WARNING!** All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice. This kit contains small quantities of sodium azide. Sodium azide is highly toxic and reactive in the pure form. At this product’s concentration, though not classified as hazardous, buildup of sodium azide may react with lead and copper plumbing to form highly reactive explosive metal azide. Dispose of the product in accordance with all state and local regulations.
Precautions and technical hints

- Thoroughly read this user manual and Certificate of Analysis that is included with the assay kit. The product insert may contain specific instructions for proper use of your kit.
- For Luminex™ 200™ and FLEXMAP 3D™ instruments initiate the startup protocol to warm up the lasers for at least 30 minutes. Ensure that the Luminex™ machine is calibrated according to the manufacturer's instructions. MAGPIX™ instrument doesn’t require additional warm up.
- When working with samples and standards, change the pipette tips after every transfer and avoid creating bubbles when pipetting.
- During the incubation steps, cover the 96-well Flat Bottom Plate with the Black Microplate Lid provided in the kit to minimize exposure of the beads to light.
- Be careful not to invert the 96-well Flat Bottom Plate during the assay or allow contents from one well to mix with another well.
- Use a multi-channel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
- Store the reconstituted standards on ice before adding to the 96-well Flat Bottom Plate.

Required equipment and materials not supplied

- MAGPIX™, Luminex™ 200™, FLEXMAP 3D™, or Luminex™-based instrument.
- Glass-distilled or deionized water.
- Adjustable single and multichannel pipettes with disposable tips.
- Multichannel pipette reservoir.
- Beakers, flasks, and cylinders necessary for preparation of reagents.
- Hand-Held Magnetic Plate Washer, vortex mixer, and microtiter plate shaker.

Sample preparation

- For frozen samples, thaw samples on ice and mix well by vortexing followed by centrifugation at 10,000 × g for 5–10 minutes to remove particulates. Avoid multiple freeze/thaw cycles.
- If samples are high in lipid content, centrifuge at 10,000 × g for 10 minutes at 2-8°C and transfer contents to a new tube.
1. Collect samples in sodium citrate or EDTA tubes. When using heparin as an anticoagulant, no more than 10 IU of heparin per mL of blood collected should be used because an excess of heparin may give falsely high values of some of the analytes.

2. Centrifuge samples at $1,000 \times g$ at $4^\circ C$ for 10 minutes within 30 minutes of collection.

3. Collect the plasma fraction. Use immediately or aliquot and store at $-80^\circ C$.

**Serum sample preparation**

Spin down serum samples at $1,000 \times g$ for 10 minutes at $20–25^\circ C$ before running the assay.

1. Allow blood to clot for 20–30 minutes at $20–25^\circ C$.

2. Centrifuge at $1,000 \times g$ for 10 minutes at $20–25^\circ C$.

3. Collect the serum fraction. (Alternatively, use any standard serum separator tube following the manufacturer's instructions.)

4. Use immediately or aliquot and store at $-80^\circ C$.

**Dilute samples with high concentration of target analytes**

The analytes included in the kit have high serum or plasma concentrations. Dilute each sample according to the following scheme:

- Dilute most serum and plasma samples 1:20,000 in Universal Assay Buffer (1X) according to the following table.

<table>
<thead>
<tr>
<th>1:20,000 Dilution</th>
<th>Volume of Sample</th>
<th>Volume of UAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 1 (1:200)</td>
<td>10 μL</td>
<td>1990 μL</td>
</tr>
<tr>
<td>Dilution 2 (1:100)</td>
<td>10 μL</td>
<td>990 μL</td>
</tr>
</tbody>
</table>

- Dilute cell culture supernatant 1:200 in cell culture medium (CCM) according to the following table.

<table>
<thead>
<tr>
<th>1:200 Dilution</th>
<th>Volume of Sample</th>
<th>Volume of CCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution (1:200)</td>
<td>10 μL</td>
<td>1990 μL</td>
</tr>
</tbody>
</table>

For samples that yield results outside the range of the standard curve, a lower or higher dilution might be required.
Assay protocol overview

Prepare Antigen Standard

Add Magnetic Beads
Vortex beads for 30 seconds. Add 50 µL of the beads to each well. Remove liquid.

Serum and Plasma Samples
Add to the beads:
• 25 µL of Universal Assay Buffer
• 25 µL of standards, controls, or samples

Cell Culture Supernatant Samples
Add to the beads:
• 50 µL of standards, controls, or samples

Wash the plate.

Seal Plate. Shake at room temperature for 60-120 min or overnight incubation for assays that require higher sensitivity.
Wash beads (2X).

Detection Antibody Preparation

Add Detection Antibody
Add 25 µL of Detection Antibody Mix (1X)
Seal Plate. Incubate with shaking at room temperature for 30 min.
Wash beads (2X).

Add Streptavidin-PE
Add 50 µL of Streptavidin-PE
Seal Plate. Incubate with shaking at room temperature for 30 min.
Wash beads (2X).

Resuspend Beads
Add 120 µL of Reading Buffer
Seal Plate. Shake at room temperature for 5 min.

Acquire Data on Luminex™ 200™, Magpix™, or FlexMAP3D™ system

Preparation of reagents

Prepare 1X Detection Antibody Mixture
Detection antibody is provided at a 50X concentration and requires dilution prior to use.

1. Add 60 µL of each Detection Antibody concentrate to a mixing bottle.
2. Fill up with Detection Antibody Diluent to a final volume of 3 mL if using the whole plate.
<table>
<thead>
<tr>
<th>Reagents</th>
<th>48-well Plate</th>
<th>96-well Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection Antibody</td>
<td>30 µL</td>
<td>60 µL</td>
</tr>
<tr>
<td>Detection Antibody Diluent</td>
<td>1470 µL</td>
<td>2940 µL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1500 µL</td>
<td>3000 µL</td>
</tr>
</tbody>
</table>

**Prepare 1X Wash Buffer**

Bring the Wash Buffer Concentrate (10X) to room temperature and vortex for 15 seconds. Mix 20 mL of the Wash Buffer Concentrate (10X) with 180 mL ddH₂O. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

**Note:** Wash Buffer Concentrate volume might not be sufficient if using automated plate washer. For bulk orders use Cat. No. EPX-66666-001.

**Prepare Universal Assay Buffer (1X)**

Prepare a 1X working concentration of Universal Assay Buffer by mixing 10 mL of the 10X Universal Assay Buffer with 90 mL ddH₂O.

Store the Universal Assay Buffer Concentrate (10X) at 2-8°C. When stored properly the Universal Assay Buffer (1X) is stable for 30 days.

**Prepare antigen standard**

Carefully read the Certificate of Analysis for lot-specific information on the kit components. This kit is supplied with lyophilized multistandards containing a mix of multiple standard proteins. Each kit is shipped with 2 identical vials of each premixed antigen standard set from the same lot to permit the user to run the assay twice if running a partial plate.

**Reconstitute standards**

1. Centrifuge the antigen standard set vial at 2,000 x g for 10 seconds.

2. Add 250 µL of sample type specific buffer into the standard vial. If you want to measure serum or plasma samples, use Universal Assay Buffer (1X) to reconstitute the standard, if you want to analyze cell culture supernatant samples, use the cell culture medium that was used to culture the cells to dissolve the standard.

3. Gently vortex the vial for 30 seconds and centrifuge at 2,000 x g for 10 seconds to collect contents at the bottom of the vial.

4. Incubate on ice for 10 min to ensure complete reconstitution.

**Prepare 3-fold serial dilution**

1. Prepare a 3-fold serial dilution of the reconstituted standard(s) using the 8-tube strip provided. Label tubes Std1, Std2, Std3, Std4, Std5, Std6, and Std7.

2. Add 200 µL of the reconstituted antigen standard into the first tube of the strip and label as Standard 1 (Std1).

3. Add 150 µL of sample type specific standard buffer into Std tubes 2–7. Use Universal Assay Buffer for serum or plasma samples and cell culture media for culture supernatant samples.

4. Transfer 75 µL of the reconstituted and prediluted antigen standard from Tube 1 into Tube 2.
5. Mix by pipetting up and down for a total of 10 times.

6. Transfer 75 μL of the mixed standards from Tube 2 into Tube 3.

7. Mix by pipetting up and down for a total of 10 times.


9. Add 200 μL of Universal Assay Buffer or cell culture medium into tube 8, which serves as a blank. Keep on ice until ready to use.

Assay protocol

1. Define the plate map.
   Mark the standard, sample, and blank wells using the plate map at the end of this manual.

2. Add magnetic beads to the plate.
   a. Vortex the Magnetic Bead vial for 30 seconds.
   b. Add 50 μL of the Magnetic Bead solution to each well of the plate. Use a multichannel pipette for this step as well as for the steps below.

3. Wash magnetic beads.
   a. Securely insert the 96-well Flat Bottom Plate into the Hand-Held Magnetic Plate Washer, ensure that the plate is held in place by the tabs, and wait 2 minutes to allow the beads to accumulate on the bottom of each well.
   b. Remove the liquid in the wells by quickly inverting the Hand-Held Magnetic Plate Washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the Hand-Held Magnetic Plate Washer. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.
   c. Add 150 μL of Wash Buffer (1X) into each well and wait 30 seconds to allow the beads to accumulate on the bottom of each well.
d. Remove the Wash Buffer in the wells by quickly inverting the Hand-Held Magnetic Plate Washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the Hand-Held Magnetic Plate Washer. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.

e. Remove the 96-well Flat Bottom Plate from the Hand Held Magnetic Plate Washer and proceed to the next step.

4. Add sample type-specific buffer, samples, standards, and blanks, and then incubate.
   a. For serum and plasma: Add 25 μL of Universal Assay Buffer (1X) to each well followed by 25 μL of prepared standards or samples into dedicated wells. For cell culture supernatants: Add 50 μL prepared standards or samples into dedicated wells.
   b. For wells designated as blanks: Add an additional 25 μL of Universal Assay Buffer for serum or plasma samples. For cell culture supernatant samples, add 50 μL of cell culture medium.
   c. Seal the plate with the provided Plate Seal. Cover the plate with the Black Microplate Lid and shake at 500 rpm for 60 to 120 minutes at room temperature.
      Alternatively, the 96-well plate can be incubated overnight. Shake the 96-well plate for 30 minutes at room temperature at 500 rpm, then transfer the plate to 4°C and store on a level surface. After overnight incubation, shake the plate for an additional 30 minutes at room temperature at 500 rpm.

5. Wash the 96-well plate twice following step 3.

6. Add Detection Antibody Mixture and incubate.
   a. Add 25 μL of Detection Antibody Mixture (1X) to each well.
   b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 500 rpm.

7. Wash the 96-well plate twice following step 3.

8. Add SAPE and incubate.
   a. Add 50 μL of SAPE solution to each well.
   b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 500 rpm.

9. Wash the 96-well plate twice following step 3.

10. Prepare the 96-well plate for analysis on a Luminex™ instrument.
    a. Add 120 μL of Reading Buffer into each well.
b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 5 minutes on a plate shaker at room temperature at 500 rpm.

c. Remove Plate Seal and run the plate on a Luminex™ instrument.

Setup of the instruments

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Sample size</th>
<th>DD gate</th>
<th>Timeout</th>
<th>Bead event/bead region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminex™ 200™</td>
<td>50 μL</td>
<td>5,000–25,000</td>
<td>60 seconds</td>
<td>50–100</td>
</tr>
<tr>
<td>FLEXMAP 3D™</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAGPIX™</td>
<td>50 μL</td>
<td>N/A</td>
<td>N/A</td>
<td>50–100</td>
</tr>
</tbody>
</table>

Prior to running the assay, ensure that the probe height has been calibrated with 96-well Flat Bottom Plate supplied with the kit. Failure to adjust the probe height can cause damage to the instrument or low bead count. The Luminex™ system allows for calibration of low and high RP1 target values. We recommend RP1 low target value settings for ProcartaPlex™ immunoassays. When entering the information into the Luminex™ Acquisition Software, refer to the Certificate of Analysis provided with the kit for bead region and S1 values for each analyte of the current lot.

**Note:** If there is a malfunction of the Luminex™ instrument or software during the run, the 96-well Flat Bottom Plate can be re-read. Remove the 96-well Flat Bottom Plate from the instrument, insert the 96-well Flat Bottom Plate into the Hand-Held Magnetic Plate Washer, wait 2 minutes, then remove the buffer in the wells by quickly inverting the 96-well Flat Bottom Plate over a sink or waste container. Blot the assembly onto several layers of paper towels to remove any residual solution. Resuspend the beads in 120 μL of Reading Buffer, remove from the Hand-Held Magnetic Plate Washer, seal the 96-well Flat Bottom Plate with a new Plate Seal and Lid and shake at 500 rpm for 5 minutes at room temperature. The assayed samples may take longer to read since there will be less beads in the well.

Analyzing results

The concentration of the samples can be calculated by plotting the expected concentration of the standards against the MFI generated by each standard. A 4PL or 5PL algorithm is recommended for the best curve fit. Analyze the assayed samples according to the operation manual for the Luminex™ instrument (e.g., MAGPIX™, Luminex™ 200™, FLEXMAP 3D™). We offer a free and robust analysis software package for data analysis. For download information visit our website or contact our technical support.
## Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Probable cause</th>
<th>Recommend solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Flow Rate</td>
<td>Samples/beads are stuck in flow cell</td>
<td>Remove the 96-well Plate and perform a wash and rinse cycle.</td>
</tr>
<tr>
<td>High CVs</td>
<td>Samples and antigen standards not stored on ice</td>
<td>Prepare the samples and standards on ice before setting up the assay.</td>
</tr>
<tr>
<td>Contamination from reusing the Plate Seal</td>
<td>Use a new Plate Seal for each incubation step.</td>
<td></td>
</tr>
<tr>
<td>Incomplete washing</td>
<td>After adding the standards and samples, it is very important that any excess standards are removed during the wash step.</td>
<td></td>
</tr>
<tr>
<td>Contamination from contents from adjacent wells</td>
<td>Avoid splashing the Wash Buffer during wash steps into adjacent wells.</td>
<td></td>
</tr>
<tr>
<td>Poor pipetting techniques</td>
<td>Use a multichannel pipettor and careful pipette techniques. Avoid touching pipette tips to sides of the wells when adding Wash Buffer.</td>
<td></td>
</tr>
<tr>
<td>Limited dynamic range for BioPlex software users</td>
<td>Instrument calibrated at high PMT settings</td>
<td>Calibrate the instrument using the CAL2 Low RP1 target value.</td>
</tr>
<tr>
<td>Low bead count</td>
<td>Volume of bead solution is too low</td>
<td>Add 120 μL Reading Buffer into each well and shake at 500 rpm for 5 minutes at room temperature to resuspend beads prior to reading on the Luminex™ instrument.</td>
</tr>
<tr>
<td>High bead aggregation</td>
<td>Vortex the bead suspension well before using in the assay and ensure that the beads are properly mixed during the incubation steps.</td>
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<tr>
<td>Dyes contained in the beads are photo-bleached from overexposure to light</td>
<td>Store bead solution and the 96-well plate in the dark.</td>
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<tr>
<td>Samples causing the instrument to clog</td>
<td>Remove the 96-well Flat Bottom Plate and perform a wash and rinse to the instrument. Rerun the assay with further dilution of samples</td>
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<tr>
<td>Probe height is incorrect</td>
<td>Refer to the Luminex™ Manual for proper adjustment of the needle height.</td>
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<tr>
<td>Observation</td>
<td>Probable cause</td>
<td>Recommend solution</td>
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<tr>
<td>Low bead count</td>
<td>Instrument needle is partially clogged</td>
<td>Replace or clean needle according to the manufacturer’s recommendations.</td>
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<td></td>
<td>Beads stuck to the bottom of the plate</td>
<td>Confirm that the plate shaker is set to 500 rpm and shaking for at least 5 minutes before reading.</td>
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<td></td>
<td>Air bubble in the sample loop</td>
<td>Refer to the Luminex™ manual for proper removal of the air bubble.</td>
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<tr>
<td>Low signal or sensitivity</td>
<td>Standards not reconstituted and diluted correctly</td>
<td>Prepare fresh antigen standards following the instructions in “Prepare antigen standard” on page 10</td>
</tr>
<tr>
<td>Poor recovery</td>
<td>Did not use appropriate cell culture media to prepare the standards</td>
<td>Use the same cell culture media that is used to culture the cells.</td>
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<tr>
<td></td>
<td>Samples and antigen standards were not stored on ice</td>
<td>Prepare the samples and standards on ice before setting up the assay.</td>
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## Recommended and blank plate layout

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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