IMPORTANT! Pay close attention to vortexing, shaking, and incubation instructions. Deviation from the protocol may result in low assay signal and assay variability.

### Initial Preparation

1. Plan the plate layout.

2. Start up/warm up the Bio-Plex Multiplex Immunoassay System **(30 min)**.
   - Bring diluents, including wash buffer, assay buffer, detection antibody diluent HB, and sample diluent, to room temperature (RT). Keep the other items on ice until needed
   - Begin to thaw frozen samples
   - Prepare 1x wash buffer
     - Mix by inversion to ensure all salts are in solution
     - Dilute 1 part 10x wash buffer (60 ml) with 9 parts distilled water (540 ml)

3. Calibrate the Bio-Plex System by following the prompts within Bio-Plex Manager Software. This can be done now or during an assay incubation step.
Bio-Plex Pro SARS-CoV-2 Serology Assay

4. Prepare sample dilution according to the guidelines provided in the table. It is important to centrifuge serum or plasma samples at 1,000 x g for 10 min at 4°C to remove particulates from all samples prior to use.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Recommended Dilution Factor</th>
<th>Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum and plasma</td>
<td>1:100</td>
<td>Sample diluent</td>
</tr>
</tbody>
</table>

5. Vortex coupled beads at medium speed for 30 sec and dilute to 1x in Bio-Plex Assay Buffer as shown. Protect from light.

<table>
<thead>
<tr>
<th>Number of Wells</th>
<th>20x Beads, µl</th>
<th>Assay Buffer, µl</th>
<th>Total Volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>288</td>
<td>5462</td>
<td>5,750</td>
</tr>
</tbody>
</table>

Running the Assay

Note: Make sure all assay components are at RT before pipetting.

1. Vortex the diluted (1x) beads. Dispense 50 µl to each well of the assay plate.

2. Wash the plate two times with 100 µl Bio-Plex Wash Buffer.

3. Vortex samples, blank, and controls. Add 50 µl to each well.

4. Cover plate with sealing tape and protect from light with aluminum foil. Incubate on shaker at 850 ± 50 rpm at RT for 30 min.

5. With 10 min left in the incubation, vortex detection antibodies for 15 sec and quick-spin to collect liquid. Dilute to 1x as shown in the table.

<table>
<thead>
<tr>
<th>Number of Wells</th>
<th>20x Ab, µl</th>
<th>Detection Ab Diluent HB, µl</th>
<th>Total Volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>150</td>
<td>2,850</td>
<td>3,000</td>
</tr>
</tbody>
</table>

6. After the first 30 min incubation is completed, wash the plate three times with 100 µl wash buffer.

7. Vortex the diluted (1x) detection antibodies. Add 25 µl to each well.

8. Cover plate with sealing tape, protect from light with aluminum foil, and incubate at 850 ± 50 rpm in the dark for 30 min at RT. Meanwhile, prepare Bio-Plex Manager Software protocol.
9. With 10 min left in the incubation, **vortex** 100x SA-PE for 5 sec and quick-spin to collect liquid. **Dilute to 1x** as shown in the table and protect from light.

<table>
<thead>
<tr>
<th>Number of Wells</th>
<th>100x SA-PE, µl</th>
<th>Assay Buffer, µl</th>
<th>Total Volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>96</td>
<td>60</td>
<td>5,940</td>
<td>6,000</td>
</tr>
</tbody>
</table>

10. After the second 30 min incubation is completed, wash the plate three times with 100 µl wash buffer.

11. **Vortex** the diluted (1x) SA-PE. Dispense 50 µl to each well

12. Cover plate with sealing tape, protect from light with aluminum foil, and incubate at **850 ± 50 rpm** in the dark for **10 min** at RT.

13. After the 10 min incubation is completed, wash the plate three times with 100 µl wash buffer.

14. Resuspend the beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm** for **30 sec**.

15. Remove the sealing tape and **read plate** using the settings in the table.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>RP1 (PMT)</th>
<th>DD Gates</th>
<th>Bead Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Plex 100, 200</td>
<td>Low</td>
<td>5,000 (low); 25,000 (high)</td>
<td>50</td>
</tr>
<tr>
<td>MAGPIX</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>use default</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>instrument</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>settings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-Plex 3D</td>
<td>Standard</td>
<td>Select Mag Plex Beads</td>
<td>50</td>
</tr>
</tbody>
</table>
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