Bio-Plex Pro SARS-CoV-2 Serology Assay

Quick Guide

For research use only. Not for use in diagnostic procedures.

For Use with	Instruction Manual #
Bio-Plex Pro SARS-CoV-2 Serology Assays	10000133853

This guide can be used to prepare and run a full 1 x 96-well assay plate. For more information on a given step, refer to the corresponding section of the complete instruction manual. New users can download the manual, which includes detailed instructions and a list of kit components, at bio-rad.com/bio-plex.

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.

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.

Sample Type	Recommended Dilution Factor	Diluent
Serum and plasma	1:100	Sample diluent

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

Number of Wells	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	288	5462	5,750

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.

Number of Wells	20x Ab, µl	Detection Ab Diluent HB, µl	Total Volume, µl
96	150	2,850	3,000

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.

Number of Wells	100x SA-PE, µl	Assay Buffer, µl	Total Volume, µl
96	60	5,940	6,000

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.

Instrument	RP1 (PMT)	DD Gates	Bead Events
Bio-Plex 100, 200	Low	5,000 (low); 25,000 (high)	50
MAGPIX	N/A use default instrument settings	N/A	N/A
Bio-Plex 3D	Standard	Select MagPlex Beads	50

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