

# 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 Human 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](https://www.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.

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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, $\mu$ l	Assay Buffer, $\mu$ l	Total Volume, $\mu$ l
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  $\mu$ l** to each well of the assay plate.
2. Wash the plate two times with **100  $\mu$ l** Bio-Plex Wash Buffer.
3. Vortex samples, blank, and controls. Add **50  $\mu$ l** to each well.
4. Cover plate with sealing tape and protect from light with aluminum foil. Incubate on shaker at **850  $\pm$  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, $\mu$ l	Detection Ab Diluent HB, $\mu$ l	Total Volume, $\mu$ l
96	150	2,850	3,000

6. After the first 30 min incubation is completed, wash the plate three times with **100  $\mu$ l** wash buffer.
7. **Vortex** the diluted (1x) detection antibodies. Add **25  $\mu$ l** to each well.
8. Cover plate with sealing tape, protect from light with aluminum foil, and incubate at **850  $\pm$  50 rpm** in the dark for **30 min** at RT. Meanwhile, prepare Bio-Plex Manager Software protocol.

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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, $\mu$ l	Assay Buffer, $\mu$ l	Total Volume, $\mu$ l
96	60	5,940	6,000

10. After the second 30 min incubation is completed, wash the plate three times with **100  $\mu$ l** wash buffer.
11. **Vortex** the diluted (1x) SA-PE. Dispense 50  $\mu$ l to each well
12. Cover plate with sealing tape, protect from light with aluminum foil, and incubate at **850  $\pm$  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  $\mu$ l** wash buffer.
14. Resuspend the beads in **125  $\mu$ l** assay buffer. Cover and shake at **850  $\pm$  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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