

Bio-Plex™ Pro Human SARS-CoV-2 Serology Assays

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 go to [bio-rad.com/SARS-CoV-2Serology](https://www.bio-rad.com/SARS-CoV-2Serology) to download the manual, which includes detailed instructions and a list of kit components.

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 in Bio-Plex Manager Software. This can be done now or during an assay incubation step.

Bio-Plex Pro Human SARS-CoV-2 Serology Assays

4. Prepare the 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

* Adjust the sample dilution scheme as needed for very high- and low-level anti-SARS-CoV-2 N/RBD/S1/S2 IgA, IgG, and IgM samples. Dilutions of 1:100 to 1:1,000 have worked, depending on the antibody concentration in samples.

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

Number of Wells	20x Beads, μ l	Assay Buffer, μ l	Total Volume, μ l
96	288	5,462	5,750

Running the Assay

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

1. Vortex the diluted (1x) beads. Dispense **50 μ l** to each well of the assay plate.
2. Wash the plate two times with **100 μ l** Bio-Plex Wash Buffer.
3. Vortex samples, blank, and controls. Add **50 μ l** to each well.
4. Cover the 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 Detection Antibody, μ l	Detection Antibody Diluent HB, μ l	Total Volume, μ l
96	150	2,850	3,000

6. After the first 30 min incubation is completed, wash the plate three times with **100 μ l** wash buffer.
7. Vortex the diluted (1x) detection antibodies. Add **25 μ l** to each well.

Bio-Plex Pro Human SARS-CoV-2 Serology Assays

8. Cover the 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 the Bio-Plex Manager Software protocol.
9. With 10 min left in the incubation, vortex 100x streptavidin-phycoerythrin (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, μ l	Assay Buffer, μ l	Total Volume, μ l
96	60	5,940	6,000

10. After the second 30 min incubation is completed, wash the plate three times with **100 μ l** wash buffer.
11. Vortex the diluted (1x) SA-PE. Dispense **50 μ l** to each well
12. Cover the 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 μ l** wash buffer.
14. Resuspend the beads in **125 μ l** 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.

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

* Or similar Luminex System.

** Discontinued.

BIO-RAD and BIO-PLEX are trademarks of Bio-Rad Laboratories, Inc. in certain jurisdictions. Luminex is a trademark of Luminex Corporation. All trademarks used herein are the property of their respective owner. © 2023 Bio-Rad Laboratories, Inc.

The Bio-Plex Suspension Array System includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Website bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800
Austria 00 800 00 24 67 23 **Belgium** 00 800 00 24 67 23 **Brazil** 4003 0399
Canada 1 905 364 3435 **China** 86 21 6169 8500
Czech Republic 00 800 00 24 67 23 **Denmark** 00 800 00 24 67 23
Finland 00 800 00 24 67 23 **France** 00 800 00 24 67 23
Germany 00 800 00 24 67 23 **Hong Kong** 852 2789 3300
Hungary 00 800 00 24 67 23 **India** 91 124 4029300 **Israel** 0 3 9636050
Italy 00 800 00 24 67 23 **Japan** 81 3 6361 7000 **Korea** 82 080 007 7373
Luxembourg 00 800 00 24 67 23 **Mexico** 52 555 488 7670
The Netherlands 00 800 00 24 67 23 **New Zealand** 64 9 415 2280
Norway 00 800 00 24 67 23 **Poland** 00 800 00 24 67 23
Portugal 00 800 00 24 67 23 **Russian Federation** 00 800 00 24 67 23
Singapore 65 6415 3188 **South Africa** 00 800 00 24 67 23
Spain 00 800 00 24 67 23 **Sweden** 00 800 00 24 67 23
Switzerland 00 800 00 24 67 23 **Taiwan** 886 2 2578 7189
Thailand 66 2 651 8311 **United Arab Emirates** 36 1 459 6150
United Kingdom 00 800 00 24 67 23

