

Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 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 Neutralization Antibody Assays	10000147006

This guide can be used to prepare and run a full 1 x 96-well assay plate. Refer to the complete instruction manual for more information on a given step. New users can go to [bio-rad.com/bio-plex](https://www.bio-rad.com/bio-plex) and download the manual, which includes detailed instructions and a list of kit components.

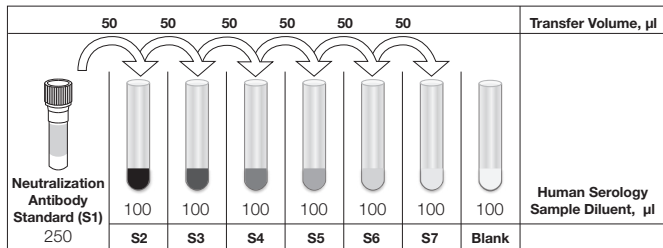
IMPORTANT! Pay close attention to **vortexing**, **shaking**, and **incubation** instructions. Deviation from the protocol may result in low assay signal and assay variability.

Initial Preparation

1. Plan the plate layout.
2. Start up/warm up the Bio-Plex Multiplex Immunoassay System (**30 min**).
3. Bring the following Bio-Plex Pro reagents to room temperature: Assay Buffer (1x), Human Serology Sample Diluent, and Wash Buffer (10x).
4. Keep the following Bio-Plex Pro reagents on ice until needed: SARS-CoV-2 Coupled Beads (20x), Biotinylated Detection ACE2 Receptor (20x), Neutralization Assay Positive Control, SARS-CoV-2 Neutralization Antibody Standard, and Streptavidin-Phycoerythrin (SA-PE) (100x).
5. Begin to thaw frozen samples.
6. Prepare 1x wash buffer. Mix 10x stock by inversion to ensure all salts are in solution. Dilute **1 part** 10x wash buffer (60 ml) with **9 parts** distilled water (540 ml).
7. 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 Neutralization Antibody Assays

8. Prepare a seven-point standard curve as shown. Add 100 μ l of human serology sample diluent to seven tubes. The last tube will be the blank.



Note: Change tips between each dilution.

9. 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.
10. Prepare sample dilutions in 0.5 or 1.0 ml polypropylene tubes as required for the assay according to the following guidelines.
- For serum and plasma samples, dilute in serology sample diluent. We recommend grouping samples by predicted/estimated neutralization antibody level (for example, samples from vaccinated donors grouped by time since date of vaccination) and performing a dilution series test to identify the optimal dilution factor for your samples. Recommended testing range: 1:5 (for example, 12 μ l sample + 48 μ l serology sample diluent) to 1:100 dilution
 - For other fluids, dilute in serology sample diluent. Dilution factor to be determined by the user
11. **Vortex** the 20x coupled beads at medium speed for **30 sec** and dilute to 1x in assay buffer as shown. Protect from light.

Number of Wells	20x Beads, μ l	Assay Buffer, μ l	Total Volume, μ l
96	285	5,415	5,700

Running the Assay

Note: Make sure all assay components are at room temperature (RT) before pipetting.

- Vortex** the diluted (1x) beads for about **20 seconds**. Add **50 µl** to each well of the assay plate.
- Wash the plate two times** with **100 µl** wash buffer.
- Gently vortex** standard, control, and samples. Add **25 µl** to each well. Add **25 µl** of sample diluent to the blank and negative control wells.
- Cover the plate with sealing tape to protect from light. Incubate on shaker at **850 ± 50 rpm** at RT for **30 min**.
- With 10 min left in the incubation, **vortex** the 20x biotinylated detection ACE2 receptor for **15 sec** and quick-spin to collect liquid. Dilute to 1x as shown.

Number of Wells	20x Biotinylated ACE2, µl	Serology Sample Diluent, µl	Total Volume, µl
96	150	2,850	3,000

- After the first 30 min incubation is completed, **do not wash the plate**.
- Vortex** the diluted (1x) biotinylated detection ACE2 receptor. Add **25 µl** to each well. **Do not dispense biotinylated ACE2 into the well that you will use for your blank. Dispense 25 µl of sample diluent instead.**

Note: The blank well will receive only coupled beads, sample diluent, and SA-PE through the assay workflow. The negative control well will receive only coupled beads, sample diluent, biotinylated detection ACE2 receptor, and SA-PE through the workflow.

- Cover the plate with sealing tape to protect from light. Incubate at **850 ± 50 rpm** in the dark for **30 min** at RT. Meanwhile, prepare the Bio-Plex Manager Software protocol; enter standard S1 values provided in the assay kit.
- With 10 min left in the incubation, **vortex** the 100x SA-PE for **5 sec** and quick-spin to collect liquid. Dilute to 1x as shown and protect from light.

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

Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays

- After the second 30 min incubation is complete, **wash the plate three times** with **100 µl** wash buffer.
- Vortex** the diluted (1x) SA-PE. Dispense **50 µl** to each well.
- Cover the plate with sealing tape to protect from light. Incubate at **850 ± 50 rpm** in the dark for **10 min** at RT.
- After the 10 min incubation is completed, **wash the plate three times** with **100 µl** wash buffer.
- Resuspend the beads in **125 µl** assay buffer. Cover and shake at **850 ± 50 rpm** for **30 sec**.
- Remove the sealing tape and **read plate** using the following settings.

Instrument	RP1 (PMT)	DD Gates	Bead Events
Bio-Plex 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		
Luminex xMAP INTELLIFLEX	Low	7,500 (low); 19,500 (high)	50

* Or similar Luminex System.

Ordering Information

Catalog #	Description
12016897	Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Assay
12016848	Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Assay
17007632	Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Custom Assay Developer Kit
Various	Bio-Plex Pro SARS-CoV-2 Coupled Beads , available with the following SARS-CoV-2 antigens: Alpha S1, Beta S1, Gamma RBD, D614G S1, Delta RBD, Delta Spike Trimer, E484K RBD, Epsilon RBD, K417N RBD, Kappa RBD, and N501Y RBD

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

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

BIO-RAD

**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 2 3473 4460
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

