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

Instruction Manual

For research use only. Not for diagnostic procedures.

BIO-RAD

Table of Contents

Section 1 Description 1

Section 2 Principle 1

Section 3 Kit Contents and Storage 3

Section 4 Recommended Materials 4

Section 5 Assay Workflow 5

Section 6 Important Considerations 6

Section 7 Detailed Instructions 7

1. Plan Plate Layout 7
2. Prepare Instrument 8
3. Prepare Wash Buffer 8
4. Prepare Wash Method 9
5. Prepare Samples 9
6. Prepare Standard Curve 10
7. Prepare Coupled Beads 10
8. Run Assay 11
9. Read Plate 13

Section 8 Troubleshooting Guide 17

Section 9 Plate Layout Template 18

Section 10 Safety Considerations 19

Section 11 Ordering Information 19

Section 1
Description

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a member of a highly diverse family of enveloped, positive-sense, single-stranded RNA viruses. Although much is known about the transmission of and mortality related to this virus, little is known about its long-term pathogenic characteristics. Tracking the impact of the viral variants on transmissibility and vaccine effectiveness is key to further understand the infection and to protect against it. The Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays are magnetic bead–based multiplex assays that allow the detection of anti–SARS-CoV-2 neutralizing antibodies specific for the viral receptor binding domain (RBD) and spike 1 (S1) antigens located on the spike protein. These assays permit the simultaneous evaluation of neutralizing antibodies that inhibit wild-type RBD and S1 and also key variants of SARS-CoV-2 in less than 15 µl of serum and plasma. The multiplexing feature makes it possible to quantify multiple targets in a single well of a 96-well microplate in just 2 hours. Table 1 lists the assays in the panel and their respective bead regions.

Table 1. Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays and bead regions.

Assay	Mutation Site	Bead Region
RBD wild type	N/A	36
S1 wild type	N/A	28
SARS-CoV-2 Alpha S1	HV69-70 del, Y144 del, N501Y, A570D, D614G, P681H	43
SARS-CoV-2 Beta S1	K417N, E484K, N501Y, D614G	47
SARS-CoV-2 Gamma RBD	K417T, E484K, N501Y	37
SARS-CoV-2 D614G S1	D614G	25
SARS-Cov-2 Delta RBD	L452R, T478K	42
SARS-Cov-2 Delta Spike Trimer	Spike trimer (aa 16-1213), T19R, G142D, EF156-157 del, R158G, L452R, T478K, D614G, P681R, D950N	51
SARS-CoV-2 E484K RBD	E484K	45
SARS-CoV-2 Epsilon RBD	L452R	26
SARS-CoV-2 K417N RBD	K417N	15
SARS-CoV-2 Kappa RBD	L452R, E484Q	56
SARS-CoV-2 N501Y RBD	N501Y	30

aa, amino acid; del, deletion; N/A, not applicable; RBD, receptor binding domain; S1, spike 1.

Section 2
Principle

Technology

There are three core elements of the Bio-Plex Multiplex Immunoassay System:

- Fluorescently dyed magnetic microspheres (also called beads), each with a distinct color code or spectral address to permit discrimination of individual tests in a multiplex suspension. This allows simultaneous detection of up to 500 different molecules in a single well of a 96-well microplate on the Bio-Plex 3D Suspension Array System, up to 100 different molecules on the Bio-Plex 200 System, and up to 50 different molecules on the Luminex MAGPIX Multiplex Reader. Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays are also compatible with the Luminex xMAP INTELLIFLEX System
- A dedicated plate reader. The Bio-Plex 200 and Bio-Plex 3D Systems are flow cytometry–based instruments with 2 lasers and associated optics to measure the different molecules bound to the surface of the beads
- A high-speed digital signal processor that efficiently manages the fluorescence data

Assay Format

The Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays are competitive immunofluorescence assays formatted on magnetic beads. The assay principle is described in Figure 1. SARS-CoV-2 viral antigens are covalently coupled to the beads. Coupled beads react with the sample containing anti–SARS-CoV-2 neutralizing antibodies. After a 30 min incubation with samples, standards, controls, and blanks, a biotinylated ACE2 receptor is added directly to the wells (without washing) to compete with anti–SARS-CoV-2 neutralization antibodies for binding to the viral antigen coated to the beads. The final detection complex is formed with the addition of streptavidin-phycoerythrin (SA-PE) conjugate. Phycoerythrin serves as a fluorescent indicator, or reporter.

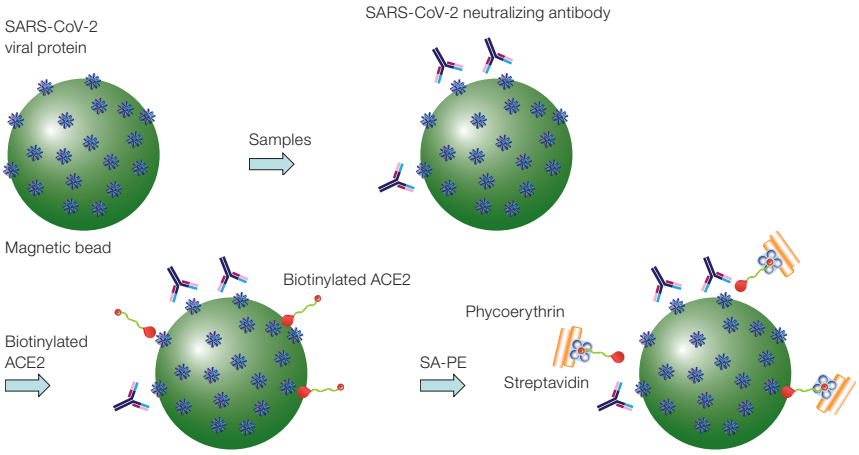


Fig. 1. Assay principle.

Data Acquisition and Analysis

Data from the reactions are acquired using a Bio-Plex System or a comparable Luminex Reader. For example, when a multiplex bead suspension is drawn into the Bio-Plex 200 Reader, a red (635 nm) laser illuminates the fluorescent dyes within each bead to provide bead classification and thus assay identification. At the same time, a green (532 nm) laser excites PE to generate a reporter signal, which is detected by a photomultiplier tube (PMT). A high-speed digital processor manages data output, and Bio-Plex Manager Software presents data as median fluorescence intensity (MFI) as well as concentration (ng/ml) for quantitative assays. All Bio-Plex Pro SARS-CoV-2 Neutralization Antibody Assays provide qualitative data; the amount of neutralization antibodies bound to each bead is inversely proportional to the MFI of reporter signal. The signal decreases with more neutralization antibodies bound because they compete with the biotinylated ACE2 receptor for binding to SARS-CoV-2 antigen–coupled beads. It's the bound biotinylated ACE2 interacting with SA-PE that results in an output of MFI signal. The percentage inhibition can be calculated from the MFI using any spreadsheet program.

Section 3
Kit Contents and Storage

Reagents Supplied

The Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays are available in a flexible format to select from 2 wild-type and 11 variant targets. Choose between complete kits or create your own multiplex kit by purchasing SARS-CoV-2 variant beads and adding them to the complete kits. The assays are performed with assay reagents, diluents, and buffers. An example of a complete kit is shown in Table 2. The assay quick guide (10000147009) can be found on **bio-rad.com/bio-plex**.

Table 2. Contents of a 1 x 96-well kit.

Component	Quantity
Bio-Plex Pro SARS-CoV-2 Neutralization Antibody Standard	1 tube
Bio-Plex Pro SARS-CoV-2 Coupled Beads (20x)	1 tube
Bio-Plex Pro Biotinylated Detection ACE2 Receptor (20x)	1 tube
Bio-Plex Pro Neutralization Assay Positive Control	1 tube
Bio-Plex Pro Human Serology Sample Diluent	1 bottle (55 ml)
Bio-Plex Pro Assay Buffer (1x)	1 bottle (50 ml)
Bio-Plex Pro Assays 10x Wash Buffer	1 bottle
Streptavidin-Phycoerythrin (100x)	1 tube
Assay plate (96-well flat bottom plate)	1 plate
Sealing tape	1 pack of 4
Product data sheet	1 sheet

Storage and Stability

Kit contents should be stored at 4°C and never frozen. Coupled magnetic beads and SA-PE should be stored in the dark. All components are guaranteed for a minimum of 6 months from the date of purchase when stored as specified.

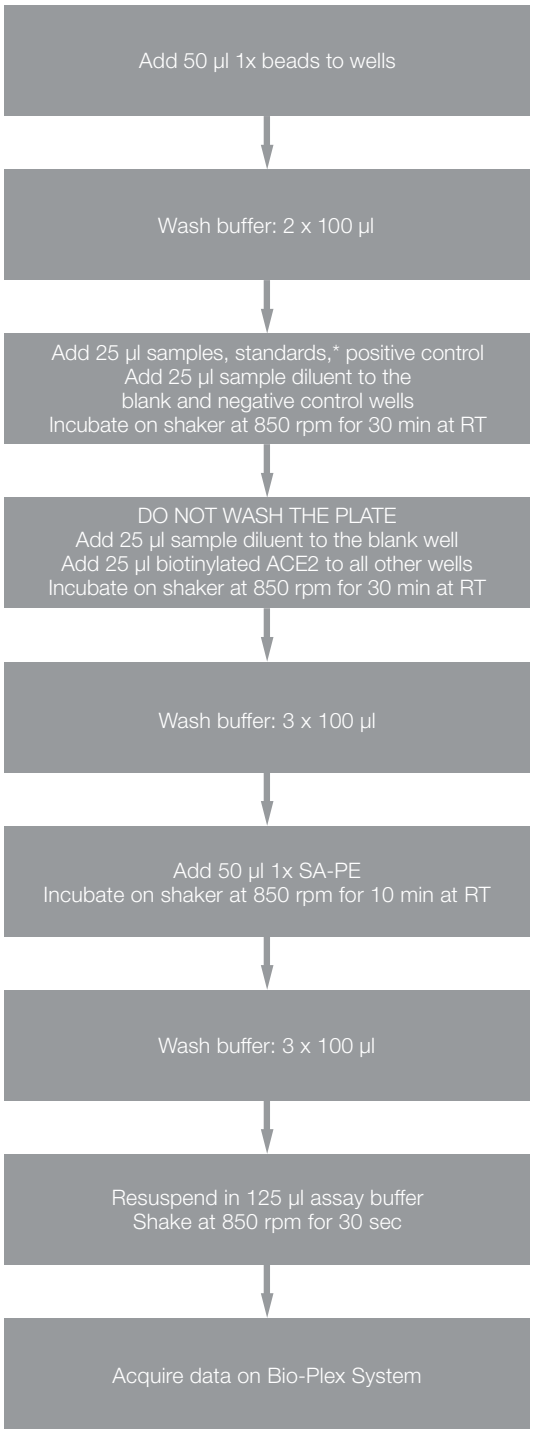
Section 4
Recommended Materials

For recommended materials, see Table 3.

Table 3. Recommended materials.

Item	Ordering Information
Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays Quick Guide	10000147009 (download at **bio-rad.com/bio-plex**)
Bio-Plex Validation Kit 4.0 **Note:** Run the validation kit monthly to ensure optimal performance of fluidics and optics systems	Bio-Rad catalog #171203001
Bio-Plex Calibration Kit **Note:** Run the calibration kit daily to standardize the fluorescence signal	Bio-Rad catalog #171203060
Bio-Plex Pro Wash Station For use with magnetic bead–based assays only	Bio-Rad catalog #30034376
Bio-Plex Handheld Magnetic Washer For use with magnetic bead–based assays only	Bio-Rad catalog #171020100
Bio-Plex Pro Flat Bottom Plates, 40 x 96-well For magnetic separation on the Bio-Plex Pro Wash Station	Bio-Rad catalog #171025001
Titertube Micro Test Tubes For preparing replicate samples, standards, and positive control prior to loading the plate	Bio-Rad catalog #2239390
Microtiter Plate Shaker IKA MTS 2/4 Shaker for 2 or 4 microplates or Barnstead/Lab-Line Model 4625 Titer Plate Shaker (or equivalent capable of 300–1,100 rpm)	IKA catalog #3208000 VWR catalog #57019-600
BR-2000 Vortexer	Bio-Rad catalog #1660610
Reagent Reservoirs, 25 ml For capture beads and detection antibodies	VistaLab catalog #3054-1002 or VistaLab catalog #3054-1004
Acrodisc PF Syringe Filter (25 mm) with Supor Membrane (0.8/0.2 µm), sterile	Pall Corporation catalog #4187
Other materials: 5 or 15 ml polypropylene tubes for reagent dilutions, calibrated pipets, pipet tips, sterile distilled water, absorbent paper towels, aluminum foil, and 1.5 or 2 ml microcentrifuge tubes.	

Section 5
Assay Workflow



Note: Once thawed, keep samples on ice. Prepare dilutions just prior to the start of the assay and equilibrate to room temperature (RT) before use.

* Standard is not included with the qualitative Bio-Plex Pro SARS-CoV-2 Delta RBD and Spike Trimer 2-Plex Assays.

Section 6
Important Considerations

Instruments and Software

The Bio-Plex Pro Assays described in this manual are compatible with all currently available Luminex life science research instruments. Assays can be read and analyzed with either Bio-Plex Manager Software or Luminex xPONENT Software (see Read Plate in section 7).

Assay Procedures

Pay close attention to specific assay reagents and incubation times, as they have been specifically optimized for each assay panel. These assay panels use the Bio-Plex Pro Human SARS-Cov-2 Neutralization Antibody Reagent Kit. Bead regions for all analytes are listed in Table 1.

Assay Quick Guide

The Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Assays Quick Guide (10000147009) can be used to prepare and run a full 1 x 96-well assay plate. Users can download a copy at **bio-rad.com/bio-plex**.

Section 7
Detailed Instructions

The following pages provide detailed instructions for each step of the assay procedure, including preparation, running the assay, and reading the plate with Bio-Plex Manager Software.

1. Plan Plate Layout

Determine the total number of wells in the experiment using the plate layout template on page 18 or the Plate Formatting tab in Bio-Plex Manager Software. A suggested plate layout is shown in Figure 2, with all conditions in duplicate.

Note: For detailed instructions about plate formatting in Bio-Plex Manager Software, see Read Plate in this section.

1. Assign the sample diluent blank to wells A1 and A2. The blank wells will receive coupled beads, sample diluent, and SA-PE. Data from the blank wells will act as a reagent control.

2. Assign the rest of the wells in columns 1 and 2 to a seven-point standard curve.

3. Assign the negative control to wells A3 and A4. **The negative control wells will receive coupled beads, sample diluent, biotinylated detection ACE2 receptor, and SA-PE. The data from these wells will be used to determine the percentage inhibition.**

4. Assign the positive control to wells B3 and B4. **The positive control wells will receive coupled beads, sample diluent, neutralization assay positive control, biotinylated detection ACE2 receptor, and SA-PE. The positive control is used to determine if the assay worked as intended and the values generated can be used to assess plate-to-plate variability.**

5. The remainder of the plate is available for samples.

6. Calculate the required volumes of beads, biotinylated ACE2 receptor, and SA-PE using Tables 5–6, 8, and 9, respectively.

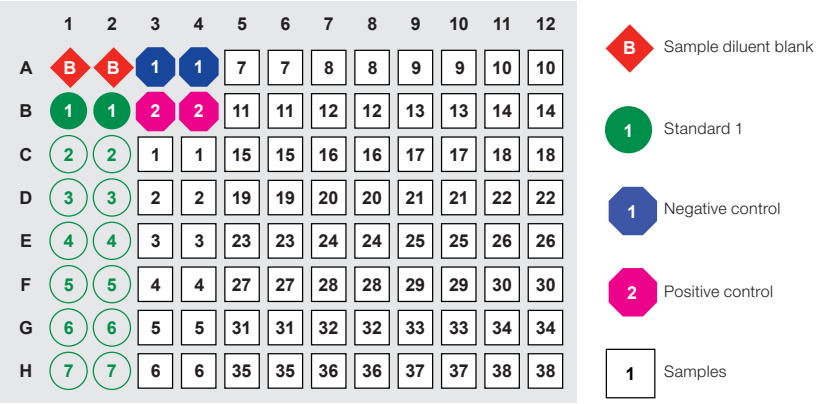


Fig. 2. Suggested plate layout.

2. Prepare Instrument

These directions are specific to the Bio-Plex 200 Reader. To prepare a Bio-Plex 3D Reader, Luminex MAGPIX Multiplex Reader, or Luminex xMAP INTELLIFLEX System, consult their respective user manuals.

Note: While the instrument is warming up, bring the 10x wash buffer, assay buffer, and diluents to room temperature. Keep other items on ice until needed. Also, begin to thaw frozen samples.

Start up and calibrate the Bio-Plex System with Bio-Plex Manager Software prior to setting up the assay. The calibration kit should be run daily or before each use of the instrument to standardize the fluorescence signal. For instructions on using other xMAP System software packages, contact Bio-Rad Technical Support.

The validation kit should be run monthly to ensure optimal performance of fluidics and optics systems. Refer to either the software manual or online Help for directions on how to conduct validation.

Starting Up and Calibrating the System (Bio-Plex 200 or similar)

1. Empty the waste bottle and fill the sheath fluid bottle before starting if the high-throughput fluidics (HTF) unit is not used. This will prevent fluidics system backup and potential data loss.
2. Turn on the reader, XY platform, and HTF (if included). Allow the system to warm up for 30 min.
3. Select **Start up and Calibrate** and follow the instructions. If the system is idle for 4 hr without acquiring data, the lasers will automatically turn off. To reset the 4 hr countdown, select **Warm up** and wait for the lasers/optics to reach operational temperature.

Calibrating the System

1. Confirm that the default values for CAL1 and CAL2 are the same as the values printed on the bottle of Bio-Plex Calibration Beads. Use the Bio-Plex System low RP1 target value.
2. Select **OK** and follow the software prompts and step-by-step instructions for CAL1 and CAL2 calibration.

Note: In Bio-Plex Manager Software version 6.1 and higher, startup, warm-up, and calibration can be performed together by selecting **Start up and calibrate**.

3. Prepare Wash Buffer

1. Bring the 10x stock solution to room temperature.
2. Due to high concentration, crystals may still be present after the buffer has been warmed up to room temperature. Hold the bottle with one hand and slowly swirl it to help solubilize the remaining crystals. Mix the 10x stock solution by inversion before preparing the 1x wash buffer.
3. To prepare 1x wash buffer, dilute 1 part 10x stock solution with 9 parts deionized water.

4. Prepare Wash Method

Compatible wash stations and plates are listed in Table 4.

Table 4. Summary of compatible wash stations and plate type.

Wash Method	Wash Station	Assay Plate
Magnetic separation	Bio-Plex Pro Wash Station Bio-Plex Handheld Magnetic Washer	Flat bottom plate

Setting Up the Bio-Plex Pro Wash Station

The wash station should be primed before use. For more information, refer to the Bio-Plex Pro Wash Station Quick Guide (bulletin 5826).

1. Install the appropriate plate carrier on the wash station.
2. Use the Prime procedure to prime channel 1 with 1x wash buffer.

Setting Up the Bio-Plex Handheld Magnetic Washer

Place an empty flat bottom plate on the magnetic washer by sliding it under the retaining clips. Push the clips inward to secure the plate. Make sure the plate is held securely. If needed, the clips can be adjusted for height and tension. For detailed instructions, refer to the user guide (M10023087).

Warning for Bio-Plex Handheld Magnetic Washer (#171020100) users: Magnetic field can be harmful to pacemaker wearers. Pacemaker wearers should stay back at least 30 cm (12 in.).

5. Prepare Samples

General guidelines for preparing different sample types are provided here. For more information, consult the publications listed in Bio-Rad bulletin 5297, available for download at **bio-rad.com**, or contact Bio-Rad Technical Support.

- Once thawed, keep samples on ice. Prepare the sample dilutions just prior to the start of the assay. Do not freeze diluted samples. Pipet carefully using calibrated pipets and a new pipet tip for every volume transfer

Serum and Plasma

Note: Avoid using hemolyzed samples. Steps 1–4 are intended as a guideline for preparing whole blood serum and plasma. If you are starting with serum and/or plasma, skip to step 5.

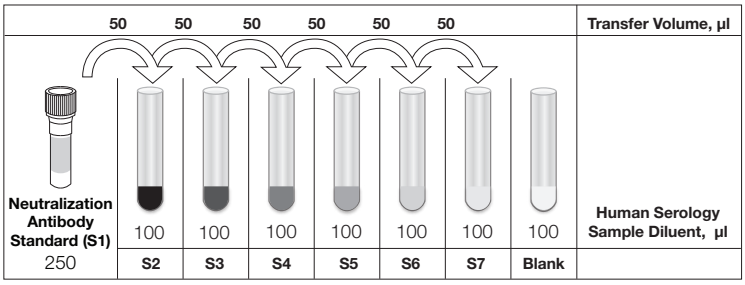
1. Draw whole blood into collection tubes containing anticoagulant. Invert tubes several times to mix.
2. For serum, allow the blood to clot at room temperature for 30 to 45 min, then transfer to a clean polypropylene tube. For plasma, proceed directly to the centrifugation steps.
3. For plasma samples, perform centrifugation at 1,000 x g for 15 min at 4°C and transfer the plasma to a clean polypropylene tube.
4. If precipitates are seen in the plasma, centrifuge again at 10,000 x g for 10 min at 4°C. Alternatively, filter the samples with a 0.8/0.2 µm dual filter to prevent clogging.

   Note: SARS-CoV-2 viral inactivation can be achieved through heat treatment at 60°C for 15 to 30 min.

5. Prepare the sample dilutions in microcentrifuge tubes.
   - 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 µl sample + 48 µl serology sample diluent) to 1:100 dilution
   - For other fluids, dilute in serology sample diluent. Dilution factor to be determined by the user
6. Assay samples immediately. Aliquot neat samples into single-use tubes and store at –70°C. Avoid repeated sample freeze-thaw cycles.

6. Prepare Standard Curve

Prepare a seven-point standard curve as shown. The standard comes in liquid form. This is S1. Add 100 µl of human serology sample diluent to seven tubes. The last tube will be the blank.



Note: Change tips between each dilution.

7. Prepare Coupled Beads

1. Use Tables 5 and 6 to calculate the volume of coupled beads and assay buffer needed to prepare a 1x working solution.
2. Add the required volume of assay buffer to a 15 ml polypropylene tube.
3. Vortex the 20x stock coupled beads at medium speed for 30 sec. Carefully open the cap and pipet any liquid trapped in the cap back into the tube. This is important to ensure maximum bead recovery. Do not centrifuge the vial; doing so will cause the beads to pellet.
4. Dilute coupled beads to 1x by pipetting the required volume into the 15 ml conical centrifuge tube. Vortex the tube.
5. Protect the beads from light with aluminum foil.

Note: To minimize volume loss, use a 200–300 µl capacity pipet to remove beads from the 20x stock tube. If necessary, perform the volume transfer in two steps. Avoid using a 1,000 µl capacity pipet and/or wide bore pipet tip.

Preparing 1x Coupled Beads from Stock (includes 20% excess volume)

Table 5. Premixed panel.

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

Table 6. Mixing singleplex assays.

	Singleplex #1*	Singleplex #2*		
Number of Wells	20x Beads, µl	20x Beads, µl	Assay Buffer, µl	Total Volume, µl
96	285	285	5,130	5,700

* For each additional singleplex assay, add 285 µl of the singleplex beads and subtract 285 µl from the amount of assay buffer added so that the total volume is 5,700 µl.

8. Run Assay

Considerations

- Bring all assay components and samples to RT before use
- Use calibrated pipets and pipet carefully, avoiding bubbles
- Pay close attention to vortexing, shaking, and incubation instructions; deviating from the protocol may result in assay variability
- Cover the plate with sealing tape during assay incubations to avoid reagent evaporation and spillover
- Select the appropriate Bio-Plex Pro Wash Station program, or perform the appropriate manual wash step noted in Table 7, after each assay step

Table 7. Summary of wash options and protocols.

	Bio-Plex Pro Wash Station	Magnetic Washer
Assay Step	Magnetic Program	Manual Wash Steps
Add beads to plate	MAG x2	2 x 100 µl
Sample incubation	–	No wash after this step
Biotinylated ACE2 incubation	MAG x3	3 x 100 µl
SA-PE incubation	MAG x3	3 x 100 µl

Adding Coupled Beads, Samples, Controls, and Sample Diluent Blank

1. Cover the unused wells of the assay plate with sealing tape.
2. Vortex the diluted (1x) beads for 30 sec at medium speed. Pour into a reagent reservoir and transfer 50 µl to each well of the assay plate.

   Tip: A multichannel pipet is highly recommended for ease of use and efficiency.
3. Wash the plate two times with 100 µl wash buffer per well using the wash method of choice.
4. Vortex the diluted samples, standards,* and positive control at medium speed for 5 sec. Transfer 25 µl of each to the appropriate well of the assay plate, changing the pipet tip after every volume transfer. **Dispense 25 µl of sample diluent into the blank and negative control wells.**

5. Cover the plate with a new sheet of sealing tape. Incubate on shaker at 850 ± 50 rpm for 30 min at RT.

Note: Be consistent with the incubation time and shaker setting used here for optimal assay performance and reproducibility.

* Standard is only included with the quantitative Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel and Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel. Standard is not included with the qualitative Bio-Plex Pro SARS-CoV-2 Delta RBD and Spike Trimer 2-Plex Assays.

Preparing and Adding Biotinylated ACE2 Receptor

1. While the samples are incubating, use Table 8 to calculate the volume of biotinylated ACE2 stock and sample diluent needed to prepare a 1x working solution. Prepare this reagent 10 min before use.

Table 8. Premixed panel.

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

2. Add the required volume of sample diluent to a 5 ml polypropylene tube.
3. Vortex the 20x stock biotinylated ACE2 for 5 sec at medium speed, then perform a 30 sec spin to collect the entire volume at the bottom of the tube.
4. Dilute the stock biotinylated ACE2 to 1x by pipetting the required volume into the 5 ml tube. Vortex the tube.
5. After incubating the beads with samples, blanks, and controls, slowly remove and discard the sealing tape.
6. **Do not wash the plate after this step.**
7. Vortex the diluted (1x) biotinylated ACE2 receptor, at medium speed for 5 sec. Transfer into a reagent reservoir and dispense 25 µl to each well (**except for the blank well**) of the assay plate using a multichannel pipet. **Dispense 25 µl of sample diluent into the blank well.**
8. Cover the plate with a new sheet of sealing tape. Incubate on shaker at 850 ± 50 rpm for 30 min at RT.

Preparing and Adding Streptavidin-Phycoerythrin (SA-PE)

1. While biotinylated ACE2 receptor is incubating, use Table 9 to calculate the volume of SA-PE and assay buffer needed to prepare a 1x stock. SA-PE should be prepared 10 min before use.

Table 9. Preparation of 1x SA-PE from 100x stock.

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

2. Add the required volume of assay buffer to a 15 ml polypropylene tube.
3. Vortex the 100x stock SA-PE for 5 sec at medium speed. Perform a 30 sec spin to collect the entire volume at the bottom of the tube.
4. Dilute SA-PE to 1x by pipetting the required volume into the 15 ml tube. Vortex and protect from light until ready to use.
5. After the biotinylated ACE2 incubation, slowly remove and discard the sealing tape.
6. Wash the plate three times with 100 µl of wash buffer per well.

7. Vortex the diluted (1x) SA-PE at medium speed for 5 sec. Pour into a reagent reservoir and transfer 50 µl to each well using a multichannel pipet.
8. Cover the plate with a new sheet of sealing tape. Incubate on shaker at 850 ± 50 rpm for 10 min at RT.
9. After the SA-PE incubation step, slowly remove and discard the sealing tape.
10. Wash the plate three times with 100 µl of wash buffer per well.
11. To resuspend beads for plate reading, add 125 µl assay buffer to each well.
12. Cover the plate with a new sheet of sealing tape. Shake at room temperature at 850 ± 50 rpm for 30 sec. Slowly remove and discard the sealing tape before placing the plate on the reader.
13. Refer to Table 10 for instrument settings.

Table 10. Instrument settings for reading the plate.

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.

9. Read Plate

Bio-Plex Manager Software is recommended for all Bio-Plex Pro Assay data acquisition and analysis. Instructions for Luminex xPONENT Software are also included. For instructions using other xMAP System Software packages, contact Bio-Rad Technical Support or your regional Bio-Rad field applications specialist.

Preparing the Protocol in Bio-Plex Manager Software Version 6.0 and Higher

The protocol should be prepared in advance so that the plate is read as soon as the experiment is complete.

A protocol file specifies the analytes in the assay, the plate wells to be read, sample information, the values of controls, and instrument settings.

Bio-Plex Manager Software version 6.0 and higher contains protocols for most Bio-Plex Pro Assays. Choose from available protocols or create a new protocol. To create a new protocol, select **File**, then **New** from the main menu. Locate and follow the steps under Protocol Settings.

1. Click **Describe Protocol** and enter information about the assay (optional).
2. Click **Select Analytes** and create a new panel. Visually confirm the selected analytes and proceed to step 3.
   a. Click **Add Panel** in the **Select Analytes** toolbar. Enter a new panel name. Select **Bio-Plex Pro Assay Magnetic** from the assay dropdown list. If using Bio-Plex Manager Software version 5.0 or lower, select **MagPlex** from the assay dropdown list.
   b. Click **Add**. Enter the bead region number and name for the first analyte. Click **Add Continue** to repeat for each analyte in the assay. Refer to the bead regions in parentheses ( ) listed on the product data sheet included in the kit.

c. Click **Add** when the last analyte has been added and click **OK** to save the new panel.

d. Highlight analytes from the Available list (left) and move to the Selected list (right) using the Add button. To move all analytes at once, click **Add All**.

e. If some of the analytes need to be removed from the Selected list, highlight them and select **Remove**. It is possible to rename the panel by clicking **Rename Panel** and entering a new panel name.

Note: Do not use preset panels found in Bio-Plex Manager Software version 5.0 or earlier because the bead regions are not up to date.

1. Click **Format Plate** and format the plate according to the plate layout created in the first step of Section 7 (page 7). To modify the plate layout, perform the following steps (see Figure 2 for reference).

   a. Select **Plate Formatting** tab.

   b. Select the Blank icon ◇B and drag the cursor to wells that contain sample blank.

   c. Select the Standard icon (S) and drag the cursor to wells that contain standards.

   d. Repeat this process for Controls (C) and Unknown Samples [X]. In order to include a negative and positive control, create them on the plate map, select the **Plate Groupings** tab, then click the **Group Samples** icon (9) and drag to select the negative and positive controls. Note that Bio-Plex Manager Software automatically subtracts the blank MFI value from all other assay wells.

2. Click **Enter Controls Info**.

   a. For user-specified controls, select an analyte from the dropdown menu, then enter a description and/or concentration. Repeat for each additional analyte in the assay.

   b. For the positive control supplied, format the appropriate wells as control, entering descriptions but leaving the concentrations blank.

3. Click **Enter Sample Info** and enter sample information and the appropriate dilution factor.

4. Click **Run Protocol** and confirm that the assay settings are correct.

   a. Refer to Table 10 for the recommended RP1 (PMT) setting. Protocols using alternative PMT settings should be validated by the end user.

   b. Confirm that data acquisition is set to 50 beads per region. In Advanced Settings, confirm that the bead map is set to 100 regions, the sample size is set to 50 µl (this is intake volume for each well), and the doublet discriminator (DD) gates are set to 5,000 (Low) and 25,000 (High). Select **Start**, save the .rbx file, and begin data acquisition. The Run Protocol pop-up screen will appear. Click **Eject/Retract** to eject the plate carrier.

Acquiring the Data

1. Shake the assay plate at 850 ± 50 rpm for 30 sec and visually inspect the plate to ensure that the assay wells are filled with buffer. Slowly remove the sealing tape before placing the plate on the plate carrier.

2. Click **Run Protocol**. On the pop-up screen, select **Load Plate** and click **OK** to start acquiring data.

3. Select **Wash Between Plates** after every plate run to reduce the possibility of clogging the instrument.

4. If data are acquired from more than one plate, empty the waste bottle and refill the sheath bottle after each plate (if HTF are not present). Select **Wash Between Plates** and follow the instructions. Then repeat the **Preparing the Protocol** and **Acquiring the Data** instructions.

5. When data acquisition is complete, select **Shut Down** and follow the instructions.

Reacquiring the Data

Data from a well or plate can be acquired a second time using the Rerun/Recovery mode located below Start in the Run Protocol step of the software process. Any previous data will be overwritten.

1. Check the wells from which data will be reacquired.

2. Aspirate the buffer with the wash method of choice, but do not perform the wash step.

3. Add 100 µl of assay buffer to each well. Cover the plate with a new sheet of sealing tape. Shake the plate at 850 ± 50 rpm for 30 sec. Carefully remove the sealing tape before placing the plate on the plate reader.

4. Repeat the **Acquiring the Data** steps to reacquire data. The data acquired should be similar to those acquired initially; however, the acquisition time will be extended because the wells have fewer beads.

Data Analysis

Controls

When reporting the results of the positive control, click **Report Table** on the result file (.rbx) and locate the control wells. Visually compare the observed concentration of the positive control in the Report Table against the lot-specific concentration values shown in the product data sheet.

Note: Bio-Plex Pro SARS-CoV-2 Delta RBD and Spike Trimer 2-Plex Assays are qualitative and should not be analyzed with the standard curve for concentration determination. Percentage inhibition can be calculated using MFI in the qualitative assay.

Samples

Inhibition of the SARS-CoV-2 protein–ACE2 receptor interaction by neutralizing antibodies in a SARS-CoV-2–positive plasma sample can be calculated by using the MFI response of the sample and of the negative control (ACE2 receptor blank). These results can be reported as percentage inhibition where higher percentage inhibition indicates high levels of neutralization antibodies. An example of percentage inhibition for sample X3 is shown.

Description	D614G S1 MFI
X1	9,244
X2	5,723
X3	3,870
Negative control	11,370

$$\text{Percentage inhibition} = \left(1 - \frac{\text{X3}}{\text{negative control}}\right) \times 100$$

$$= \left(1 - \frac{3{,}870}{11{,}370}\right)$$

$$= 66\%$$

For analysis, reference the sample interpretation guidance in the product data sheet to compare the sample values (concentration for quantitative assays and percentage inhibition for qualitative assays) to the cutoff value for each assay. If the value is above the cutoff, then there are detectable levels of neutralizing antibodies in the sample against that SARS-CoV-2 protein.

Previous Versions of Bio-Plex Manager Software

For instructions on using previous versions of Bio-Plex Manager Software, please contact Bio-Rad Technical Support.

Luminex xPONENT Software

Although guidelines are provided here, consult the xPONENT Software manual for more details. Perform a system initialization with the Luminex Calibration and Performance Verification Kit, as directed by Luminex. Select **Batches** to set up the protocol and follow the information under Settings.

Note: The instrument settings described below apply to Luminex 100/200 and FLEXMAP 3D or Bio-Plex 3D Systems. For the Luminex MAGPIX Multiplex Reader, use the default instrument settings.

1. Select **MagPlex** as the bead type for magnetic beads, which automatically sets the DD gates.
2. Volume: 50 µl.
3. Refer to Table 10 to select the appropriate PMT setting for your instrument.
4. Plate name: 96-well plate.
5. Analysis type: Qualitative.

Select **Analytes** to set up the panel.

1. Enter 50 in the Count field.
2. Select the bead region and enter the analyte name.
3. Click **Apply All** for Count.

Select **Controls**. Enter lot number and other information as applicable.

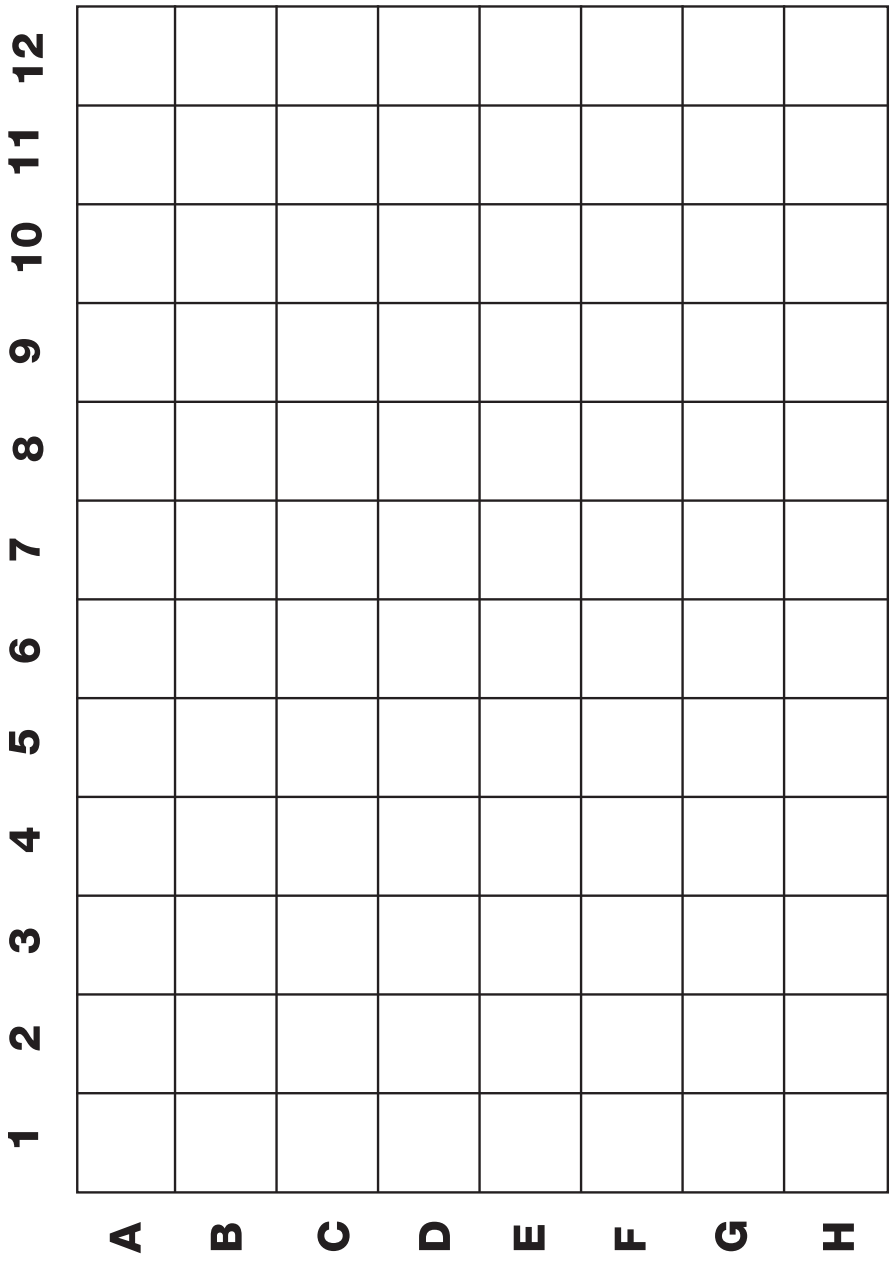
After the assay is complete, select **Results**, then select **Saved Batches**.

Section 8
Troubleshooting Guide

This troubleshooting guide addresses problems that may be encountered with Bio-Plex Pro Assays. If you experience any of the problems listed below, review the possible causes and solutions provided. Poor assay performance may also be due to the Bio-Plex Suspension Array Reader. To eliminate this possibility, use the validation kit to determine whether the array reader is functioning properly.

Problem and Possible Causes	Possible Solutions
High Intra-Assay CV	
Improper pipetting technique	Pipet carefully when adding standards, controls, samples, biotinylated ACE2 receptor, and SA-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer
Reagents and assay components not equilibrated to room temperature prior to pipetting	All reagents and assay components should be equilibrated to room temperature prior to pipetting
Contamination with wash buffer during wash steps	During the wash steps, be careful not to splash wash buffer from one well to another. Be sure to monitor residual volume after each wash cycle. Ensure that the microplate shaker setting is not too high. Reduce the microplate shaker speed to minimize splashing
Slow pipetting of samples and reagents across the plate	Sample pipetting across the entire plate should take less than 4 minutes. Reagent pipetting across the entire plate should take less than 1 minute
Bio-Plex Wash Station: insufficient washing due to clogged pins	Clean dispensing pins with the thicker of the two cleaning needles provided with washer. Perform regular rinses to minimize salt buildup
Low Bead Count	
Miscalculation of bead dilution	Check your calculations and be careful to add the correct volumes
Beads clumped in multiplex bead stock tube	Vortex for 30 sec at medium speed before aliquoting beads
Assay plate not shaken enough during incubation steps and prior to reading	Shake the plate at 850 ± 50 rpm during incubation steps and for 30 sec immediately before reading the plate
Reader is clogged	Refer to the troubleshooting guide in the Bio-Plex 200 System Hardware Instruction Manual (10005042)
Incorrect needle height on the reader	Adjust the needle height to coincide with the plate type provided in the kit
Low Signal or Poor Sensitivity	
Biotinylated ACE2 receptor or SA-PE diluted incorrectly	Check your calculations and be careful to add the correct volumes
High Background Signal	
Accidentally spiked blank wells	Make sure the blank well is represented by the sample diluent provided in the kit and no ACE2 receptor has been added to this well
Impact of Sample Matrix	
Poor precision in serum and plasma sample measurements	Check whether any interfering components, additives, or gel from separators were introduced into the samples. Avoid using hemolyzed and heavily lipemic samples. Remove visible particulate in samples by centrifugation. Avoid multiple freeze-thaw cycles of samples

Section 9
Plate Layout Template



Section 10
Safety Considerations

Eye protection and gloves are recommended when using these products. Consult the safety data sheet for additional information. Bio-Plex Pro Assays contain components of animal origin. These assays should be handled as if capable of transmitting infectious agents. Use universal precautions. These components should be handled at Biosafety Level 2 containment as defined by the U.S. Department of Health and Human Services publication, Biosafety in Microbiological and Biomedical Laboratories, 6th Edition (Centers for Disease Control and Prevention No. 300859, 2020).

Warning for Bio-Plex Handheld Magnetic Washer (#171020100) users: Magnetic field can be harmful to pacemaker wearers. Pacemaker wearers should stay back at least 30 cm (12 in.).

Section 11
Ordering Information

Catalog #	Description
Multiplex Kits	
12016897	**Bio-Plex Pro Human SARS-CoV-2 Variant Neutralization Antibody 11-Plex Panel**, 1 x 96-well
12016848	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody 2-Plex Panel**, 1 x 96-well
Singleplex Variants	
12016868	**Bio-Plex Pro SARS-CoV-2 Alpha S1 Coupled Beads**
12016849	**Bio-Plex Pro SARS-CoV-2 Beta S1 Coupled Beads**
12016898	**Bio-Plex Pro SARS-CoV-2 Gamma RBD Coupled Beads**
12016838	**Bio-Plex Pro SARS-CoV-2 D614G S1 Coupled Beads**
12017225	**Bio-Plex Pro SARS-CoV-2 Delta RBD and Spike Trimer 2-Plex Coupled Beads**
12016943	**Bio-Plex Pro SARS-CoV-2 E484K RBD Coupled Beads**
12016875	**Bio-Plex Pro SARS-CoV-2 Epsilon RBD Coupled Beads**
12016942	**Bio-Plex Pro SARS-CoV-2 K417N RBD Coupled Beads**
12016850	**Bio-Plex Pro SARS-CoV-2 Kappa RBD Coupled Beads**
12016869	**Bio-Plex Pro SARS-CoV-2 N501Y RBD Coupled Beads**
Reagents	
12017037	**Bio-Plex Pro Human SARS-CoV-2 Neutralization Antibody Reagent Kit**
12016945	**Bio-Plex Pro SARS-CoV-2 Neutralization Antibody Standard**
12016944	**Bio-Plex Pro Biotinylated Detection ACE2 Receptor**
12016837	**Bio-Plex Pro Serology Beads Storage Buffer**

Bio-Plex Manager Software, Bio-Plex Data Pro Software, Bio-Plex Pro Flat Bottom Plates, Bio-Plex Pro Assays 10x Wash Buffer, and Bio-Plex Streptavidin-Phycoerythrin are also available individually.

Bio-Rad Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific time.

Phone: 1-800-424-6723, option 2
Email: Support@Bio-Rad.com (U.S./Canada only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at **bio-rad.com**.

Visit **bio-rad.com/bio-plex** for more information.

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*

10000147006 Ver B US/EG *22-0122 0222 Sig 0122*