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ProcartaPlex™ Human SARS-CoV2 Variants Neutralizing Antibody Panel

USER GUIDE

Using Magnetic Beads for Serum and Plasma

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Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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A.0 (30)	4 March 2022	New manual for the ProcartaPlex™ Human SARS-CoV2 Variants Neutralizing Antibody Panel (Cat. No. EPX060-16018-901).

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Contents

- **CHAPTER 1** Product information 5
  - Product description 5
  - Technology overview 5
  - Contents and storage 6
  - Required materials not supplied 7
- **CHAPTER 2** Methods 8
  - Workflow 8
  - Procedural guidelines 9
  - Prepare samples 10
    - Prepare plasma samples 10
    - Prepare serum samples 10
    - Dilute samples 10
  - Prepare reagents 11
    - Prepare 1X wash buffer 11
    - Prepare the positive control 11
    - Prepare 1X Biotinylated Detection Reagent 11
  - Perform the assay 12
    - Define the plate map 12
    - Add magnetic beads to the 96-well Flat Bottom plate 12
    - Wash the magnetic beads 12
    - Add Assay Diluent, controls, and samples, then incubate 12
    - Add the 1X Biotinylated Detection Reagent, then incubate 13
    - Add Streptavidin-PE (SA-PE), then incubate 13
    - Add Reading Buffer, then run the plate on a Luminex™ instrument 13
  - Set up the instrument 14
  - Re-read the plate after failed run 14
  - Analyze the results 15
- **APPENDIX A** Troubleshooting 16
  - Troubleshooting 16

APPENDIX B Performance characteristics 18

Performance characteristics 18

APPENDIX C Recommended plate layouts 19

Recommended plate layouts 19

APPENDIX D Documentation and support 20

Customer and technical support 20

Limited product warranty 20

Product information

Product description

This user manual is for a ProcartaPlex™ Immunoassay Kit to perform multiplexed protein measurements from serum and plasma samples using Luminex™ magnetic bead technology. Other biological samples may be suitable for use in the assay.

IMPORTANT! ProcartaPlex™ Human SARS-CoV2 Variants Neutralizing Antibody Panel **cannot** be combined with other ProcartaPlex™ Panels or Simplex Kits.

For the most current version of user documentation, visit our website **www.thermofisher.com**.

Technology overview

ProcartaPlex™ Immunoassays incorporate magnetic microsphere technology licensed from Luminex™ to enable the simultaneous detection and quantitation of multiple protein targets in diverse matrices. The platform allows the simultaneous detection from a single sample of up to 80 protein targets on the Luminex™ 200™ and FLEXMAP 3D™ platforms, and 50 protein targets on the MAGPIX™ platform.

This assay is designed to detect the level of SARS-CoV2 neutralizing antibodies in serum and plasma. It allows direct comparison of neutralizing potential of antibodies towards five described variants of SARS-CoV2, namely wild-type and variants B.1.1.7 (α, originating in the United Kingdom), B.1.351 (β, originating in South Africa), P1 (γ, originating in Brazil), B.1.617.2 (δ, originating in India), and B.1.1.529 (ο, originated in South Africa). The capture beads are coupled with spike S1 protein from the different SARS-CoV2 variants containing the known relevant mutations in the spike protein. Samples with neutralizing antibodies compete with an excess amount of biotinylated ACE2 (Biotinylated Detection Reagent). Any biotinylated ACE2 that binds to the proteins on the beads will produce a median fluorescence intensity (MFI) signal. Signals are inversely proportional to the level of neutralizing antibodies since this is a competitive immunoassay. The negative control (Assay Diluent without neutralizing antibodies) will give the highest MFI values. Neutralizing antibodies in the sample or in the positive control will reduce the signal. The neutralization (%) for samples are calculated from the MFI of the samples and the MFI of the negative control, see “Analyze the results” on page 15.

Contents and storage

ProcartaPlex™ Immunoassay Kits contain the components listed below. For quantities and details of components supplied, see the Certificate of Analysis and Table 1. Store kit at 2°C–8°C. See the expiration date on the label. Do not use expired product.

Contents	Amount
Bead Mix Neutr Ab (magnetic beads, 1X) [1]	1 x 5 mL
Biotinylated Detection Reagent (lyophilized)	2 vials
Detection AB Diluent	1 x 3 mL
Control Neutr Ab (lyophilized)	2 vials
Streptavidin-PE (SA-PE, 1X)	1 x 5 mL
Wash Buffer (10X) [1]	1 x 25 mL
Assay Diluent [1]	1 x 60 mL
Reading Buffer[1]	1 x 40 mL
96-well Flat Bottom Plate	1 plate
Black Microplate Lid	1 lid
Plate Seals	8 seals

[1] Contains sodium azide. See WARNING.

WARNING! All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice. This kit contains small quantities of sodium azide. Sodium azide is highly toxic and reactive in the pure form. At this product's concentration, though not classified as hazardous, build up of sodium azide may react with lead and copper plumbing to form highly reactive explosive metal azide. Dispose of the product in accordance with all state and local regulations.

Table 1 Bead mix neutralizing antibodies

Analyte	Beadsets
SARS-CoV2 wild type neutralizing antibodies	15
SARS-CoV2 α B.1.1.7 neutralizing antibodies	29
SARS-CoV2 β B.1.351 neutralizing antibodies	33
SARS-CoV2 γ P.1 neutralizing antibodies	53
SARS-CoV2 δ B.1.617.2 neutralizing antibodies	56
SARS-CoV2 ο B.1.1.529 neutralizing antibodies	57

Required materials not supplied

- MAGPIX™, Luminex™ 200™, FLEXMAP 3D™, or equivalent Luminex™-based instrument
- Glass-distilled or deionized water
- Adjustable single and multichannel pipettes with disposable tips
- Multichannel pipette reservoir
- Beakers, flasks, and cylinders for preparation of reagents
- Hand-held magnetic plate washer
- Vortex mixer
- Microtiter™ plate shaker

2

Methods

Workflow

Assay protocol

Prepare samples

Prepare the plasma samples and serum samples.

Prepare reagents

Prepare the 1X wash buffer and positive control.

Define the plate map

Mark sample and control wells using page 19.

Add magnetic beads to the 96-well Flat Bottom plate

1. Vortex the capture beads for 30 seconds, then add 50 µL of the capture beads to each well.
2. Remove the liquid.

   Note: Wash the plate after adding the beads.

Add Assay Diluent, controls, and samples, then incubate

1. Add the following according to sample type.
   a. Positive control: 25 µL of Assay Diluent, followed by 25 µL of the reconstituted positive control.
   b. Negative control: 50 µL of 1X Assay Diluent.
   c. Serum and plasma samples: 25 µL of 1X Assay Diluent, followed by 25 µL of pre-diluted samples.
2. Seal the plate, then incubate with shaking at room temperature for 120 minutes.
3. Wash the plate twice.

Add the 1X Biotinylated Detection Reagent, then incubate

1. Add 25 µL of prediluted 1X Detection Antibody mixture to each well.
2. Seal the plate, then incubate with shaking at room temperature for 30 minutes.
3. Wash the plate twice.

Assay protocol

Add Streptavidin-PE (SA-PE), then incubate

1. Add 50 µL of SA-PE solution to each well.
2. Seal the plate, then incubate with shaking at room temperature for 30 minutes.
3. Wash the plate twice.

Add Reading Buffer, then run the plate on a Luminex™ instrument

1. Add 120 µL of Reading Buffer into each well.
2. Seal the plate, then incubate with shaking at room temperature for 5 minutes.
3. Remove the Plate Seal, then run the plate on a Luminex™ instrument.

Analyze the results

Analyze the results using the neutralization (%) equation.

Procedural guidelines

- Thoroughly read this user manual and the product insert that is included with the assay kit. The product insert contains specific instructions for proper use of your kit.
- For Luminex™ 200™ and FLEXMAP 3D™ instruments, initiate the startup protocol to warm up the lasers for at least 30 minutes.
  - Ensure that the Luminex™ machine is calibrated according to the manufacturer's instructions.
  - The MAGPIX™ instrument does not require additional warm up.
- When working with samples and standards, change the pipette tips after every transfer and avoid creating bubbles when pipetting.
- During the incubation steps, cover the 96-well Flat Bottom Plate with the Black Microplate Lid provided in the kit to minimize exposure of the beads to light.
- Do not to invert the 96-well Flat Bottom Plate during the assay or allow contents from one well to mix with another well.
- Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.

Prepare samples

Prepare samples using one of the following procedures:

- For frozen samples: thaw samples on ice and mix well by vortexing, followed by centrifugation at 10,000 × *g* for 5–10 minutes to remove particulates.

  IMPORTANT! Avoid multiple freeze/thaw cycles.

- For samples with high lipid content: centrifuge at 10,000 x *g* for 10 minutes and transfer contents to a new tube.

Prepare plasma samples

1. Collect samples in sodium citrate or EDTA tubes.

   When using heparin as an anticoagulant, no more than 10 IU of heparin per mL of blood collected should be used because an excess of heparin may give falsely high values of some of the analytes.

2. Centrifuge samples at 1,000 × *g* at 4°C for 10 minutes within 30 min of collection.

3. Collect the plasma fraction. Use immediately or aliquot and store at –80°C.

Prepare serum samples

For best results, centrifuge serum samples immediately before running the assay.

1. Allow blood to clot for 20–30 minutes at 20°C–25°C.

2. Centrifuge at 1,000 × *g* for 10 minutes at 20°C–25°C.

3. Use immediately or aliquot and store at –80°C.

Dilute samples

Levels may vary between different samples. You may need to further dilute your samples if the analyte concentration exceeds the assay upper limit of quantitation (ULOQ). When preparing dilution of serum and plasma samples, use Assay Diluent. The suggested dilution for serum/plasma is 1:100.

1. Prepare a 1:100 external dilution of serum or plasma samples in Assay Diluent (e.g., add 5 µL of sample to 495 µL of Assay Diluent).

2. Mix diluted samples gently.

Prepare reagents

Prepare 1X wash buffer

1. Bring the Wash Buffer Concentrate (10X) to room temperature, then vortex for 15 seconds.
2. Mix 20 mL of the Wash Buffer Concentrate (10X) with 180 mL ddH_2O.

Note: Store 1X wash buffer at 2°C to 8°C for up to 6 months if needed. Wash Buffer Concentrate (10X) volume might not be sufficient if using an automated plate washer. For bulk orders use Cat. No. EPX-66666-001.

Prepare the positive control

Lyophilized neutralizing antibodies are used as the positive control. Assay Diluent is used as the negative control.

Note: After use, discard any remaining positive controls.

1. Centrifuge the control vial at 2,000 x *g* for 10 seconds.
2. Add Assay Diluent to the control vial (see the label for the appropriate reconstitution volume).
3. Gently vortex the vial for 10 seconds and centrifuge at 2,000 × *g* for 10 seconds to collect contents at the bottom of the vials.
4. Incubate at room temperature for 10 minutes to ensure complete reconstitution.
   The reconstituted positive control is ready to use, no pre-dilution is needed.

Prepare 1X Biotinylated Detection Reagent

Reconstitute the Biotinylated Detection Reagent (lyophilized) 15 minutes prior to usage.

Note: The reconstituted Biotinylated Detection Reagent should be kept on ice.

1. Centrifuge the Biotinylated Detection Reagent (lyophilized) vial at 2,000 × *g* for 10 seconds.
2. Add Assay Diluent into the vial (see the label for the appropriate reconstitution volume).
3. Gently vortex the vial for 10 seconds and centrifuge at 2,000 × *g* for 10 seconds to collect contents at the bottom of the vials.
4. Incubate for 10 minutes to ensure complete reconstitution.
5. Add 60 µL of reconstituted Biotinylated Detection Reagent concentrate (50X) to the mixing bottle, then add the Detection AB Diluent to a final volume of 3 mL if assaying a whole plate (otherwise scale the volume accordingly).

Perform the assay

Define the plate map

Mark sample and control wells using the plate map at the end of this manual (see page 19).

Add magnetic beads to the 96-well Flat Bottom plate

The panel is provided with magnetic beads at a 1X working concentration.

1. Vortex the bead vials for 30 seconds.
2. Pour the bead mix into a disposable reservoir, then add 50 µL of bead solution to each well of the plate. Use a multichannel pipette for this step as well as for the steps that follow.

Wash the magnetic beads

1. Securely insert the 96-well Flat Bottom Plate into the hand-held magnetic plate washer, ensure that the plate is held in place by the tabs, and wait 2 minutes to allow the beads to accumulate on the bottom of each well.
2. Remove the liquid in the wells by quickly inverting the hand-held magnetic plate washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the hand-held magnetic plate washer.
3. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.
4. Add 150 µL of 1X wash buffer into each well and wait 30 seconds to allow the beads to accumulate on the bottom of each well.
5. Remove the wash buffer in the wells by quickly inverting the hand-held magnetic plate washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the hand-held magnetic plate washer.
6. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.
7. Remove the 96-well Flat Bottom Plate from the hand-held magnetic plate washer and proceed to the next step.

Add Assay Diluent, controls, and samples, then incubate

1. For the positive control, add 25 µL of Assay Diluent to the dedicated wells of the plate, followed by 25 µL of the reconstituted positive control.

   Note: The reconstituted positive control is ready to use, no pre-dilution is needed.

2. For the negative control, add 50 µL of 1X Assay Diluent to the dedicated wells.

3. For serum and plasma samples, add 25 µL of 1X Assay Diluent to the appropriate wells, followed by 25 µL of pre-diluted samples.

4. Seal the plate with the provided Plate Seal. Cover the plate with the Black Microplate Lid, then incubate at room temperature for 120 minutes on a plate shaker set at 500 rpm.

5. Wash the 96-well plate twice. See "Wash the magnetic beads" on page 12.

Add the 1X Biotinylated Detection Reagent, then incubate

1. Add 25 µL of prediluted 1X Biotinylated Detection Reagent mixture to each well.

2. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, then incubate at room temperature for 30 minutes on a plate shaker set at 500 rpm.

3. Wash the 96-well plate twice. See "Wash the magnetic beads" on page 12.

Add Streptavidin-PE (SA-PE), then incubate

1. Add 50 µL of SA-PE solution to each well.

2. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, then incubate at room temperature for 30 minutes on a plate shaker set at 500 rpm.

3. Wash the 96-well plate twice. See "Wash the magnetic beads" on page 12.

Add Reading Buffer, then run the plate on a Luminex™ instrument

1. Add 120 µL of Reading Buffer into each well.

2. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, then incubate at room temperature for 5 minutes on a plate shaker set at 500 rpm.

3. Remove the Plate Seal, then run the plate on a Luminex™ instrument.

   Note: For details about instrument settings, see "Set up the instrument" on page 14.

Set up the instrument

Instrument	Acquistion volume	Timeout (optional)	Bead type	DD gate	Reporter gain	Min. bead count
MAGPIX™	50 µL	N/A	N/A	N/A	Standard PMT	50
INTELLIFLEX™	30 µL	40 seconds	MagPlex™	4,000–13,000	Standard PMT	50
Luminex™ 200™ FLEXMAP 3D™	50 µL	60 seconds	MagPlex™	7,500–25,000	Standard PMT	50
Bio-Rad™ BioPlex™	50 µL	60 seconds	MagPlex™	5,000–25,000	Standard PMT	50

Prior to running the assay, ensure that the probe height has been calibrated with 96-well Flat Bottom Plate supplied with the kit. Failure to adjust the probe height can cause damage to the instrument or low bead count. The Luminex™ system allows for calibration of low and high RP1 target values. We recommend RP1 low target value settings for ProcartaPlex™ immunoassays. When entering the information into the Luminex™ Acquisition Software, refer to the Certificate of Analysis provided with the kit for bead region.

For Luminex™ 200™ and FLEXMAP 3D™ instruments, initiate the startup protocol to warm up the lasers for at least 30 minutes.

- Ensure that the Luminex™ instrument is calibrated according to the manufacturer's instructions.
- The MAGPIX™ instrument does not require additional warm up.

Re-read the plate after failed run

If there is a malfunction of the Luminex™ instrument or software during the run, the 96-well Flat Bottom Plate can be re-read using the following instructions:

1. Remove the 96-well Flat Bottom Plate from the instrument, then insert the 96-well Flat Bottom Plate into the hand-held magnetic plate washer.
2. Wait 2 minutes.
3. Remove the buffer in the wells by quickly inverting the 96-well Flat Bottom Plate over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the hand-held magnetic plate washer.
4. Blot the assembly onto several layers of paper towels to remove any residual solution.
5. Resuspend the beads in 120 µL of Reading Buffer.
6. Remove from the hand-held magnetic plate washer.

7. Seal the 96-well Flat Bottom Plate with a new Plate Seal and lid.

8. Shake at 500 rpm for 5 minutes at room temperature.

Note: The assayed samples may take longer to read because there will be less beads in the wells.

Analyze the results

Since this is a competitive assay, the negative control will give the highest MFI values without any reduction of the signal due to neutralizing antibodies. Expected MFI levels for the negative and positive controls are shown in “Performance characteristics” on page 18.

Calculate the ratio of values using the following equation:

Neutralization (%) = (1 – (MFI of samples / MFI of negative control)) × 100

- >20% = Positive
- <20% = Negative

The cut-off level was determined at 20% after screening 160 healthy samples.

The cut-off level for SARS-CoV2 o B.1.1.529 variant was determined at 25% after screening 160 healthy samples.

Troubleshooting

Troubleshooting

Observation	Probable cause	Recommend solution
Low flow rate	Samples/beads are stuck in flow cell	Remove the 96-well plate and perform a wash and rinse cycle.
High CVs	Contamination from re-using the Plate Seal	Use a new Plate Seal for each incubation step.
	Incomplete washing	After adding the controls and samples, it is very important that any excess controls are removed during the wash step.
	Contamination from contents from adjacent wells	Avoid splashing the wash buffer during wash steps into adjacent wells.
	Poor pipetting techniques	Use a multichannel pipettor and careful pipette techniques. Avoid touching pipette tips to sides of the wells when adding wash buffer.
Limited dynamic range for BioPlex™ software users	Instrument calibrated at high PMT settings	Calibrate the instrument using the CAL2 Low RP1 target value.
Low bead count	Volume of bead solution is too low	Add 120 µL Reading Buffer into each well and shake at 500 rpm for 5 minutes at room temperature to resuspend beads prior to reading on the Luminex™ instrument.
	High bead aggregation	Vortex the bead suspension well before using in the assay and ensure that the beads are properly mixed during the incubation steps.
	Dyes contained in the beads are photo-bleached from overexposure to light	Store bead solution and the 96-well plate in the dark.

(continued)

Observation	Probable cause	Recommend solution
Low bead count	Samples causing the instrument to clog	Remove the 96-well Flat Bottom Plate and perform a wash and rinse to the instrument. Rerun the assay with further dilution of samples
	Probe height is incorrect	Refer to the Luminex™ manual for proper adjustment of the needle height.
	Instrument needle is partially clogged	Replace or clean needle according to the manufacturer's recommendations.
	Beads stuck to the bottom of the plate	Confirm that the plate shaker is set to 500 rpm and shaking for at least 5 minutes before reading.
	Air bubble in the sample loop	Refer to the Luminex™ manual for proper removal of the air bubble.
Low signal or sensitivity of the negative control	Low shaking, or the Biotinylated Detection Reagent was not reconstituted and diluted properly	Prepare fresh Biotinylated Detection Reagent following the instructions provided (see “Prepare 1X Biotinylated Detection Reagent” on page 11).
High signal or sensitivity of the positive control, or no difference compared to the negative control	Positive control was not reconstituted and diluted properly	Prepare fresh controls following the instructions provided (see “Prepare the positive control” on page 11).

Performance characteristics

Performance characteristics

The data presented in this section are examples of typical data, and levels can vary depending upon the instrument used for measurement, and according to the conditions under which the assay is performed (e.g., operator, pipetting technique, washing technique, or temperature effects).

The positive control (Neutralizing Antibodies (lyophilized) reconstituted with Assay Diluent) and the negative control (Assay Diluent) provided in the kit were run in duplicate according to the protocol. The results were measured with a Luminex™ 200™ instrument.

Table 2 Performance of positive control and negative control (assay diluent)

Analyte	Negative control MFI	Positive control MFI	Neutralization
SARS-CoV2 wild type neutralizing antibodies	6726	33	100%
SARS-CoV2 α B.1.1.7 neutralizing antibodies	17144	604	96%
SARS-CoV2 β B.1.351 neutralizing antibodies	7410	3468	53%
SARS-CoV2 γ P.1 neutralizing antibodies	10195	3122	69%
SARS-CoV2 δ B.1.617.2 neutralizing antibodies	14178	4224	70%
SARS-CoV2 ο B.1.1.529 neutralizing antibodies	2321	784	66%

Neutralization of the positive control is expected to be at least 30%.

Recommended plate layouts

Recommended plate layouts

Table 3 Layout for using the negative control (no competition, high MFI signal) and positive control

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg. Control	Neg. Control										
B	Pos. Control	Pos. Control										
C												
D												
E												
F												
G												
H												

Documentation and support

Customer and technical support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.