

ProcartaPlex™ Human Coronavirus Ig Total USER GUIDE

for Simplex Kits

Using Magnetic Beads for Serum and Plasma

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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 Coronavirus Simplex Kits **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.

Contents and storage

ProcartaPlex™ Immunoassay Kits contain the components listed below. Refer to the Certificate of Analysis for quantities and details of components supplied. Store kit at 2°C–8°C. Expiration date is stated on the kit. Do not use after the expiration date.

Contents	Amount
Simplex Kit	
Antibody Coupled Magnetic Beads, Simplexes (50X) ^[1]	1 x 120 µL
Reagent kit	
Detection Antibody (50X) ^[1]	1 x 70 µL
High Control	2
Medium Control	2
Low Control	2
Wash Buffer Concentrate (10X) ^[1]	1 x 25 mL
Detection Antibody Diluent ^[1]	1 x 3 mL
Assay Diluent ^[1]	1 x 60 mL
Reading Buffer ^[1]	1 x 40 mL
8-Tube Strips	2
96-well Flat Bottom Plate	1
Black Microplate Lid	1
Plate Seals	8

^[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.

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



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Workflow

Assay protocol

Prepare samples

Prepare the plasma samples and serum samples.

Prepare the reagents

Prepare the 1X wash buffer, 1X simplex beads, 1X Detection Antibody, and controls.

Define the plate map

Mark sample and background wells using “Recommended plate layouts” on page 21.

Add magnetic beads to the 96-well Flat Bottom plate

1. Vortex the bead vials for 30 seconds, then add 50 μ L of the bead solution to each well.
2. Wash the plate once.

Add Assay Diluent, controls (Tubes 1–7), samples, standards, and backgrounds, then incubate

1. Add 25 μ L of Assay Diluent to each well.
2. Add 25 μ L of the reconstituted controls and pre-diluted samples to the dedicated wells. Add an additional 25 μ L of Assay Diluent for background wells.
3. Seal the plate, then incubate with shaking at room temperature for 120 minutes.
4. Wash the plate twice.

Add the Detection Antibody mixture, then incubate

1. Add 25 μ L 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.

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

1. Add 120 μ L 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 via the manual method or with the ProcartaPlex™ Analysis App.

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 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

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

IMPORTANT! Avoid multiple freeze/thaw cycles.

- For samples with high lipid content: centrifuge at $10,000 \times g$ 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 $1,000 \times g$ 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 $1,000 \times g$ 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 1:1,000.

1. Prepare a 1:1,000 external dilution of serum or plasma samples in Assay Diluent.
 - a. Make a 1:10 dilution of sample in Assay Diluent (e.g., add 5 μ L of sample to 45 μ L of Assay Diluent).
 - a. Make a 1:100 dilution of 1:10 diluted sample in Assay Diluent (e.g., add 5 μ L of 1:10 diluted sample to 495 μ L of Assay Diluent).
2. Mix diluted samples gently.

Prepare the reagents

Prepare 1X wash buffer

Bring the Wash Buffer Concentrate (10X) to room temperature and vortex for 15 seconds. Mix 20 mL of the Wash Buffer Concentrate (10X) with 180 mL ddH₂O. Store 1X wash buffer at 2°C to 8°C for up to 6 months if needed.

Note: 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 1X simplex beads

Simplex kits are provided with concentrated 50X beads. Therefore, dilution of concentrated simplex beads is required.

1. Vortex each simplex bead vial (50X) for 30 seconds, then add 100 μ L of each simplex bead vial (50X) to a mixing bottle if using a whole plate (otherwise adjust the volume accordingly).
2. Fill up with 1X wash buffer to a final volume of 5 mL. To combine 2 or more different bead vials follow the table below (using a whole plate):

Number of different simplex bead vials to be mixed	Total volume of mixed beads	Volume of 1X wash buffer
1	100 μ L	4,900 μ L
2	200 μ L	4,800 μ L
3	300 μ L	4,700 μ L
4	400 μ L	4,600 μ L
5	500 μ L	4,500 μ L
6	600 μ L	4,400 μ L

Prepare 1X Detection Antibody

Detection Antibody is provided at 50X concentration. Add 60 μL of Detection Antibody (50X) concentrate to the mixing bottle, then add Detection Antibody Diluent to a final volume of 3 mL if assaying a whole plate (otherwise scale the volume accordingly).

Prepare Controls

High, Medium, and Low Controls are provided in the kit. Controls serve to give a positive signal for all targets with the exception of MERS and the negative control, which do not yield any positive signal, and show a signal near background. High, Medium, and Low Controls are to be used in the assay for qualitative results.

Note: After usage any remaining controls cannot be stored and must be discarded.

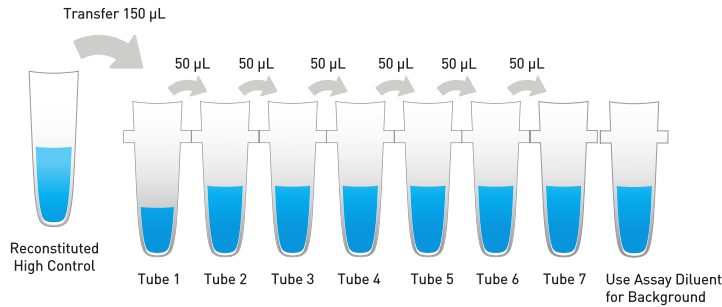
Reconstitute controls

1. Centrifuge each control vial at $2,000 \times g$ for 10 seconds.
2. Add Assay Diluent into each control vial (see the label for appropriate reconstitution volume).
3. Gently vortex each vial for 10 seconds and centrifuge at $2,000 \times g$ for 10 seconds to collect contents at the bottom of the vials.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.

(Optional) Prepare High Control as a standard curve

For relative quantification, the High Control can be prepared as a standard curve using a 4-fold serial dilution. The Medium and Low Controls can be compared on the standard curve as positive controls.

1. Prepare a 4-fold serial dilution of the reconstituted High Control using the tube strip provided.
2. Add 150 μL of reconstituted High Control into the first tube of the strip.
3. Add 150 μL of 1X Assay Diluent into Tubes 2–7.
4. Transfer 50 μL of the High Control from Tube 1 into Tube 2.
5. Mix by pipetting up and down for a total of 10 times.
6. Transfer 50 μL of High Control from Tube 2 into Tube 3.
7. Mix by pipetting up and down for a total of 10 times.
8. Repeat steps 4–7 for Tubes 4–7.
9. Add 150 μL of Assay Diluent into Tube 8 which serves as a background.



Perform the assay

Define the plate map

Mark sample and background wells using the plate map at the end of this manual (see “Recommended plate layouts” on page 21).

Add magnetic beads to the 96-well Flat Bottom plate

Add 50 µL of diluted 1X simplex beads per well prepared according to the instructions provided (see “Prepare 1X simplex beads” on page 11).

1. Vortex the bead vials for 30 seconds.
2. Pour the bead mix to a disposable reservoir and 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 (Tubes 1–7), samples, standards, and backgrounds, then incubate

1. Add 25 μL of Assay Diluent to each well of the plate.
2. Add 25 μL of the reconstituted controls and pre-diluted samples to the dedicated wells. For wells designated as background, add an additional 25 μL of Assay Diluent.

Note: Reconstituted High, Medium, and Low Controls are ready to use, no pre-dilution is needed.

3. (Optional) If a 4-fold dilution of the High Control is used as a standard curve (see “(Optional) Prepare High Control as a standard curve” on page 12), add 25 μL of Assay Diluent followed by 25 μL of prepared standard into the dedicated wells.
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. Follow “Wash the magnetic beads” on page 13.

Add the Detection Antibody mixture, then incubate

1. Add 25 μL of prediluted 1X Detection Antibody 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. Follow “Wash the magnetic beads” on page 13.

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: See, “Set up the instrument” on page 15 for details about instrument settings.

Set up the instrument

Instrument	Sample size	DD gate	Timeout	Bead event/bead region
Luminex™ 200™ FLEXMAP 3D™	50 µL	7,500–25,000	60 seconds	50–100
MAGPIX™	50 µL	N/A	N/A	50–100

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 a 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

Analyze the results (manual method)

To use the measured MFI values for evaluation, it is recommended to include healthy control samples in the assay run to determine cut-off values between healthy PCR(-) control samples vs PCR (+) samples.

In addition, the Low Control value was established based on 160 healthy PCR(-) samples and 39 PCR (+) SARS-CoV2 samples.

Calculate the ratio of values using the following equation:

$$\text{Ratio} = \frac{\text{MFI sample}}{\text{MFI low control}}$$

- If the ratio is <1, the target Ig is absent.
- If the ratio is 1–1.3, the result is indeterminate.
- If the ratio is >1.3, the target Ig is present.

This ratio calculation can be applied to the following SARS-CoV2 targets1:

- SARS-CoV2 Spike protein (trimer) Ig
- SARS-CoV2 S1 protein Ig
- SARS-CoV2 RBD Ig
- SARS-CoV2 Nucleocapsid protein Ig

(Optional) If the High Control was used to generate a standard curve, all targets with the exception of MERS and the negative control can be interpolated off the standard curves for relative quantitative results in units/mL using an appropriate standard curve analysis software.

Analyze the results with the ProcartaPlex™ Analysis App

We offer a free and robust analysis software package for analyzing High Controls used to generate a standard curve (except for MERS and the negative control). To analyze the data, follow the instructions below or contact Technical Support (see “Customer and technical support” on page 22).

1. Export the run data in CSV format and navigate to the ProcartaPlex™ Analysis App on Thermo Fisher Connect: <https://apps.thermofisher.com/apps/procartaplex>

Note: Before exporting CSV raw data from BioPlex™ Manager, make sure to set **Analytes Labels** under **Document Export Properties** to **Name (Region)**. The CSV raw data exported as Report Type 'xPONENT' from INTELLIFLEX™ instruments are supported.

2. Upload the CSV files to the ProcartaPlex™ Analysis App to analyze the run data. The intuitive software features 4PL/5PL curve fit optimization, group-wise statistical and heat map analysis. Users can export detailed reports including images for presentations and publications.



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	Controls not reconstituted and diluted correctly	Prepare fresh controls following the instructions provided (see "Reconstitute controls" on page 12).



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 controls provided in the kit were reconstituted with Assay Diluent and run in duplicate according to the protocol. The results were measured with a Luminex™ 200™ instrument.

Table 1 Performance of Controls (mean MFI)

Analyte	High Control	Medium Control	Low Control	Background
SARS-CoV2 Spike (trimer) Ig Total	9292	2729	329	8
SARS-CoV2 S1 protein Ig Total	8723	2021	250	9
SARS-CoV2 RBD Ig Total	13637	4446	555	19
SARS-CoV2 Nucleocapsid Ig Total	13679	4335	571	12
SARS S1 Ig Total	7028	1104	121	14
CoV-NL63 S1 Ig Total	3842	603	84	14
CoV-HKU1 S1 Ig Total	7148	1070	128	14
CoV-229E S1 Ig Total	9182	1668	192	8
CoV-OC43 S1 Ig Total	9986	2238	258	14
MERS S1 protein Ig Total	86	24	10	9
Negative control	90	27	13	10
SARS-CoV2 S1 α (B.1.1.7) Ig Total	8590	1888	228	18
SARS-CoV2 S1 β (B.1.351) Ig Total	6724	1329	157	8
SARS-CoV2 S1 γ (P.1) Ig Total	7946	3191	419	12
SARS-CoV2 Spike δ (B.1.617.2) Ig Total	10480	6447	888	10

The High Control was reconstituted and diluted with Assay Diluent and run in duplicate according to the protocol. The results were measured with a Luminex™ 200™ instrument.

Medium Control MFI levels are expected between the S2 and S3 MFI standard curve ranges. Low Control MFI levels are expected to fall between the S4 and S5 MFI standard curve ranges. MERS S1 and the Negative control are expected to have low MFI signals near background levels and are thus removed from the following table. MERS and the negative control results are not recommended for use in data extrapolation.

Table 2 Performance of High Control after 4-fold serial dilution (mean MFI)

Analyte	S1	S2	S3	S4	S5	S6	S7	Bkgrnd
SARS-CoV2 Spike (trimer) Ig Total	9292	5833	2350	805	292	128	64	8
SARS-CoV2 S1 protein Ig Total	8723	4278	1561	542	192	87	48	9
SARS-CoV2 RBD Ig Total	13637	8338	3374	1188	422	197	98	19
SARS-CoV2 Nucleocapsid Ig Total	13679	8687	3444	1211	459	197	95	12
SARS S1 Ig Total	7028	2870	902	276	105	53	32	14
CoV-NL63 S1 Ig Total	3842	1479	487	165	68	38	25	14
CoV-HKU1 S1 Ig Total	7148	2745	847	277	106	51	31	14
CoV-229E S1 Ig Total	9182	4091	1364	432	159	72	37	8
CoV-OC43 S1 Ig Total	9986	4734	1681	551	210	95	51	14
SARS-CoV2 S1 α (B.1.1.7) Ig Total	8590	3933	1254	367	127	55	31	18
SARS-CoV2 S1 β (B.1.351) Ig Total	6724	2929	923	263	89	35	19	8
SARS-CoV2 S1 γ (P.1) Ig Total	7946	5711	2220	676	220	87	41	12
SARS-CoV2 Spike δ (B.1.617.2) Ig Total	10480	9700	4466	1381	410	128	45	10



Recommended plate layouts

Recommended plate layouts

Table 3 Layout for using High, Medium, and Low Controls

	1	2	3	4	5	6	7	8	9	10	11	12
A	High	High										
B	Med	Med										
C	Low	Low										
D	Bkgrnd	Bkgrnd										
E												
F												
G												
H												

Table 4 Layout for using High Control to create a standard curve

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	Med	Med								
B	S2	S2	Low	Low								
C	S3	S3										
D	S4	S4										
E	S5	S5										
F	S6	S6										
G	S7	S7										
H	Bkgrnd	Bkgrnd										



Documentation and support

Customer and technical support

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- Product documentation
 - User guides, manuals, and protocols
 - 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.

