invitrogen

ProcartaPlex™ Human Coronavirus Ig Total Panel 11-Plex

USER GUIDE

Catalog Number EPX110-16000-901

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Revision A.0 (30)

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0024729

Revision	Date	Description
A.0 (30)	26 February 2021	new manual

Contents

ProcartaPlex™ Human Coronavirus Ig Total Panel 11-Plex

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Introduction

The ProcartaPlex™ Coronavirus Ig Total Panel 11-Plex has been optimized for detection of multiple analytes from serum and plasma.

The panel is provided with individual vials of 1X capture and 50X detection reagents and is not combinable with simplexes or other panels.

ProcartaPlex™ preconfigured panels are extensively tested for analyte combinability, interference and cross-reactivity to provide the highest level of validation and precision. All ProcartaPlex™ panels are supplied with the necessary reagents to perform the assay.

Analytes		
SARS-CoV2 Spike (trimer) Ig Total	SARS-CoV2 S1 protein Ig Total	SARS-CoV2 RBD Ig Total
SARS-CoV2 Nucleocapsid Ig Total	SARS S1 Ig Total	CoV-NL63 Ig Total
CoV-HKU1 Ig Total	CoV-229E Ig Total	MERS S1 Ig Total
CoV-OC43 Ig Total	Negative Control	

For detailed product information, visit thermofisher.com/procartaplex

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. When stored as indicated, all reagents are stable until the expiration date.

Contents	Amount
High Control	2 each
Medium Control	2 each
Low Control	2 each
Detection Antibody Mix (50X)	1 x 70 µL
Capture Bead Mix (1X)	1 x 5 mL
Wash Buffer (10X)	1 x 25 mL
Reading Buffer (1X)	1 x 40 mL
Assay Diluent (1X)	1 x 60 mL
Detection Antibody Diluent (1X)	1 x 3 mL
8-Tube Strip	2 each
Flat Bottom 96-well Plate, black	1 each
Microplate Lid	1 each
Plate Seals	8 each

Retain the lot-specific Certificate of Analysis that contains the product expiration date. The Certificate of Analysis also contains important information such as bead number, analyte names and highest standard concentration required for the assay setup on the xMAP instrument.

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Required materials not supplied

- xMAP™ instrument
- Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000)
- Deionized water
- Vortex mixer (e.g., Cat. No. 88882010)
- Microcentrifuge

- Adjustable single and multichannel pipettes with disposable tips and low volume reservoirs (e.g., Cat. No. 95128093)
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Orbital microplate shaker with at least 1.5 mm or 0.059 inch orbit diameter capable of maintaining a speed of 600 ± 50 rpm (e.g., Cat. No. 88882006)

Note: The use of rockers or large orbit shakers may cause adverse results.

Precautions and technical hints

1. Thoroughly read this User Guide and Certificate of Analysis prior to using the kit.
2. All chemicals should be considered potentially hazardous.
3. To avoid cross-contamination, do not invert the assay plate during the assay or allow contents from one well to mix with another well.
4. Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
5. Ensure that the xMAP™ instrument has been properly calibrated and set up prior to preparing and running the assay.

Workflow

Assay protocol

Prepare controls

Add capture beads

1. Vortex capture beads for 30 sec. Add 50 µL of the capture beads to each well.
2. Remove liquid.

Note: Wash the plate after adding the beads.

Add samples and controls

1. Add the following according to sample type
   -**For serum and plasma samples:** Add 25 µL of Assay Diluent, then add 25 µL of controls or prediluted samples. For background wells, add 50 µL of Assay Diluent.
2. Seal the plage and incubate with shaking at room temp for 2 hr.
3. Wash plate twice.

Prepare and add detection antibody

1. Add 25 µL of Detection Antibody Mix (1X).
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate twice.

Resuspend beads

1. Add 120 µL of Reading Buffer.
2. Seal the plate and shake at room temp for 5 min.

Acquire data on xMAP™system

2 Methods

Sample preparation

Thaw frozen serum and plasma samples on ice and mix well by vortexing. Centrifuge at 10,000 × *g* for 5–10 minutes to pellet out particulates. Avoid multiple freeze/thaw cycles. If samples are high in lipid content, centrifuge at 10,000 × *g* for 10 minutes and transfer contents to a new tube.

Plasma sample preparation

1. Collect samples in sodium citrate or EDTA tubes. If using heparin as an anticoagulant, no more than 10 IU of heparin per mL of blood collected should be used to prevent assay interference that can result in a false positive signal.
2. Centrifuge samples at 1,000 × *g* at 4°C for 10 minutes within 30 minutes of collection.
3. Collect the plasma fraction. Use immediately or store aliquots at –80°C.

Serum sample preparation

1. Allow blood to clot for 20–30 minutes at 20–25°C.
2. Centrifuge at 1,000 × *g* for 10 minutes at 20–25°C.
3. Collect the serum fraction. Alternatively, a serum separator tube can be used following the manufacturer's instructions.
4. Use immediately or store aliquots at –80°C. Avoid multiple freeze/thaw cycles.

Dilution of plasma and serum samples

The analytes included in the panel typically have high serum and plasma concentrations. We recommend that you dilute samples 1:1,000 in 1X Assay Diluent to ensure that they fall within range of the assay.

Tube	Sample volume	Assay diluent volume
Dilution 1 (1:10)	10 µL	90 µL
Dilution 2 (1:10)	10 µL of Dilution 1	90 µL
Dilution 3 (1:10)	10 µL of Dilution 2	90 µL

Preparation of reagents

Before starting with the assay protocol, define the plate map. Mark the standard, sample and background wells using the plate map found in Appendix A, “Recommended plate layout” to determine the number of wells used.

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 ddH_2O. Mix gently to avoid foaming. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

Note: Additional Wash Buffer Concentrate (200 mL, Cat. No. EPX-66666-001) can be purchased separately for automated plate washers.

Prepare 1X Detection Antibody Mix

Detection Antibody is provided at a 50X concentration and requires dilution prior to use. The steps below provide diluted detection antibody mix for a 96-well plate.

1. Add 60 µL of the detection antibody concentrate to the mixing bottle.
2. Add Detection Antibody Diluent (1X) to a final volume of 3 mL if using the entire 96-well plate (otherwise adjust 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: Change pipette tips after each dilution step and avoid air bubbles.

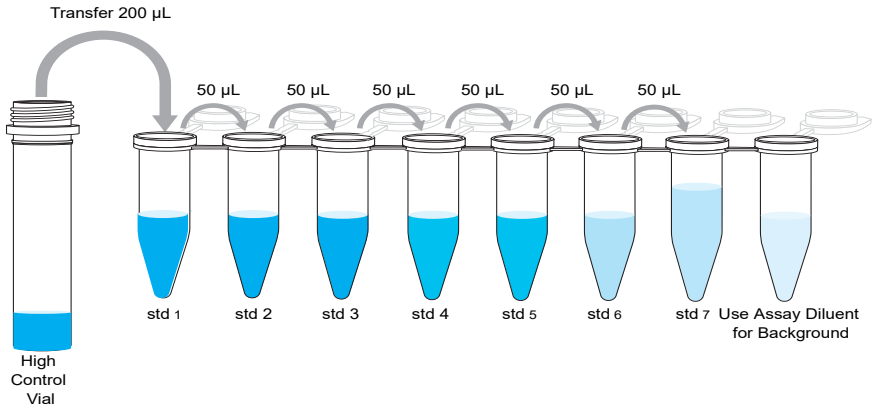
1. Centrifuge each Control stock vial at 2,000 x *g* for 10 seconds.
2. Add Assay Diluent to each Control stock vial (See the label for appropriate reconstitution volume).
3. Vortex the vial at high speed for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.

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

Prepare High Control as standard curve (optional)

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. Label the tubes in the 8-Tube Strip: Std1, Std2, Std3, Std4, Std5, Std6 and Std7.
2. Add 200 µL of the reconstituted High Control into Std1 tube.
3. Add 150 µL of diluent into Std2–Std7 tubes.
4. Transfer 50 µL from Std1 tube into Std2 tube.
5. Mix by pipetting up and down 10 times.
6. Transfer 50 µL of the mixed control from Std2 tube into Std3 tube using new pipette tip.
7. Mix by pipetting up and down 10 times.
8. Repeat steps 4–7 for tubes Std4–Std7, changing pipette tips between dilution steps.
9. Add 150 µL of diluent to the last tube of the 8-Tube Strip to serve as a background.
10. Keep tubes on ice until ready to use.



Assay protocol

1. Add Capture Bead Mix to the plate.
   a. Vortex the 1X Capture Bead Mix vial for 30 seconds at high speed.
   b. Using a multichannel pipette, add 50 µL of the Capture Bead Mix to each well of the plate.
2. Wash beads using a Hand-Held Magnetic Plate Washer.

   Note: To avoid loss of beads, secure the plate using the clamps on both sides of the Hand-Held Magnetic Plate Washer during this procedure.

   Note: This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000). Other washers should be validated by the end user.

   a. Place the plate on the Hand-Held Magnetic Plate Washer and wait 2 minutes to allow the beads to settle on the bottom of each well.
   b. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
   c. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
   d. Add 150 µL of 1X Wash Buffer into each well and wait 30 seconds.
   e. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
   f. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
   g. Remove the plate from the magnet and proceed to step 3.
3. Add controls, samples, and standards (optional) to the plate.
   a. **For Controls**: Add 25 µL of Assay Diluent to dedicated wells followed by 25 µL of reconstituted Controls.

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

   b. **Serum and plasma**: Add 25 µL of Assay Diluent to each well followed by 25 µL of prepared standards or prediluted samples as defined on the plate layout. Add an additional 25 µL of Assay Diluent to the wells designated as backgrounds.
   c. (Optional) If a 4-fold dilution of the High Control is used as a standard curve (see “Prepare High Control as standard curve (optional)” on page 10): Add 25 µL of Assay Diluent followed by 25 µL of prepared control into dedicated wells.
   d. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm for 2 hours at room temperature.

4. Remove and discard the Plate Seal. Wash the plate following the steps below.
    a. Place the plate on the Hand-Held Magnetic Plate Washer and wait 2 minutes to allow particles to settle on the bottom of each well.
    b. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
    c. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
    d. Add 150 µL of 1X Wash Buffer into each well and wait 30 seconds.
    e. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
    f. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
    g. Repeat steps 4d-4f once more for a total of two washes.
    h. Remove the plate from the magnet and proceed to the next step.
5. Add Detection Antibody Mix (1X) to the plate.
    a. Using a multichannel pipette, add 25 µL of the detection antibody solution (1X) to each well of the plate. Gently tap the plate to evenly distribute the solution in the wells.

    Note: A narrow trough reservoir for multichannel pipetting is recommended to be used to prevent volume loss.

    b. Seal the plate using a new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.
6. Wash the plate following step 4.
7. Prepare the plate for analysis on a xMAP™ instrument.
    a. Add 120 µL of reading buffer into each well.
    b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 5 minutes at room temperature.
8. Remove the Plate Seal and run the plate on a xMAP™ instrument.

Instrument settings

Follow the recommended guidelines and procedures for calibration and verification of the instrument. Laser-based systems require 30 minutes to warm up prior to use.

Instrument	Acquisition volume	Timeout (optional)	Bead type	DD gate	Reporter gain	Min. bead count
MAGPIX™	50 µL[1]	N/A	N/A	N/A	Standard PMT	50
INTELLIFLEX™	30 µL	40 sec	MagPlex™	4,000–13,000	Standard PMT	50
FLEXMAP 3D™ Luminex™ 100/200™	50 µL	60 sec	MagPlex™	7,500–25,000	Standard PMT	50
Bio-Rad™ Bio-Plex™	50 µL	60 sec	MagPlex™	5,000–25,000	Standard PMT	50

[1] MAGPIX volume can be changed during the run to optimize bead count.

Note: To assure a good bead count, the probe height must be adjusted to the plate provided in the kit. We recommend using two 5.08 mm spacer disks to adjust the sample probe height for Mylar-bottom plates.

Analyze results

The concentration of the samples can be calculated by plotting the expected concentration of the standards against the NET MFI generated by each standard. For Bio-Plex™ Manager, plot standard concentrations against FI-Bkgd. A 4PL or 5PL algorithm is recommended for the best curve fit. Analyze the assayed samples according to the operation manual for the Luminex™ or Bio-Plex™ instrument.

We offer a free and robust analysis software package for data analysis. To analyze the data, follow the instructions below or contact our technical support.

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 Bio-Plex™ Manager, please 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.

   Note: The samples have been diluted 1:1,000, which must be accounted for in the software analysis.

IMPORTANT! For ProcartaPlex™ getting started guides, technical literature, protocol support tools, and common troubleshooting questions visit thermofisher.com/procartaplexsupport
For more complete troubleshooting questions and answers, visit our FAQ database at thermofisher.com/procartaplexfaqs

Data evaluation

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 equation.

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

- 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 targets.

- 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.

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

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 Control High 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

A

Recommended plate layout

Recommended plate layout

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

Samples											
1	1	5	5	13	13	21	21	29	29	37	37
2	2	6	6	14	14	22	22	30	30	38	38
3	3	7	7	15	15	23	23	31	31	39	39
4	4	8	8	16	16	24	24	32	32	40	40
CH [1]	CH	9	9	17	17	25	25	33	33	41	41
CM [2]	CM	10	10	18	18	26	26	34	34	42	42
CL [3]	CL	11	11	19	19	27	27	35	35	43	43
Bkgd[4]	Bkgd	12	12	20	20	28	28	36	36	44	44

[1] Control High
[2] Control Medium
[3] Control Low
[4] Background

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	CM	CM								
B	S2	S2	CL	CL								
C	S3	S3										
D	S4	S4										
E	S5	S5										
F	S6	S6										
G	S7	S7										
H	Bkgd	Bkgd										