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ProcartaPlex™ Human Immune Response Panel 80-Plex

USER GUIDE

Catalog Number EPX800-10080-901

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Revision B (31)

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.

Revision history: MAN0028065 B (31) (English)

Revision	Date	Description
B (31)	13 May 2024	MAGPIX™ instrument was removed from the document and replaced by the xMAP™ INTELLIFLEX DR-SE and xMAP™ INTELLIFLEX™ instruments.
A.0 (30)	29 June 2022	New document for the ProcartaPlex™ Human Immune Response Panel 80-Plex.

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

Contents

1 Product information

Product description

The ProcartaPlex™ Human Immune Response Panel 80-Plex has been optimized for detection of multiple analytes from serum, plasma, and cell culture supernatants.

The panel is provided in a ready-to-use format with individual vials of 1X capture and detection reagents that require less pipetting and experimental setup. These reagents are 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				
APRIL	FGF-2	IL-4	IL-23	MIP-1α
BAFF	Fractalkine	IL-5	IL-27	MIP-1β
BLC	Gal-3	IL-6	IL-31	MIP-2α (CXCL2)
bNGF	G-CSF	IL-7	IL-34	MIP-3β (CCL19)
CCL1 (I-309)	GM-CSF	IL-8	IL-37	MIP-3α
CCL17 (TARC)	Granzyme A	IL-9	IP-10	MMP-1
CCL21 (6Ckine/SLC)	Granzyme B	IL-10	I-TAC	PTX3
CCL23 (MPIF)	GRO α	IL-12p70	LIF	SCF
CCL25 (TECK)	HGF	IL-13	MCP-1	TNF-β
CD30	IFN-α	IL-15	MCP-2	TNF-α
CD40L	IFN-γ	IL-16	MCP-3	TNF-R2
CXCL6 (GCP-2)	IL-1α	IL-17A	MCP-4 (CCL13)	TRAIL
ENA-78	IL-1β	IL-18	M-CSF	TREM-1
Eotaxin	IL-2	IL-20	MDC	TSLP
Eotaxin-2	IL-2R	IL-21	MIF	TWEAK
Eotaxin-3	IL-3	IL-22	MIG	VEGF-A

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.

Table 1 ProcartaPlex™ Human Immune Response Panel 80-Plex (Cat. No. EPX800-10080-901)

Contents	Amount
Standard Mix M (lyophilized)	2 each
Standard Mix M2 (lyophilized)	2 each
Immune Response Control High	2 each
Immune Response Control Low	2 each
Immune Monitoring Control High	2 each
Immune Monitoring Control Low	2 each
Biotinylated Detection Antibody Mix (1X)	1 × 3.5 mL
Capture Bead Mix (1X)	1 × 5 mL
Streptavidin-PE (SA-PE) (1X)	1 × 5 mL
Wash Buffer (10X)	1 × 25 mL
Reading Buffer (1X)	1 × 40 mL
Universal Assay Buffer (1X)	1 × 10 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, names of analytes, and highest standard concentration required for the assay setup on the xMAP™ instrument.

Required materials not supplied

Catalog numbers that appear as links open the web pages for those products.

- xMAP™ instrument
- Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000)
- Deionized water
- Fresh cell culture medium for running cell culture supernatant samples

- 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)
- For cell signaling assays, we recommend using ProcartaPlex™ Cell Lysis Buffer (Cat. No. EPX-999999-000) or Cell Extraction Buffer (Cat. No. FNN0011) supplemented with Halt™ Protease Inhibitor Cocktail (100X) (Cat. No. 78442), however, other buffers can be used as well

Note: Do not use rockers or large orbital shakers with ProcartaPlex™ assays.

Workflow

Assay protocol

Prepare antigen standard

Add capture beads

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

   Note: Wash the plate after adding the beads.

Add samples and standards

1. Add the following according to sample type:
   - For serum and plasma samples—Add 25 µL of Universal Assay Buffer, then add 25 µL of standards, controls, or samples. For background wells, add 50 µL of 1X UAB.
   - For cell culture supernatant samples—Add 50 µL of standards, controls, or samples. For background wells, add 50 µL of cell culture medium.
2. Seal the plate, then incubate with shaking at room temperature for 2 hours.
3. Wash the plate twice.

Add detection antibody

1. Add 25 µL of Detection Antibody Mix (1X).
2. Seal the plate, then incubate with shaking at room temperature for 30 minutes.
3. Wash the plate twice.

Add Streptavidin-PE

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

Resuspend the beads

1. Add 120 µL of Reading Buffer.
2. Seal the plate, then shake at room temperature for 5 minutes.

Acquire data on the xMAP™ system

2 Methods

Procedural guidelines

- Thoroughly read this user guide and the certificate of analysis before using the kit.
- All chemicals should be considered potentially hazardous.
- To avoid cross-contamination, do not invert the assay 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.
- 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.
- Ensure that the xMAP™ instrument has been properly calibrated and set up before preparing and running the assay.

Prepare samples

1. Thaw frozen serum and plasma samples on ice, then mix well by vortexing.

   Note: Avoid multiple freeze/thaw cycles.

2. Centrifuge at 10,000 × *g* for 5–10 minutes to remove particulates.

   For samples high in lipid content, centrifuge at 10,000 × *g* for 10 minutes, then transfer the contents to a new tube.

Prepare plasma samples

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.

Prepare serum samples

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.

Prepare cell culture supernatants

1. Centrifuge samples at 1,400 rpm for 10 minutes at 4°C to remove particulates.
2. Aliquot the clarified medium into clean polypropylene microcentrifuge tubes.
3. Use immediately or store aliquots at –80°C. Avoid multiple freeze/thaw cycles.

Prepare the reagents

Before starting with the assay protocol, define the plate map. Mark the standard, sample, and background wells to determine the number of wells used (see Appendix A, "Recommended plate layout").

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 Standard Mixes, High Controls, and Low Controls

The kit is supplied with two lyophilized Standard Mixes (for the generation of standard curves), two High Controls, and two Low Controls. Two vials of each Standard Mix, High Control, and Low Control are provided to allow the user to run the assay twice if running a partial plate. For experiments measuring serum or plasma samples, use 1X UAB as the diluent to reconstitute and dilute the standards and

controls. For experiments measuring cell culture supernatant samples, use fresh cell culture medium as the diluent.

Note: Change pipette tips after each dilution step and avoid air bubbles.

1. Centrifuge each Standard Mix and control at 2,000 × *g* for 10 seconds.
2. Add 125 µL of diluent to each vial.
3. Vortex the vials at high speed for 30 seconds, then centrifuge at 2,000 × *g* for 10 seconds to collect the contents at the bottom of the vials.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.
5. For the Standard Mix and each control, combine the contents of each of the two vials into one vial.

Note: After reconstitution, the controls are ready to be used in the assay protocol. The Standard Mix will be further diluted as described in the following section.

Prepare 4-fold serial dilution

1. Label the tubes in the 8-Tube Strip: Std1, Std2, Std3, Std4, Std5, Std6 and Std7.
2. Add 200 µL of the reconstituted Standard Mix into Std1 tube (see Figure 1).
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. Use a new pipette tip to transfer 50 µL of the mixed standards from Std2 tube into Std3 tube.
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 the tubes on ice until ready to use.

Note: Use the reconstituted standards immediately. The reconstituted standards cannot be stored.

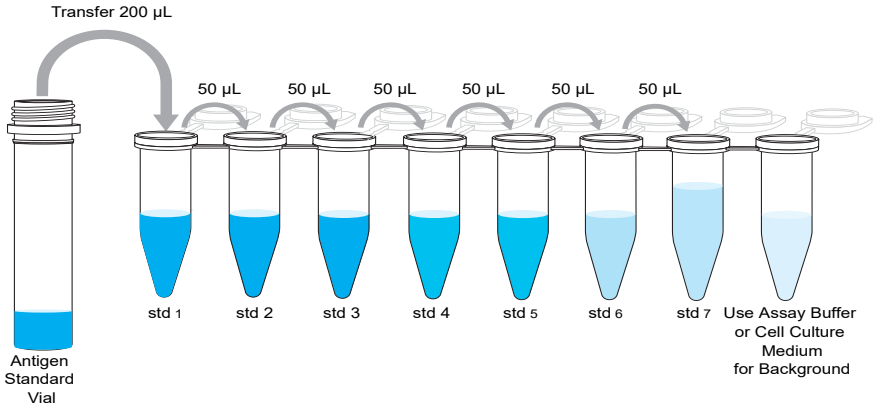

Figure 1 4-fold serial dilution

Expected values of controls

- Control High: S2–S3
- Control Low: S5–S6

Note: All control ranges were evaluated in Universal Assay Buffer and 2 hours incubation at room temperature.

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.
- 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, then 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 to each well, then 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 the next step.

3. Add samples and standards to the plate.
   a. **Serum and plasma**: Add 25 µL of 1X UAB to each well followed by 25 µL of prepared standards, controls, or samples as defined on the plate layout. Add an additional 25 µL of 1X UAB to the wells designated as background wells. **Cell culture supernatants**: Add 50 µL of prepared standards, controls, or samples as defined on the plate layout. Add 50 µL of cell culture medium to the wells designated as background wells.

   b. Seal the plate using one of the provided Plate Seals, then cover with the provided Microplate Lid. Shake at 600 rpm for 2 hours at room temperature.

4. Remove and discard the Plate Seal, then wash the plate.
   a. Place the plate on the Hand-Held Magnetic Plate Washer, then 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 to each well, then 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 substep 4d–substep 4f once for a total of two washes.

   h. Remove the plate from the magnet, then proceed to the next step.

5. Add Biotinylated Detection Antibody Mix to the plate.
   a. Using a multichannel pipette, add 25 µL of the mix to each well of the plate. Gently tap the plate to evenly distribute the solution in the wells.

   Note: We recommend using a narrow trough reservoir for multichannel pipetting to prevent volume loss.

b. Seal the plate using a new Plate Seal, then cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.

6. Wash the plate as described (see step 4).

7. Add Streptavidin-PE (SA-PE) to the plate.
   a. Add 50 µL of SA-PE solution to each well.

   b. Seal the plate using a new Plate Seal, then cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.

8. Wash the plate as described (see step 4).

9. Prepare the plate for analysis on an xMAP™ instrument.
   a. Add 120 µL of Reading Buffer to each well.

   b. Seal the plate using a new Plate Seal, then cover with the provided Microplate Lid. Shake at 600 rpm for 5 minutes at room temperature.

10. Remove the Plate Seal, then run the plate on an xMAP™ instrument.

    Alternatively, the sealed plate can be stored overnight at 2–8°C before running on an xMAP™ instrument. Shake the plate at 600 rpm for 5 minutes at room temperature, then remove the plate seal and run the plate.

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
xMAP™ INTELLIFLEX™ DR-SE xMAP™ INTELLIFLEX™	30 µL	40 seconds	MagPlex™	7,000–17,000	Standard PMT	50
FLEXMAP 3D™ Luminex™ 100/200™	50 µL	60 seconds	MagPlex™	7,500–25,000	Standard PMT	50
Bio-Rad™ Bio-Plex™	50 µL	60 seconds	MagPlex™	5,000–25,000	Standard PMT	50

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. A 4PL or 5PL algorithm is recommended for the best curve fit. Analyze the assayed samples according to the operation manual for the Luminex™ 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 the Thermo Fisher™ Connect Platform: **https://apps.thermofisher.com/apps/procartaplex**

   Note: 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.

   IMPORTANT! For ProcartaPlex™ getting started guides, technical literature, protocol support tools, and common troubleshooting questions visit **http://thermofisher.com/procartaplexsupport**

   For more complete troubleshooting questions and answers, visit our FAQ database at **http://thermofisher.com/procartaplexfaqs**

A Recommended plate layout

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

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

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

B

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

Limited product warranty

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