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ProcartaPlex™ Porcine Cytokine & Chemokine Panel 1 9-Plex

USER GUIDE

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ThermoFisher SCIENTIFIC

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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. MAN0024754

Revision	Date	Description
A.0 (30)	9 February 2021	new manual

Contents

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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™ Porcine Cytokine & Chemokine Panel 9-Plex has been optimized for detection of multiple analytes from serum, plasma, and cell culture supernatants.

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		
IFN alpha	IFN gamma	IL-1 beta
IL-10	IL-12/IL-23p40	IL-4
IL-6	IL-8 (CXCL8)	TNF alpha

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
Standard Mix (lyophilized)	2 each
Biotinylated Detection Antibody Mix (20X)	150 µL
Capture Bead Mix (1X)	1 x 5 mL
Streptavidin-PE (SA-PE) (1X)	1 x 5 mL
Wash Buffer (10X)	1 x 25 mL
Reading Buffer (1X)	1 x 40 mL
Porcine Specific Universal Assay Buffer (1X)[1]	1 x 10 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

[1] Note: Porcine Specfic Universal Assay Buffer will be called UAB throughout this user guide. Do not substitute this buffer with the Universal Assay Buffer found in our other kits. They are not the same formulation.

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
- 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)

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 antigen standard

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 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 or samples. For background wells, add 50 µL of 1X UAB.

-**For cell culture supernatant samples:** Add 50 µL of standards or samples. For background wells, add 50 µL of cell culture medium.

2. Seal the plage and incubate with shaking at room temp for 30 min.
3. Store overnight at 4°C.

Add detection antibody

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

Add Streptavidin-PE

1. Add 50 µL of Streptavidin-PE.
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.

Cell culture supernatant preparation

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.

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 20X concentration and requires dilution prior to use. The steps below provide diluted Detection Antibody Mix for a 96-well plate.

1. Add 150 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 Standard Mix

This kit is supplied with one lyophilized Standard Mix for generation of standard curves. Two vials of each Standard Mix are provided to permit 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 standard. 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.

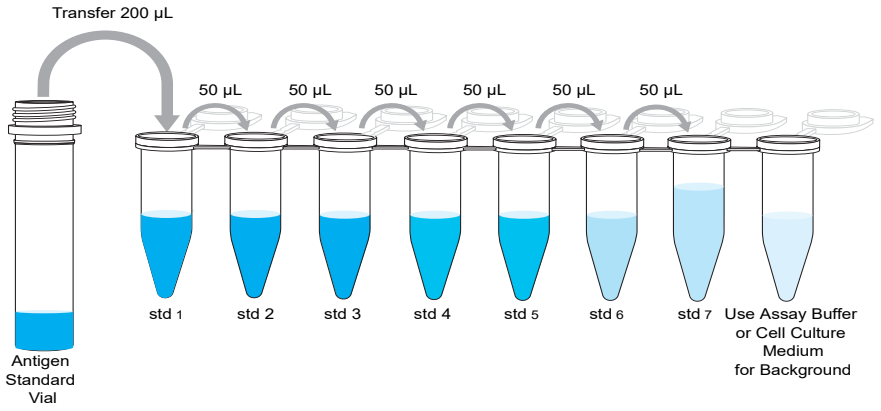
1. Centrifuge the standard mix stock vial at 2,000 x *g* for 10 seconds.
2. Add 250 µL of diluent to the stock vial.
3. Vortex the vial at high speed for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.

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.
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 standards 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.

Note: Use the reconstituted standard immediately. The reconstituted standard cannot be stored. Discard unopened standard vials if the entire plate was used in a single experiment.



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 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 or samples as defined on the plate layout. Add an additional 25 µL of 1X UAB to the wells designated as backgrounds. **Cell culture supernatants**: Add 50 µL prepared standards or samples as defined on the plate layout. Add 50 µL of cell culture medium to the wells designated as backgrounds.
   b. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.
   c. Store plate on a level surface at 4°C overnight.
   d. After overnight incubation, shake the plate at 600 rpm at room temperature for an additional 30 minutes.

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 Biotinylated detection Antibody Mix to the plate.
    a. Using a multichannel pipette, add 25 µL of the detection antibody solution 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. Add Streptavidin-PE (SA-PE) to the plate.
    a. Add 50 µL of SA-PE solution to each well.
    b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.
8. Wash the plate following step 4.
9. 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.
10. 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.

   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

Recommended plate layout

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

[1] Background

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