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ProcartaPlex™ Canine Simplex Kits

USER GUIDE

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Revision B

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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.

Revision history: MAN0028748 B (English)

Revision	Date	Description
B	20 May 2024	Removal of reading buffer and minor updates.
01.00	6 February 2024	Initial release.

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

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1 Product information

Product description

The ProcartaPlex™ Canine Simplex Kits have been optimized for detection of multiple analytes from serum, plasma and cell culture supernatants.

ProcartaPlex™ Simplex Kits are designed to be combinable with each other so that you can create your own multiplex panel that utilizes Luminex™ xMAP™ technology for protein quantitation. When combining multiple Simplex Kits only one buffer kit (Basic Kit) is needed for each assay plate regardless of plex size. The buffer kits are sold separately and are optimized for the different species and kit formats.

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.

Components supplied	Simplex kit	Basic kit
Antigen Standards, premixed	✓	
Detection Antibody (50X)[1]	✓	
Antibody Coupled Magnetic Beads, Simplexes (50X) [1]	✓	
Streptavidin-PE (SA-PE) (1X)[1]		✓
Wash Buffer Concentrate (10X)[1]		✓
Detection Antibody Diluent		✓
Universal Assay Buffer (1X)[1]		✓
PCR 8-Tube Strip		✓
96-well Flat Bottom Plate		✓
Black Microplate Lid		✓
Plate Seals		✓

[1] Contains sodium azide.

Retain the lot-specific Certificate of Analysis (CoA) that contains the product expiration date. The Certificate of Analysis also includes information required for the assay setup on the xMAP™ instrument (such as bead number, analyte and S1 concentration).

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)

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

Procedural guidelines

- Make sure to thoroughly review this manual and the Certificate of Analysis that comes with the assay kit. For the correct use of the kit, specific instructions can be included in a product insert.
- Run the startup protocol on the FLEXMAP 3D™ and INTELLIFLEX™ instruments for a minimum of 30 minutes to warm up the lasers. Follow the manufacturer's instructions are followed when calibrating the Luminex™ instrument.
- It is important to change the pipette tips when working between samples and standards in order to prevent bubbles during pipetting.
- During the incubation steps, cover the plate with the Black Microplate Lid provided in the kit to minimize exposure of the beads to light.
- Do not invert the plate or allow contents from one well to mix with another well during the assay.
- Use a multi-channel 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.

Workflow

Assay protocol

Prepare antigen standard

Add capture beads

IMPORTANT! Simplex kits contain 50X beads. Refer to Table 1 for dilution instructions, which vary according to the number of combined Simplex Beads.

1. Vortex beads 30 sec.
2. Add 50 µL of beads to each well. 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 plate and incubate with shaking for 60–120 min at room temp.

Add detection antibody

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

Add Streptavidin-PE

1. Add 50 µL of Streptavidin-PE.
2. Seal the plate and incubate with shaking for 30 min at room temp.
3. Wash plate twice.

Resuspend beads

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

Acquire data on xMAP™ system

2 Methods

Prepare the samples

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.

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.

TGF-β (acidification of samples)

TGF-β1 has to be acid treated for proper detection of the bioactive form. Therefore, this analyte should be tested as simplex assay. An additional datasheet with the preparation instructions are included in TGF-ß kits and can be requested from technical service.

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 1X Simplex Beads

Simplex kits are provided with concentrated 50X beads, which will require dilution before use. The dilution is dependent on the number of different Simplex Beads that are combined (Table 1).

IMPORTANT! ProcartaPlex™ Simplex Kits can be mixed for enhanced flexibility. Ensure that the bead regions from your ProcartaPlex™ Simplex Kits do not overlap. Some analytes use the same bead region and cannot be combined in one multiplex assay. Check the compatibility of our analytes using our **online panel configurator** or contact our technical support.

1. Vortex each Simplex Bead vial (50X) for 30 seconds, then add 100 µL of each Simplex Bead vial (50X) to a mixing bottle if using a whole plate (otherwise adjust the volume accordingly).

2. Add Wash Buffer (1X) to a final volume of 5 mL. To combine 2 or more different Simplex Bead vials, follow Table 1 (using a whole plate):

Table 1 Dilution of Simplex Beads

Number of different Simplex Bead vials to be mixed	Total volume of mixed bead solution	Volume of Wash Buffer (1X) to add
1	100 µL	4,900 µL
2	200 µL	4,800 µL
3	300 µL	4,700 µL
4	400 µL	4,600 µL
5	500 µL	4,500 µL
6	600 µL	4,400 µL

Prepare 1X Detection Antibody Mixture

For simplex and combinable panels, detection antibody is provided at 50X concentration. Add 60 µL of each detection antibody concentrate to the mixing bottle and bring volume to a total of 3 mL using detection antibody diluent if using a whole plate (otherwise adjust the volume accordingly).

Number of vials of detection antibody	Total volume of detection antibody	Volume of diluent to add
1	60 µL	2,940 µL
2	120 µL	2,880 µL
3	180 µL	2,820 µL
4	240 µL	2,760 µL
5	300 µL	2,700 µL
6	360 µL	2,640 µL

Prepare Standard Mix

Carefully review the Certificate of Analysis for lot-specific information on the kit components. The majority of kits are supplied with lyophilized Standard Mixes that contain a mix of multiple standard proteins. Some kits contain multiple sets of standards, each with a unique lot number, that require pooling prior to use. Each kit is shipped with two identical vials of each premixed antigen standard set from the same lot to permit the user to run the assay twice if running a partial plate. When combining multiple kits, ensure that the antigen standards of your analytes of interest are only present in one of the used standard vials. For instructions for combining more than five antigen standard sets, visit **thermofisher.com/support**.

When preparing antigen standards, the final volume after reconstitution and pooling should be 250 µL. 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 each different standard mix stock vial at 2,000 x *g* for 10 seconds.
2. Add 50 µL of diluent to each stock vial.
3. Vortex the vials 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.

5. Pool entire content of each stock vial into one of the vials and fill up to a total volume of 250 µL.
6. Vortex the vial at high speed for 10 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vials.

# of standard sets	Reconstitution volume per vial	Pooled volume	Buffer to add	Total volume
1	50 µL	50 µL	200 µL	250 µL
2	50 µL	100 µL	150 µL	250 µL
3	50 µL	150 µL	100 µL	250 µL
4	50 µL	200 µL	50 µL	250 µL
5	50 µL	250 µL	0 µL	250 µL

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, see Figure 1.
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 reconstituted standards immediately. Reconstituted standards cannot be stored. Discard unopened standard vials if the entire plate was used in a single experiment.

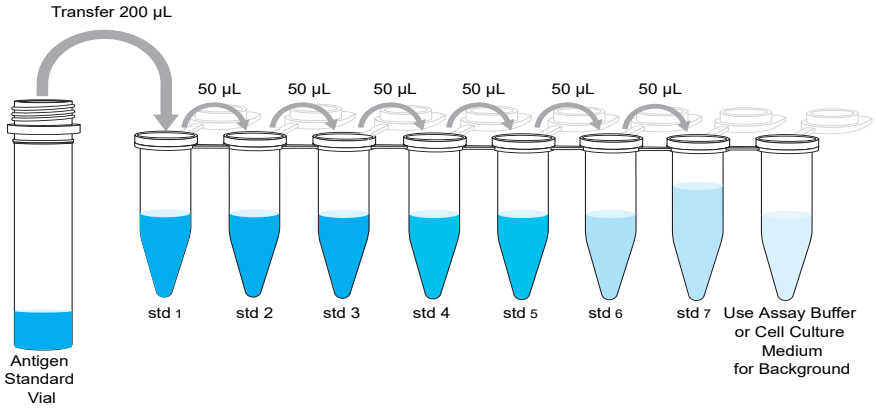

Figure 1 4-fold serial dilution

Assay protocol

1. Define the plate map by marking the standard, sample, and blank wells using the plate map in Appendix A, “Recommended plate layout”.

2. Add Simplex Bead Mix to the plate.
   a. Vortex the 1X Simplex Bead Mix vial for 30 seconds at high speed.

   b. Using a multichannel pipette, add 50 µL of the Simplex Bead Mix to each well of the plate. Mix to each well of the plate.

3. 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 4.

4. 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.

   b. **Cell culture supernatants**: Add 50 µL of prepared standards or samples as defined on the plate layout. Add 50 µL of cell culture medium to the wells designated as backgrounds.

   c. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm for 60–120 minutes at room temperature.

Note: ProcartaPlex™ assay validation is always performed at a 2-hour incubation at room temperature.

5. 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 5d-f once for a total of two washes.
   h. Remove the plate from the magnet and proceed to step 6.
6. 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.
   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.
7. Wash the plate twice following step 5.
8. 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.
9. Wash the plate twice following step 5.
10. Prepare the plate for immediate or later analysis on a xMAP™ instrument.
    a. Add 120 µL of Wash 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.

Note: Alternatively, it is also possible to store the 96-well plate overnight at 4°C. On the next day, shake the plate at 600 rpm for 5 minutes at room temperature and proceed with the next step below.

11. 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]	NA	MagPlex™	NA	Standard PMT	50
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

[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 BioPlex™ 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 BioPlex™ 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 BioPlex™ 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 sample dilution factor 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**

A 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												

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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