

ProcartaPlex™ Porcine Simplex Kits

USER GUIDE

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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| B | 20 May 2024 | Removal of reading buffer and minor updates. |
| 01.00 | 8 May 2023 | Initial release. |

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Product information

Product description

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

ProcartaPlex™ Porcine 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 <http://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 (20X) ^[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).



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

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 μ L of beads to each well.
3. 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 μ L of Universal Assay Buffer, then add 25 μ L of standards or samples. For background wells, add 50 μ L of 1X UAB.
 - For **cell culture supernatant samples:** Add 50 μ L of standards or samples. For background wells, add 50 μ L of cell culture medium.
2. Seal the plate and incubate with shaking for 30 min at room temp.
3. Store overnight at 4°C.

Add detection antibody

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

Add Streptavidin-PE

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

Resuspend beads

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

Acquire data on xMAP™ system

Prepare the samples

Thaw frozen serum and plasma samples on ice and mix well by vortexing. Centrifuge at $10,000 \times g$ for 5–10 minutes to pellet out particulates. Avoid multiple freeze/thaw cycles. If samples are high in lipid content, centrifuge at $10,000 \times g$ 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 $1,000 \times g$ 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 $20\text{--}25^{\circ}\text{C}$.
2. Centrifuge at $1,000 \times g$ for 10 minutes at $20\text{--}25^{\circ}\text{C}$.
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- β 1 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- β 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 ddH₂O. 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 Mix

For simplex kits, Detection Antibody is provided at 20X concentration. Add 150 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 | 150 μL | 2,850 μL |
| 2 | 300 μL | 2,700 μL |
| 3 | 450 μL | 2,550 μL |
| 4 | 600 μL | 2,400 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](https://www.thermofisher.com/support).

When preparing antigen standards, the final volume after reconstitution and pooling should be 250 μL . For experiments measuring serum, plasma, or CSF samples, use 1X UAB to reconstitute and dilute the standard. For experiments measuring cell culture supernatant samples, use fresh cell culture medium as 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 $\times g$ 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.

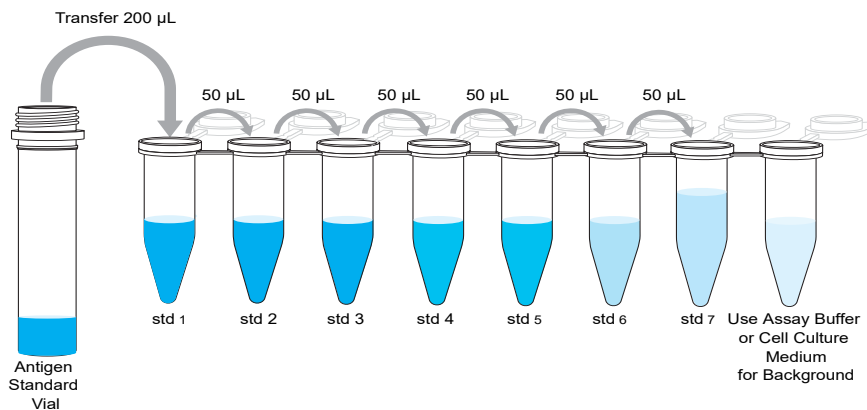


Figure 1 4-fold serial dilution

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 diluted 1X Simplex Beads vial for 30 seconds at high speed.
 - b. Using a multichannel pipette, add 50 μ L of the diluted 1X Simplex Bead 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 μ L 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 μ L of 1X UAB to each well followed by 25 μ L of prepared standards or samples as defined on the plate layout. Add an additional 25 μ L of 1X UAB to the wells designated as backgrounds.
 - b. **Cell culture supernatants:** Add 50 μ L of prepared standards or samples as defined on the plate layout. Add 50 μ L of cell culture medium to the wells designated as backgrounds.
 5. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm at room temperature for 30 minutes.
 6. Transfer the plate to 4°C and store on a level surface overnight. After overnight incubation, shake the plate at 600 rpm for an additional 30 minutes at room temperature.

7. 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 8.
8. 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 for an additional 30 minutes at room temperature.
9. Wash the plate twice following step 7.
10. 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.
11. Wash the plate twice following step 7.
12. 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.

13. 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, ensure that you 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 <http://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 | | | | | | | | | | | | |



Documentation and support

Customer and technical support

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 - 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 its affiliates 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 questions, contact Life Technologies at www.thermofisher.com/support.

