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# *ProcartaPlex™ Human, NHP and Dog Mix & Match Panels (384-Well) User Guide*

## USER GUIDE

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| D (35) | 27 May 2024 | Reading buffer was removed, and minor updates were made throughout to improve clarity and consistency of style. |
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# Product information

**IMPORTANT!** Before using this product, read and understand the information in the "Safety" appendix in this document.

## Product description

This user manual is for a ProcartaPlex™ Human, NHP and Dog Mix & Match Panels (384-Well) to perform quantitative, multiplexed protein measurements from serum, plasma, cell lysates, CSF, and cell culture supernatant samples using magnetic beads technology from Luminex™. Other biological samples might be suitable for use in the assay.

For detailed product information, visit **thermofisher.com/procartaplex**

## How it works

ProcartaPlex™ Human, NHP and Dog Mix & Match Panels (384-Well) incorporate magnetic microsphere technology licensed from the Luminex™ Corporation to enable the simultaneous detection and quantitation of multiple protein targets in diverse matrices. The platform allows the simultaneous detection from a single sample of up to 80 protein targets on the FLEXMAP 3D™ and xMAP™ INTELLIFLEX™ platforms.

# Contents and storage

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

| Contents | Amount |
|---|---|
| Antigen Standards, premixed | 2 each |
| Detection Antibody, premixed (1X) | 1 x 3.5 mL |
| Antibody-Coupled Magnetic Beads, premixed (1X) | 5 mL |
| Streptavidin-PE (SA-PE) (1X) | 1 x 5 mL |
| Wash Buffer (10X) | 1 x 100 mL |
| Universal Assay Buffer (1X) | 1 x 10 mL |
| *(Optional)* Universal Assay Buffer (10X) [1] | 1 x 10 mL |
| PCR 8-Tube Strip | 2 each |
| 384-well Flat Bottom Plate | 1 each |
| Black Microplate Lid | 1 each |
| Plate Seals | 8 each |

[1] Included for analytes that show high concentration in serum and plasma.

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 instrument.

# Required materials not supplied

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

| Item | Source |
|---|---|
| FLEXMAP 3D™, xMAP™ INTELLIFLEX™, xMAP™ INTELLIFLEX™ DR-SE instrument | (Cat. No. APX2021) |
| Deionized water | (Cat. No. 036645.K2) |
| Fresh cell culture medium for running cell culture supernatant samples | N/A |
| Vortex mixer | (Cat. No. 88882010) |
| Microcentrifuge | (Cat. No. 75002435) |
| Adjustable single and multichannel pipettes with disposable tips and low volume reservoirs | (Cat. No. 95128093) |

*(continued)*

| Item | Source |
| --- | --- |
| Beakers, flasks, and cylinders necessary for preparation of reagents | N/A |
| Orbital microplate shaker with at least 1.5 mm or 0.059 inch orbit diameter capable of maintaining a speed of 600 ± 50 rpm | (Cat. No. 88882006) |
| Cell lysis buffer | (Cat. No. EPX-99999-901)[1]<br>or<br>(Cat. No. FNN0011)[1] supplemented with 78430 |

[1] Other buffers may be suitable as well.

Automated Plate Washer suited for the washing of 384-well plates. This assay was assessed by using the Biotek EL406 using the ring magnet (Biotek Part Nr: 7102215). The correct settings for the Biotek EL406 plate washer can be found in "Settings of the Biotek EL406 plate washer on page 17" on page 18. Every other washer needs to be adjusted to the 384-well plates.

**Note:** Use of rockers or large orbit shakers can cause adverse results are therefore not recommended to be used for ProcartaPlex™ assays.

## Procedural guidelines

- Thoroughly read this user manual and Certificate of Analysis that is included with the assay kit. The product insert may contain specific instructions for proper use of your kit.
- For xMAP™ instruments initiate the startup protocol to warm up the lasers for at least 30 minutes. Ensure that the Luminex™ machine is calibrated according to the manufacturer's instructions.
- When working with samples and standards, change the pipette tips after every transfer and avoid creating bubbles when pipetting.
- During the incubation steps, cover the 384-well Flat Bottom Plate with the Black Microplate Lid provided in the kit to minimize exposure of the beads to light.
- Be careful not to invert the 384-well Flat Bottom Plate during the assay or allow contents from one well to mix with another well.
- Use a multi-channel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
- Store the reconstituted standards on ice before adding to the 384-well Flat Bottom Plate.

## Prepare the samples

- For frozen samples, thaw samples on ice and mix well by vortexing followed by centrifugation at 10,000 × *g* for 5–10 minutes to remove particulates. Avoid multiple freeze/thaw cycles.
- If samples are high in lipid content, centrifuge at 10,000 × *g* for 10 minutes at 2-8℃ and transfer contents to a new tube.

## Plasma sample preparation

1. Collect samples in sodium citrate or EDTA tubes. When using heparin as an anticoagulant, no more than 10 IU of heparin per mL of blood collected should be used because an excess of heparin may give falsely high values of some of the analytes.

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 aliquot and store at –80°C.

## Serum sample preparation

Spin down serum samples at 1,000 × *g* for 10 minutes at 20–25°C before running the assay.

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, use any standard serum separator tube following the manufacturer's instructions.)

4. Use immediately or aliquot and store at –80°C.

## Prepare cell lysate samples

If you have chosen ProcartaPlex™ Cell Lysis Buffer (Cat. No. EPX-99999-901) or Cell Extraction Buffer (Cat. No. FNN0011) supplemented with 0.5M EDTA, complement the buffer with Halt™ Protease and Phosphatase Inhibitor Single-Use Cocktail (100X) (Cat. No. 78440 or 78442). For example, add 100 µL of 0.5M EDTA Solution and 100 µL of Halt™ Protease and Phosphatase Inhibitor Single-Use Cocktail (100X) to 9.8 mL of Cell Extraction Buffer.

1. Stimulate cells as desired.
   a. Non-adherent cells:
      - Collect the cells by low speed centrifugation (400 × *g* for 10 minutes at 4℃).
      - Remove the medium from the pellet, and wash twice with ice-cold PBS by low speed centrifugation.
      - Remove the PBS, and add 0.5–1.0 × $10^7$ cells/mL Cell Lysis Buffer yielding about 500–3000µg/ml protein according to BCA test.
      - Incubate on ice for 15 minutes, vortexing every 5 minutes.

   b. Adherent cells:
      - Remove the medium from the cells, and wash twice with ice-cold PBS.
      - Remove the PBS, and add Cell Lysis Buffer to cover the surface of the culture dish.
      - Incubate on ice for 15 minutes, vortexing every 5 minutes.

   **Note:** Alternatively it is possible to trypsinize, wash and lyse adherent cells as described for non-adherent cells.

2. Collect the cell lysate.

3. Transfer the lysate to a microfuge tube, then centrifuge at 13,000 rpm for 10 minutes at 4℃.
4. Aliquot the cleared lysate into clean microfuge tubes. Then determine total protein concentration.

**IMPORTANT!**

- Proceed to analysis immediately after collection or freeze and store the cell lysates at –80°C.
- Avoid multiple freeze-thaw cycles of the frozen cell lysates. Thaw completely, mix well, then clarify by centrifugation at 18,000 × *g* for 5 minutes before analysis to prevent clogging.

## Dilution of serum and plasma samples

You may need to further dilute your samples if the analyte concentration exceeds the assay upper limit of quantitation (ULOQ). When preparing dilution of serum and plasma samples use Universal Assay Buffer (1X). For dilution of cell culture supernatant samples, use cell culture medium that was used to culture the cells. Recommended dilution factors for analytes with high normal serum or plasma concentration are listed in the table below.

**Note:** For analytes that show high concentration in serum and plasma, additional Universal Assay Buffer (10X) will be included in the kit.

| Species | Analytes | Recommended sample dilution factor |
|---|---|---|
| Human, NHP | Adiponectin | 100 |
| Human | Angiogenin | 4000 |
| Human | Angiostatin | 4000 |
| Human | Apolipoprotein E4 | 10,000 |
| Human | C3a | 100,000 |
| Human | CD14 | 100 |
| Human | CD2L (L-Selectin) | 100 |
| Human | CD44 | 100 |
| Human | CD44var (var6) | 100 |
| Human | Clusterin (Apo-J) | 10,000 |
| Human | Complement Factor H | 10,000 |
| Human | CRP | 500 |
| Human | Cystatin C | 100 |
| Human | Elafin | 100 |
| Human | Endoglin | 100 |
| Human | Endostatin | 4000 |
| Human | Fetuin-A | 10,000 |

*(continued)*

| Species | Analytes | Recommended sample dilution factor |
| --- | --- | --- |
| Human | Fibrinogen | 200,000 [1] |
| Human, NHP | ICAM-1 | 100 |
| Human | Lactoferrin | 100 |
| Human | Lp-PLA2 | 100 |
| Human | MBL | 100 |
| Human | MMP-2 | 100 |
| Human | MMP-3 | 100 |
| Human | MMP-9 | 100 |
| Human | NGAL | 100 |
| Human | NRP-1 | 100 |
| Human | Osteopontin (OPN) | 100 |
| Human, NHP | RANTES (CCL5) | 100 |
| Human | RBP4 | 100 |
| Human | REG3a | 100 |
| Human | SAA | 100 |
| Human | SAP (Pentraxin 2) | 4,000 |
| Human | SCGF-β | 100 |
| Human | TIMP-1 | 100 |
| Human, NHP | VCAM-1 | 100 |
| Human | YKL-40 (CHI3L1) | 100 |
| Human | ZAG | 100 |

[1] Dilution required only for plasma samples.

## CSF samples

Analytes validated for measuring Cerebrospinal fluid (CSF) samples including recommended dilution factors for analytes with high normal CSF concentration are listed in the table below. When preparing dilution of CSF samples, use Universal Assay Buffer (1X).

| Species | Analytes | Recommended sample dilution factor |
|---|---|---|
| Human | Amyloid beta 1-40 | — |
| Human | Amyloid beta 1-42 | — |
| Human | Apolipoprotein E4 | 100 |
| Human | BACE1 (Beta-secretase 1) | — |
| Human | BDNF | — |
| Human | BLC (CXCL13) | — |
| Human | Clusterin (Apo-J) | 100 |
| Human | CNTF | — |
| Human | Complement Factor H | 100 |
| Human | Fetuin-A | 100 |
| Human | FGF-21 | — |
| Human | GDNF | — |
| Human | GFAP | — |
| Human | IL-34 | — |
| Human | Kallikrein-6 (KLK6) | — |
| Human | MIF | — |
| Human | NCAM-1 | — |
| Human | Neurofilament heavy (NF-H) | — |
| Human | Neurogranin (NRGN) | — |
| Human | NGF beta | — |
| Human | NSE | — |
| Human | RAGE | — |
| Human | S100B | — |
| Human | Tau (Phospho) [T181] | — |
| Human | Tau (Phospho) [T231] | — |
| Human | Tau (Total) | — |

*(continued)*

| Species | Analytes | Recommended sample dilution factor |
| --- | --- | --- |
| Human | TDP-43 | — |
| Human | TREM-2 | — |
| Human | UCHL1 | — |
| Human | YKL-40 ( CHI3L1) | — |

## Recommendations for isolation and lysis of exosomes

After isolation of exosomes by precipitation with reagents–Total Exosome Isolation Reagent (from serum) Cat. No. 4478360, (from plasma) Cat. No. 4484450, or (from cell culture media) Cat. No. 4478359–ultracentrifugation, or other procedure, lyse exosomes using Exosome Resuspension Buffer provided in the Total Exosome RNA & Protein Isolation Kit (Cat. No. 4478545) or other established procedure.

Further dilute the sample in 1X Universal Assay Buffer if needed, then immediately proceed to add samples to the plate.

Resuspension volume and predilution, if needed, depends on the exosome source, volume, and sample concentration.

## Dilution of cell lysate samples

Dilution of 1:5 with Universal Assay Buffer is recommended. Higher dilution might be necessary depending upon cell-lysates used.

# Workflow

**Assay protocol**

## Prepare antigen standard

## Add capture beads

1. Vortex capture beads for 30 sec. Add 12.5 µL of the capture beads to each well.
2. Remove liquid.

   **Note:** Wash the plate once after adding the beads.

## Add samples and standards

1. Add the following according to sample type

   -For serum, plasma, cell lysates and CSF samples: Add 6.3 µL of Universal Assay Buffer, then add 6.3 µL of standards or samples.

   -For cell culture supernatant samples: Add 12.5 µL of standards or samples.
2. Seal the plate and incubate with shaking at room temp for 2 hr.
3. Wash plate twice.

## Add detection antibody

1. Add 6.3 µL of Detection Antibody Mix (1X).
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate twice.

## Add Streptavidin-PE

1. Add 12.5 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 50 µL of Wash Buffer (1X).
2. Seal the plate and shake at room temp for 5 min.

## Acquire data on FLEXMAP 3D™, xMAP™ INTELLIFLEX™ or xMAP™ INTELLIFLEX™ DR-SE instrument

# 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 (10X) to room temperature and vortex for 15 seconds. Mix 100 mL of the Wash Buffer (10X) with 900 mL dd$H_2O$. Mix gently to avoid foaming. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

**Note:** Wash Buffer volume in this kit is calculated for the use of the Biotek EL406 automated plate washer. Additional Wash Buffer Concentrate (200 mL, Cat. No. EPX-66666-001) can be purchased separately.

## Optional: Prepare 1X Universal Assay Buffer (UAB)

**IMPORTANT!** This dilution step is only required for kits containing 10X Universal Assay Buffer.

**Note:** 1X UAB is required for the preparation of standards and dilution of serum and plasma samples, CSF and cell lysates only. If working with cell culture supernatant samples, use the cell culture medium as a diluent.

Mix 10 mL of 10X Universal Assay Buffer (UAB) with 90 mL dd$H_2O$. Mix gently to avoid foaming.
1X UAB can be stored at 2–8°C for up to 30 days.

## Prepare Standard Mix

This kit is supplied with multiple lyophilized Standard Mixes 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 each 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 |

**IMPORTANT!** Standard preparation instructions for Mix & Match panels with more than 5 Standard Mixes are available under Appendix B.

## 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.

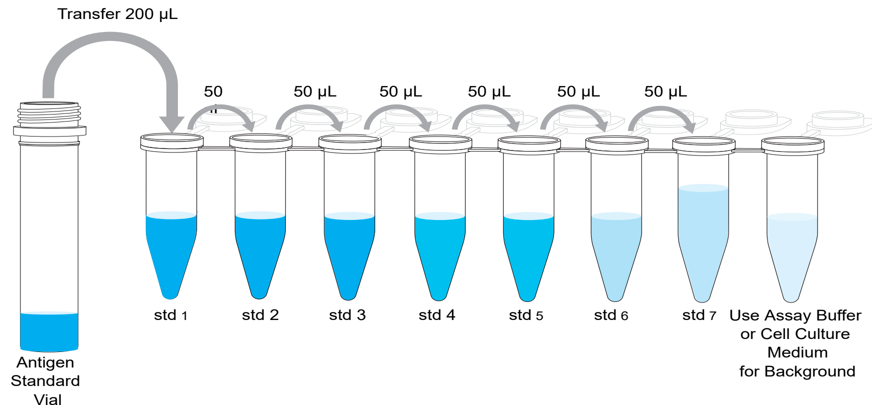


**Figure 1** 4-fold serial dilution

# Assay protocol

**IMPORTANT!** Gently touch the bottom of the well to dispense the solution.

1. Define the plate map.

   Mark the standard, sample, and blank wells using the plate map at the end of this manual.

2. Add magnetic beads to the plate.

   a. Vortex the Magnetic Bead vial for 30 seconds.

   b. Add 12.5 µL of the Magnetic Bead solution to each well of the plate. Use a multichannel pipette for this step as well as for the steps below.

3. Wash magnetic beads.

   a. Securely insert the 384-well Flat Bottom Plate into the automated plate washer.

   b. Wash the beads once with the automated plate washer. The washer should be programed in a way that the beads settle for 2 minutes on the magnet before the buffer is removed. Between each washing step should be a 1 minute pause. For more details, see "Settings of the Biotek EL406 plate washer" on page 17.

   c. Remove the 384-well Flat Bottom Plate from the Plate Washer and proceed to the next step.

   **Note:** Make sure to create two wash protocols for an Automated Plate Washer: 1st protocol for one wash; 2nd protocol for two washes.

4. Add sample type-specific buffer, samples, standards, and blanks, and then incubate.
   a. For serum, plasma, cell lysates and CSF: Add 6.3 µL of Universal Assay Buffer (1X) to each well followed by 6.3 µL of prepared standards or samples into dedicated wells.
   b. For cell culture supernatants: Add 12.5 µL prepared standards or samples into dedicated wells.
   c. For wells designated as blanks: Add an additional 6.3 µL of Universal Assay Buffer (1X) for serum, plasma, cell lysates or CSF samples. For cell culture supernatant samples, add 12.5 µL of cell culture medium.
   d. Seal the plate with the provided Plate Seal. Cover the plate with the Black Microplate Lid and shake at 1,400 ± 50 rpm for 120 minutes at room temperature.
5. Wash the 384-well plate twice following step 3.
6. Add Detection Antibody Mixture and incubate.
   a. Add 6.3 µL of Detection Antibody Mixture (1X) to each well.
   b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 1,400 ± 50 rpm.
7. Wash the 384-well plate twice following step 3.
8. Add SAPE and incubate.
   a. Add 12.5 µL of SAPE solution to each well.
   b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 1,400 ± 50 rpm.
9. Wash the 384-well plate twice following step 3.
10. Prepare the 384-well plate for immediate or later analysis on a FLEXMAP 3D™, xMAP™ INTELLIFLEX™ or INTELLIFLEX™ DR-SE.
    a. Add 50 µL of Wash Buffer (1X) into each well.
    b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 5 minutes on a plate shaker at room temperature at 1,400 ± 50 rpm.
    c. Remove Plate Seal and run the plate on a FLEXMAP 3D™, xMAP™ INTELLIFLEX™ or INTELLIFLEX™ DR-SE.

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**Note:** Alternatively, the sealed plate can be stored overnight at 2–8°C before running on a FLEXMAP 3D™, xMAP™ INTELLIFLEX™ or INTELLIFLEX™ DR-SE. Shake the plate at 1,400 rpm for 5 minutes at room temperature, then remove the plate seal and run the plate.

---

# Settings of the Biotek EL406 plate washer

The following table outlines settings for the Biotek EL406 microplate washer using the ring magnet (Biotek Cat. No. 7102215).

| Step | Description |
| --- | --- |
| Step 1: Shake/Soak | Shake: No |
| | Soak: YES 120 seconds (= before wash step begins) |
| | Home carrier: YES |
| Step 2: Washer wash[1] | **Cycles**<br>Number of cycles for bead wash: 1<br>Number of cycles for 384-well plate wash: 2 |
| | **Aspiration**<br>• TOP<br>• Travel rate: 6 CW (14.7 mm/sec)<br>• Asp Delay: 0 sec<br>• Asp x-position: 0 (center of well)<br>• Asp y-position: 0 (center of well)<br>• Asp height: 30 (3.811 mm) *<br>• Secondary Asp: NO) |
| | **Dispense**<br>• Rate: 09<br>• Volume: 50 µL/well<br>• Vacuum Delay: 0<br>• (Buffer A)<br>• Disp x-position: 0 (center of well)<br>• Disp y-position: 0 (center of well)<br>• Disp height: 120 (15.245mm) |
| | **OPTS**<br>Midcycle<br>• Wash Soak: YES 60 sec (= in between the two wash cycles)<br>• Home carrier: YES<br>Post<br>Final Asp: YES (same settings as above) (= aspiration as last step of program) |

[1] To test the setup of the washer, run the program with wash buffer. Then, using a pipette, manually check the residual volume of 10–15 wells in random positions. The residual volume in the wells should not exceed 6 µL.

# Instrument settings

| Instrument | Bead type | Acquisition volume | Reporter Gain | DD gate | Timeout | Min. bead count |
|---|---|---|---|---|---|---|
| FLEXMAP 3D™ | MagPlex™ | 40 µL | Enhanced PMT | 7,500–25,000 | 60 sec | 50 |
| xMAP™ INTELLIFLEX™ DR-SE - xMAP™ INTELLIFLEX™ instrument | MagPlex™ | 40 µL | High PMT FLEXMAP 3D™ | 7,000–17,000 | 60 sec | 50 |

Prior to running the assay, ensure that the probe height has been calibrated with a 384-well Flat Bottom Plate supplied with the kit. Failure to adjust the probe height can cause damage to the instrument or low bead count. When entering the information into the Luminex™ Acquisition Software, refer to the Certificate of Analysis provided with the kit for bead region and S1 values for each analyte of the current lot.

**Note:** If there is a malfunction of the Luminex™ instrument or software during the run, the 384-well Flat Bottom Plate can be re-read. Remove the 384-well Flat Bottom Plate from the instrument, insert the 384-well Flat Bottom Plate into the automated plate washer. Let the beads settle for 2 minutes on the magnet and then aspirate the fluid. Resuspend the beads in 50 µL of Wash Buffer (1X), seal the 384-well Flat Bottom Plate with a new Plate Seal and Lid and shake at 1,400 ± 50 rpm for 5 minutes at room temperature. The assayed samples may take longer to read since there will be less beads in the well.

# 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 Thermo Fisher Connect: **https://apps.thermofisher.com/apps/procartaplex**

   **Note:** The .csv raw data exported as Report Type ‘xPONENT’ from INTELLIFLEX™ DR-SE 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**

# Troubleshooting

| Observation | Probable cause | Recommend solution |
| --- | --- | --- |
| Low Flow Rate | Samples/beads are stuck in flow cell | Remove the 384-well Plate and perform a wash and rinse cycle. |
| High CVs | Samples and antigen standards not stored on ice | Prepare the samples and standards on ice before setting up the assay. |
| | Contamination from reusing the Plate Seal | Use a new Plate Seal for each incubation step. |
| | Incomplete washing | After adding the standards and samples, it is very important that any excess standards are removed during the wash step. |
| | Contamination from contents from adjacent wells | Avoid splashing the Wash Buffer during wash steps into adjacent wells. |
| Low bead count | Volume of bead solution is too low | Add 50 µL Wash Buffer (1X) into each well and shake at 1,400 ± 50 rpm for 5 minutes at room temperature to resuspend beads prior to reading on the Luminex™ instrument. |
| | High bead aggregation | Vortex the bead suspension well before using in the assay and ensure that the beads are properly mixed during the incubation steps. |
| | Dyes contained in the beads are photo-bleached from overexposure to light | Store bead solution and the 384-well plate in the dark. |
| | Samples causing the instrument to clog | Remove the 384-well Flat Bottom Plate and perform a wash and rinse to the instrument. Rerun the assay with further dilution of samples |
| | Probe height is incorrect | Refer to the FLEXMAP 3D™, xMAP™ INTELLIFLEX™ or INTELLIFLEX™ DR-SE Manual for proper adjustment of the needle height. |

*(continued)*

| Observation | Probable cause | Recommend solution |
|---|---|---|
| Low bead count | Instrument needle is partially clogged | Replace or clean needle according to the manufacturer's recommendations. |
| | Beads stuck to the bottom of the plate | Confirm that the plate shaker is set to 1,400 ± 50 rpm and shaking for at least 5 minutes before reading. |
| | Air bubble in the sample loop | Refer to the FLEXMAP 3D™, xMAP™ INTELLIFLEX™ or INTELLIFLEX™ DR-SE manual for proper removal of the air bubble. |
| Low signal or sensitivity | Standards not reconstituted and diluted correctly | Prepare fresh antigen standards following the instructions in "Prepare 4-fold serial dilution" on page 14. |
| Poor recovery | Did not use appropriate cell culture media to prepare the standards | Use the same cell culture media that is used to culture the cells. |
| | Samples and antigen standards were not stored on ice | Prepare the samples and standards on ice before setting up the assay. |

# Recommended plate layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | S1 | S1 | 9 | 9 | 25 | 25 | 41 | 41 | 57 | 57 | 73 | 73 | 89 | 89 | 105 | 105 | 121 | 121 | 137 | 137 | 153 | 153 | 169 | 169 |
| B | S2 | S 2 | 10 | 10 | 26 | 26 | 42 | 42 | 58 | 58 | 74 | 74 | 90 | 90 | 106 | 106 | 122 | 122 | 138 | 138 | 154 | 154 | 170 | 170 |
| C | S3 | S3 | 11 | 11 | 27 | 27 | 43 | 43 | 59 | 59 | 75 | 75 | 91 | 91 | 107 | 107 | 123 | 123 | 139 | 139 | 155 | 155 | 171 | 171 |
| D | S4 | S4 | 12 | 12 | 28 | 28 | 44 | 44 | 60 | 60 | 76 | 76 | 92 | 92 | 108 | 108 | 124 | 124 | 140 | 140 | 156 | 156 | 172 | 172 |
| E | S5 | S5 | 13 | 13 | 29 | 29 | 45 | 45 | 61 | 61 | 77 | 77 | 93 | 93 | 109 | 109 | 125 | 125 | 141 | 141 | 157 | 157 | 173 | 173 |
| F | S6 | S6 | 14 | 14 | 30 | 30 | 46 | 46 | 62 | 62 | 78 | 78 | 94 | 94 | 110 | 110 | 126 | 126 | 142 | 142 | 158 | 158 | 174 | 174 |
| G | S7 | S7 | 15 | 15 | 31 | 31 | 47 | 47 | 63 | 63 | 79 | 79 | 95 | 95 | 111 | 111 | 127 | 127 | 143 | 143 | 159 | 159 | 175 | 175 |
| H | Bkg [1] | Bkg | 16 | 16 | 32 | 32 | 48 | 48 | 64 | 64 | 80 | 80 | 96 | 96 | 112 | 112 | 128 | 128 | 144 | 144 | 160 | 160 | 176 | 176 |
| I | 1 | 1 | 17 | 17 | 33 | 33 | 49 | 49 | 65 | 65 | 81 | 81 | 97 | 97 | 113 | 113 | 129 | 129 | 145 | 145 | 161 | 161 | 177 | 177 |
| J | 2 | 2 | 18 | 18 | 34 | 34 | 50 | 50 | 66 | 66 | 82 | 82 | 98 | 98 | 114 | 114 | 130 | 130 | 146 | 146 | 162 | 162 | 178 | 178 |
| K | 3 | 3 | 19 | 19 | 35 | 35 | 51 | 51 | 67 | 67 | 83 | 83 | 99 | 99 | 115 | 115 | 131 | 131 | 147 | 147 | 163 | 163 | 179 | 179 |
| L | 4 | 4 | 20 | 20 | 36 | 36 | 52 | 52 | 68 | 68 | 84 | 84 | 100 | 100 | 116 | 116 | 132 | 132 | 148 | 148 | 164 | 164 | 180 | 180 |
| M | 5 | 5 | 21 | 21 | 37 | 37 | 53 | 53 | 69 | 69 | 85 | 85 | 101 | 101 | 117 | 117 | 133 | 133 | 149 | 149 | 165 | 165 | 181 | 181 |
| N | 6 | 6 | 22 | 22 | 38 | 38 | 54 | 54 | 70 | 70 | 86 | 86 | 102 | 102 | 118 | 118 | 134 | 134 | 150 | 150 | 166 | 166 | 182 | 182 |
| O | 7 | 7 | 23 | 23 | 39 | 39 | 55 | 55 | 71 | 71 | 87 | 87 | 103 | 103 | 119 | 119 | 135 | 135 | 151 | 151 | 167 | 167 | 183 | 183 |
| P | 8 | 8 | 24 | 24 | 40 | 40 | 56 | 56 | 72 | 72 | 88 | 88 | 104 | 104 | 120 | 120 | 136 | 136 | 152 | 152 | 168 | 168 | 184 | 184 |

[1] Background

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | | | | | | | | | | | | | | | | | | | | | | | |
| B | | | | | | | | | | | | | | | | | | | | | | | | |
| C | | | | | | | | | | | | | | | | | | | | | | | | |
| D | | | | | | | | | | | | | | | | | | | | | | | | |

*(continued)*

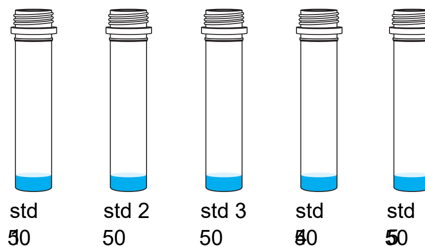
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | | | | | | | | | | | | | | | | | | | | | | | | |
| F | | | | | | | | | | | | | | | | | | | | | | | | |
| G | | | | | | | | | | | | | | | | | | | | | | | | |
| H | | | | | | | | | | | | | | | | | | | | | | | | |
| I | | | | | | | | | | | | | | | | | | | | | | | | |
| J | | | | | | | | | | | | | | | | | | | | | | | | |
| K | | | | | | | | | | | | | | | | | | | | | | | | |
| L | | | | | | | | | | | | | | | | | | | | | | | | |
| M | | | | | | | | | | | | | | | | | | | | | | | | |
| N | | | | | | | | | | | | | | | | | | | | | | | | |
| O | | | | | | | | | | | | | | | | | | | | | | | | |
| P | | | | | | | | | | | | | | | | | | | | | | | | |

# Preparation of a working standard for kits with more than 5 standards

This protocol demonstrates the procedure for reconstituting and pooling 12 antigen standard vials, but can be modified for any number of standards greater than 5. Each vial needs to be reconstituted in at least 50 µL and the total volume at the end will be 250 µL. A video demonstrating the procedure by mixing 6 antigen standard vials is available at **thermofisher.com/multivial-antigen-prep**

1. Remove one of each standard stock vial. Centrifuge each vial at 2,000 x *g* for 10 seconds.

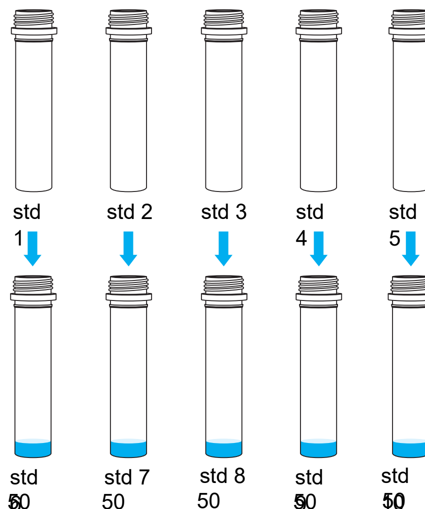
2. Choose the first 5 standard stock vials (std 1-5 below) and open carefully on the lab bench. Depending on the sample type, add 50 µL of either 1X UAB or cell culture medium. Vortex all 5 vials at high speed for 30 seconds.




3. 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.

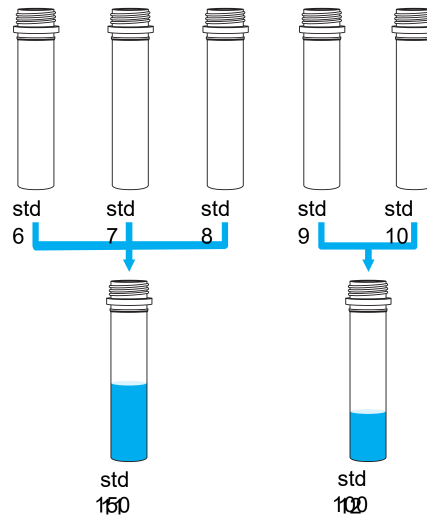
5. Transfer 50 µL from each reconstituted vial into the next 5 standard stock vials (std 6-10 below) and vortex the vials at high speed for 30 seconds.



6. Centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial.

7. Incubate on ice for 10 minutes to ensure complete reconstitution.

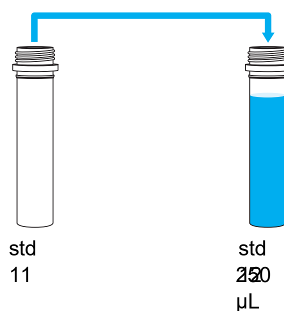
8. Transfer 50 µL of each of the 5 reconstituted standard vials into the remaining 2 standard stock vials (std 11-12 below) and vortex the vials at high speed for 30 seconds.




9. Centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vials.

10. Incubate on ice for 10 minutes to ensure complete reconstitution.

11. Pool the contents of the 2 vials (std 11-12 below) into a single vial so the final volume should be 250 µL.




12. Vortex the working antigen standard vial at high speed and then centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial.

# Documentation and support

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