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ProcartaPlex™ Mouse and Rat Mix & Match Panels (96-Well)

USER GUIDE

Publication Number MAN0025393

Revision B (31)

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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: MAN0025393 B (31) (English)

Revision	Date	Description
B (31)	27 May 2024	Reading buffer was removed, and minor updates were made throughout to improve clarity and consistency of style.
A00 (30)	26 May 2021	New document for ProcartaPlex™ Mouse and Rat Mix & Match Panels (96-Well).

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

Contents

1 Product information

Product description

The ProcartaPlex™ Mouse and Rat Mix & Match Panels (96-Well) have been optimized for detection of multiple analytes from serum, plasma, cell lysates, and cell culture supernatants.

ProcartaPlex™ Mix & Match Panels are provided in a ready-to-use format with individual vials of 1X capture and detection reagents that require less pipetting and experimental setup. These reagents are not combinable with simplexes or other panels.

All ProcartaPlex™ Mix & Match Panels are supplied with the necessary reagents to perform the assay.

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

Contents and storage

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

Contents	Amount
Standard Mixes (lyophilized)	2 each
Biotinylated Detection Antibody (1X)	1 x 3.5 mL
Capture Bead Mix (1X)	1 x 5 mL
Streptavidin-PE (SA-PE) (1X)	1 x 5 mL
Wash Buffer (10X)	1 x 25 mL
Universal Assay Buffer (1X)	1 x 10 mL
Universal Assay Buffer (10X) (optional)[1]	1 x 10 mL
8-Tube Strip	2 each
Flat Bottom 96-Well Plate, black	1 each
Microplate Lid	1 each
Plate Seals	8 each

[1] Will be 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 xMAP instrument.

Required materials not supplied

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

Item	Source
FLEXMAP 3D™	(Cat. No. APX1342)
Luminex™ 100/200™	(Cat. No. APX10031)
xMAP™ INTELLIFLEX™ / xMAP™ INTELLIFLEX™ DR-SE	(Cat. No. APX2020 / Cat. No. APX2021)
Hand-Held Magnetic Plate Washer	(Cat. No. EPX-55555-000)
Deionized water	(Cat. No. 036645.K2)
Fresh cell culture medium for running cell culture supernatant samples	N/A
Vortex mixer	(Cat. No. 88882010)

(continued)

Item	Source
Microcentrifuge	(Cat. No. 75002435)
Adjustable single and multichannel pipettes with disposable tips and low volume reservoirs	(Cat. No. 95128093)
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] or (Cat. No. FNN0011)[1] supplemented with 78430

[1] Other buffers may be suitable as well.

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 guide and the certificate of analysis before using the kit.
- All chemicals should be considered potentially hazardous.
- To avoid cross-contamination, do not invert the assay plate during the assay or allow contents from one well to mix with another well.
- Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
- This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000). Other washers should be validated by the end user.
- Ensure that the xMAP™ instrument has been properly calibrated and set up before preparing and running the assay.

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 and cell lysate 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 at room temp for 2 hr.
3. Wash plate three times.

Add detection antibody

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

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 three times.

Resuspend beads

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

Acquire data on xMAP™ instrument 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.

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°C).
      - 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°C.
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
Mouse	Adiponectin	1000
Mouse	CRP	1000
Mouse	ICAM-1	1000
Mouse	MCP-2 (CCL8)	1000
Rat	CRP	200,000
Rat	ICAM-1	200
Rat	VACM-1	200

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.

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.

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 ddH_2O. Mix gently to avoid foaming. 1X UAB can be stored at 2–8°C for up to 30 days.

Prepare Standard Mix

Carefully read the Certificate of Analysis for lot-specific information on the kit components. These kits are 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 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

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.

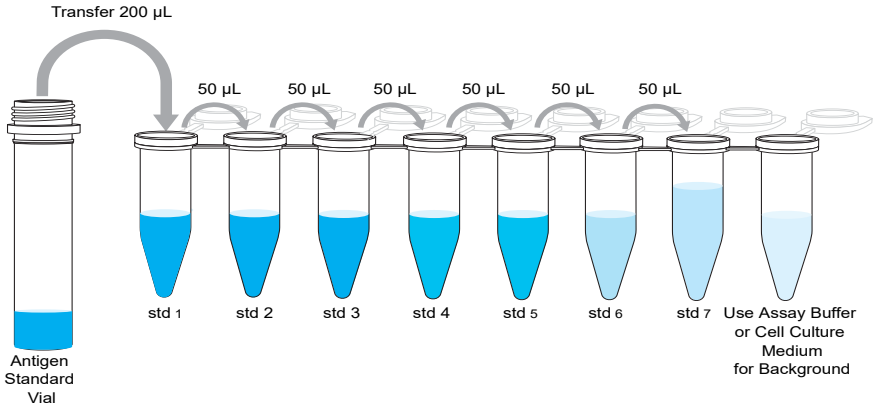


Figure 1 4-fold serial dilution

Assay protocol

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 and cell lysate**: 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 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 2 hours at room temperature.
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 for a total of three 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 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, the sealed plate can be stored overnight at 2–8°C before running on an xMAP™ instrument. Shake the plate at 600 rpm for 5 minutes at room temperature and proceed with the next step below.

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]	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 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.

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 **http://thermofisher.com/procartaplexsupport**

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

A Recommended plate layout

Standards		Samples									
1	1	1	1	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

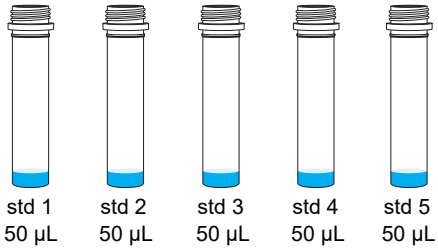
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

B 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.

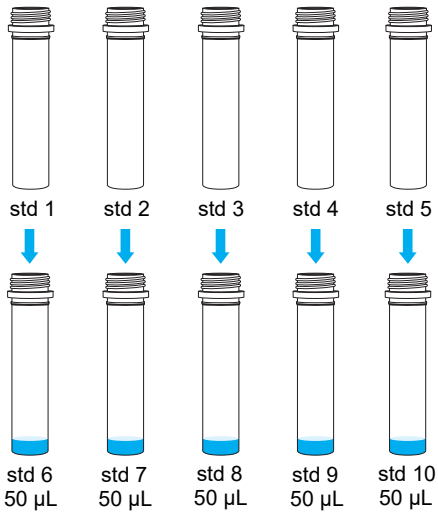
2. Choose the first 5 standard stock vials (std 1-5 below) and open carefully on the lab bench. Depending on the sample type, add 50 µL of either 1X UAB or cell culture medium. Vortex all 5 vials at high speed for 30 seconds.



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.

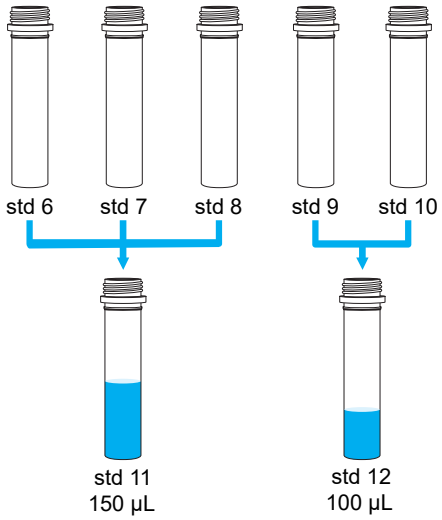
5. Transfer 50 µL from each reconstituted vial into the next 5 standard stock vials (std 6-10 below) and vortex the vials at high speed for 30 seconds.



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.

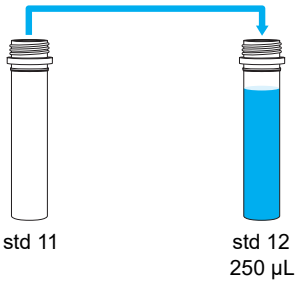
8. Transfer 50 µL of each of the 5 reconstituted standard vials into the remaining 2 standard stock vials (std 11-12 below) and vortex the vials at high speed for 30 seconds.



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.

C Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - 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**.