

ProcartaPlex™ Human, NHP, and Canine Mix & Match Panels

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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Revision	Date	Description
C.0 (32)	24 January 2022	Removed Human ZAG analyte.
B.0 (31)	06 October 2021	Addition of MIP-4 (CCL18), Haptoglobin, IGFBP-2, IGFBP-3, HGFR (c-Met), MIA, VE-Cadherin, Cathepsin D, CEA (CEACAM-5), Periostin (OSF-2), Beta-2-microglobulin (B2M), and EGFR (ErbB1) to dilution sample information.
A.0 (30)	26 May 2021	New manual.

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Product information

Product description

The ProcartaPlex™ Human, NHP, and Canine Mix & Match Panels have been optimized for detection of multiple analytes from serum, plasma, cerebrospinal fluid (CSF), 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°C to 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
Reading Buffer (1X)	1 x 40 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.



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: Use of rockers or large orbit shakers can cause adverse results.

Precautions and technical hints

- 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.
- 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 μ L 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, plasma, and CSF 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 at room temp for 2 hr.
3. Wash plate twice.

Add detection antibody

1. Add 25 μ L 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 50 μ L of Streptavidin-PE.
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate twice.

Resuspend beads

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

Acquire data on xMAP™ system



Prepare 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.

CSF sample preparation

1. Centrifuge samples at 1,400 rpm for 10 minutes at 4°C to remove particulates.
2. Use immediately or store aliquots at -80°C . Avoid multiple freeze/thaw cycles.

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	Beta-2-microglobulin (B2M)	100
Human	C3a	100,000
Human	Cathepsin D	100
Human	CEA (CEACAM-5)	100
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	EGFR (ErbB1)	100
Human	Elafin	100
Human	Endoglin	100
Human	Endostatin	4000
Human	Fetuin-A	10,000
Human	Fibrinogen	200,000 ^[1]

(continued)

Species	Analytes	Recommended sample dilution factor
Human	Haptoglobin	100
Human	HGFR (c-Met)	100
Human, NHP	ICAM-1	100
Human	IGFBP-2	100
Human	IGFBP-3	100
Human	Lactoferrin	100
Human	Lp-PLA2	100
Human	MBL	100
Human	MIA	100
Human	MIP-4 (CCL18)	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	Periostin (OSF-2)	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	VE-Cadherin	100
Human	YKL-40 (CHI3L1)	100

^[1] Dilution required only for plasma samples.

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.

Prepare 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.

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 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 ddH₂O. Mix gently to avoid foaming. 1X UAB can be stored at 2° to 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, plasma, or CSF 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.

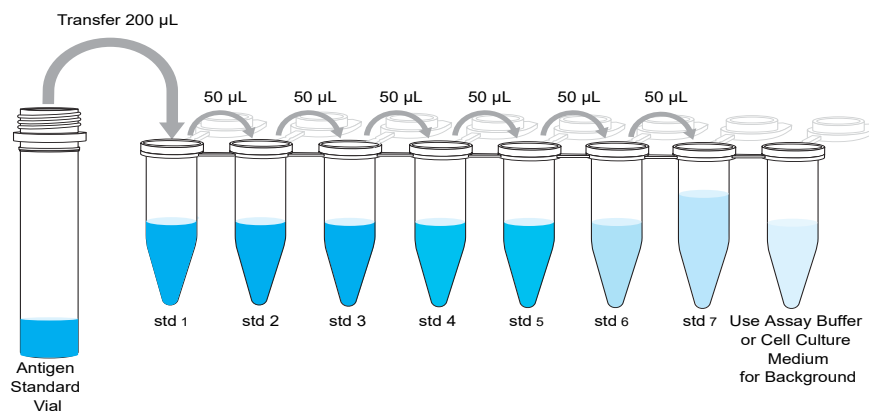


Figure 1 4-fold serial dilution

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 μ L 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 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 3.
3. Add samples and standards to the plate.
 - a. **Serum, plasma, and CSF:** 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. **Cell culture supernatants:** Add 50 μ L prepared standards or samples as defined on the plate layout. Add 50 μ L of cell culture medium to the wells designated as backgrounds.
 - b. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm for 2 hours at room temperature.

Note: For those wishing to perform the assay over two days, the 96-well plate can be incubated overnight. Shake the 96-well plate for 30 minutes at room temperature at 600 rpm, then transfer the plate to 4°C and store on a level surface. After overnight incubation, shake the plate for an additional 30 minutes at room temperature at 600 rpm.

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 two washes.
 - h. Remove the plate from the magnet and proceed to the next step.
5. Add Biotinylated detection Antibody Mix to the plate.
 - a. Using a multichannel pipette, add 25 μL of the detection antibody solution to each well of the plate. Gently tap the plate to evenly distribute the solution in the wells.

Note: A narrow trough reservoir for multichannel pipetting is recommended to be used to prevent volume loss.

 - b. Seal the plate using a new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.
6. Wash the plate following step 4.
7. Add Streptavidin-PE (SA-PE) to the plate.
 - a. Add 50 μL of SA-PE solution to each well.
 - b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.
8. Wash the plate following step 4.
9. Prepare the plate for analysis on a xMAP™ instrument.
 - a. Add 120 μL of reading buffer into each well.
 - b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 5 minutes at room temperature.
10. Remove the Plate Seal and run the plate on a xMAP™ instrument.

Instrument settings

Follow the recommended guidelines and procedures for calibration and verification of the instrument. Laser-based systems require 30 minutes to warm up prior to use.

Instrument	Acquisition volume	Timeout (optional)	Bead type	DD gate	Reporter gain	Min. bead count
MAGPIX™	50 µL ^[1]	N/A	N/A	N/A	Standard PMT	50
INTELLIFLEX™	30 µL	40 sec	MagPlex™	4,000–13,000	Standard PMT	50
FLEXMAP 3D™ Luminex™ 100/200™	50 µL	60 sec	MagPlex™	7,500–25,000	Standard PMT	50
Bio-Rad™ Bio-Plex™	50 µL	60 sec	MagPlex™	5,000–25,000	Standard PMT	50

^[1] MAGPIX volume can be changed during the run to optimize bead count.

Note: To assure a good bead count, the probe height must be adjusted to the plate provided in the kit. We recommend using two 5.08 mm spacer disks to adjust the sample probe height for Mylar-bottom plates.

Analyze results

The concentration of the samples can be calculated by plotting the expected concentration of the standards against the NET MFI generated by each standard. For Bio-Plex™ Manager, plot standard concentrations against FI-Bkgd. A 4PL or 5PL algorithm is recommended for the best curve fit. Analyze the assayed samples according to the operation manual for the Luminex™ or Bio-Plex™ instrument.

We offer a free and robust analysis software package for data analysis. To analyze the data, follow the instructions below or contact our technical support.

1. Export the run data in .csv format and navigate to the ProcartaPlex™ Analysis App on Thermo Fisher Connect: <https://apps.thermofisher.com/apps/procartaplex>

Note: Before exporting .csv raw data from Bio-Plex™ Manager, please make sure to set 'Analytes Labels' under 'Document Export Properties' to 'Name (Region)'. The .csv raw data exported as Report Type 'xPONENT' from INTELLIFLEX™ instruments are supported.

2. Upload the .csv files to the ProcartaPlex™ Analysis App to analyze the run data. The intuitive software features 4PL/5PL curve fit optimization, group-wise statistical and heat map analysis. Users can export detailed reports including images for presentations and publications.

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



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

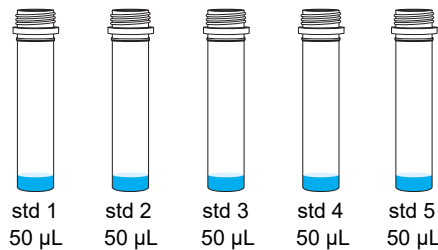
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												



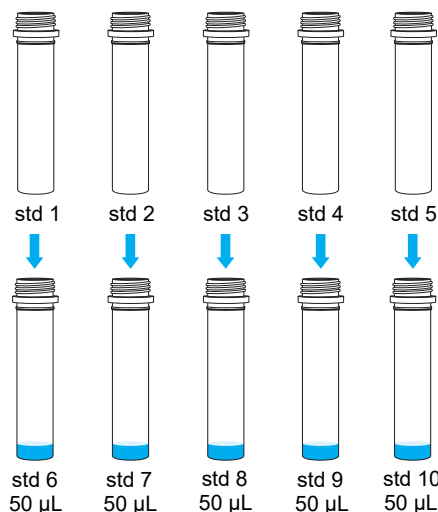
Preparation of a working standard for kits with more than 5 standards

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](https://www.thermofisher.com/multivial-antigen-prep)

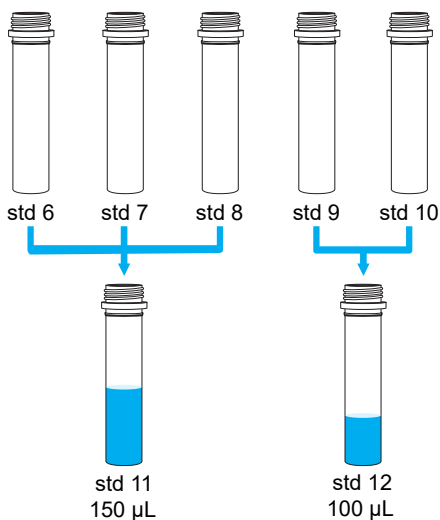
1. Remove one of each standard stock vial. Centrifuge each vial at 2,000 x g for 10 seconds.
2. Choose the first 5 standard stock vials (std 1-5 below) and open carefully on the lab bench. Depending on the sample type, add 50 μL of either 1X UAB or cell culture medium. Vortex all 5 vials at high speed for 30 seconds.



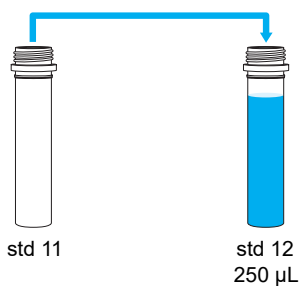
3. Centrifuge at 2,000 x g for 10 seconds to collect contents at the bottom of the vial.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.
5. Transfer 50 μL from each reconstituted vial into the next 5 standard stock vials (std 6-10 below) and vortex the vials at high speed for 30 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

Customer and technical support

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 - 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/or its affiliate(s) 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 any questions, please contact Life Technologies at www.thermofisher.com/support.

