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ProcartaPlex™ Multiplex Immunoassay

USER GUIDE

Multiplex immunoassays using magnetic bead technology for serum, plasma, CSF, and cell-culture supernatant

for use with:
Simplex Kits and Combinable Panels
Human, NHP, and dog assays

Publication Number MAN0016941

Revision K.0 (42)

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ThermoFisher SCIENTIFIC

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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Revision	Date	Description
K.0 (42)	21 January 2022	Removed an analyte—Human ZAG.
J.0 (41)	6 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.
H.0 (40)	21 April 2020	Harmonization of target names.
G.0 (39)	13 November 2019	Removal of NF-L from "CSF sample" table page 10/11.
F.0 (38)	17 June 2019	Addition of CSF samples.
E.0 (37)	1 April 2019	Removal of mentions of Luminex™ 100.
D.0 (36)	19 December 2018	Removal of draft comments at the base of every page by unchecking the draft comments box in publishing options.
C.0 (35)	25 October 2018	Addition of the following dilution sample information: Human Angiogenin, Human Angiostatin, and Human Endostatin.
B.0 (34)	29 August 2017	Added additional species to table in “Dilution of serum and plasma samples” on page 10.
A.0 (33)	28 June 2017	Baseline for this revision history.

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Contents

1 Product information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product use

This user manual is for a ProcartaPlex™ Immunoassay Kit to perform quantitative, multiplexed protein measurements from serum, plasma, CSF, and cell culture supernatant samples using magnetic beads technology from Luminex™. Other biological samples might be suitable for use in the assay.

For the most current version of user documentation, visit our website.

Technology overview

ProcartaPlex™ Immunoassays incorporate magnetic microsphere technology licensed from Luminex™ 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 Luminex™ 200™ and FLEXMAP 3D™ platforms, and 50 protein targets on the MAGPIX™ platform.

Contents and storage

ProcartaPlex™ Immunoassay Kits contain the components listed below. See the Certificate of Analysis for quantities and details of components supplied. Store kit at 2–8°C. Expiration date is stated on the kit. Do not use after expiration date.

Components supplied	Simplex kit	Basic kit	Combinable panels
Antigen Standards, premixed	✓		✓
Detection Antibody (50X)[1]	✓		✓
Antibody Coupled Magnetic Beads, Simplexes (50X)[1]	✓		
Antibody Coupled Magnetic Beads, premixed panels (1X)[1]			✓
Competitive Conjugate Solution (50X) [1],[2]	✓		✓
Streptavidin-PE (SA-PE) (1X)[1]		✓	✓

(continued)

Components supplied	Simplex kit	Basic kit	Combinable panels
Wash Buffer Concentrate (10X)[1]		✓	✓
Detection Antibody Diluent[1]		✓	✓
Universal Assay Buffer (1X)[1]		✓	✓
Universal Assay Buffer Concentrate (10X) [1](optional)	✓		✓
Reading Buffer[1]		✓	✓
PCR 8-Tube Strip		✓	✓
96-well Flat Bottom Plate		✓	✓
Black Microplate Lid		✓	✓
Plate Seals		✓	✓

[1] Contains sodium azide. See WARNING.
[2] Will be included in Competitive Assays only. Refer to the next table.

WARNING! All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice. This kit contains small quantities of sodium azide. Sodium azide is highly toxic and reactive in the pure form. At this product's concentration, though not classified as hazardous, build up of sodium azide may react with lead and copper plumbing to form highly reactive explosive metal azide. Dispose of the product in accordance with all State and local regulations.

Required materials not supplied

- MAGPIX™, Luminex™ 200™, FLEXMAP 3D™, or equivalent Luminex™-based instrument
- Glass-distilled or deionized water
- Adjustable single and multichannel pipettes with disposable tips
- Multichannel pipette reservoir
- Exosome isolation reagents, see "Recommendations for isolation and lysis of exosomes"
- Beakers, flasks, and cylinders for preparation of reagents
- Hand-held magnetic plate washer
- Vortex mixer
- Microtiter™ plate shaker

Precautions and technical hints

- Thoroughly read this user manual and the Certificate of Analysis that is included with the assay kit. The product insert may contain specific instructions for proper use of your kit.
- For Luminex™ 200™ and FLEXMAP 3D™ 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. The MAGPIX™ instrument do not require additional warm up.
- 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 96-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 96-well Flat Bottom 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.
- Store the reconstituted standards on ice before adding to the 96-well Flat Bottom Plate.

Competitive assays

Competitive Assays are based on competitive ELISA technique and require addition of Competitive Conjugate Solution (1X) to samples, standards, and blanks. Competitive Assays available are listed below:

Species	Analytes
Human	Cortisol

Preparation of Competitive Conjugate Solution (1X) is described in "Prepare 1X competitive conjugate solution for competitive assays" on page 15.

Note: The Assay Protocol for Competitive Assays differs from our standard Assay Protocol (see step 4).

2 Methods

Prepare 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.

  IMPORTANT! Avoid multiple freeze/thaw cycles.

- For samples with high lipid content: centrifuge at 10,000 x *g* for 10 minutes and transfer contents to a new tube.

Prepare plasma samples

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 min of collection.

3. Collect the plasma fraction. Use immediately or aliquot and store at –80°C.

Prepare serum samples

For best results, centrifuge serum samples immediately before running the assay.

1. Allow blood to clot for 20–30 minutes at 20°C–25°C.

2. Centrifuge at 1,000 × *g* for 10 minutes at 20°C–25°C.

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

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[1]
Human	Angiogenin	4000
Human	Angiostatin	4000
Human	Apolipoprotein E4	10,000
Human	Beta-2-microglobulin (B2M)	100
Human	Cathepsin D	100
Human	C3a	100,000
Human	CD14	100
Human	CD44	100
Human	CD44var (var6)	100
Human	CD62L (L-Selectin)	200
Human	CEA (CEACAM-5)	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[2]

(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)	4000
Human	SCGF-β	100
Human	TIMP-1	100
Human, NHP	VCAM-1	100

(continued)

Species	Analytes	Recommended sample dilution factor
Human	VE-Cadeherin	100
Human	YKL-40 (CHI3L1)	100

[1] For Metabolism/Obesity Panel 1, sample dilution factor is 200.
[2] 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.

TGF-β (acidification of samples)

TGF-β1 has to be acid treated for proper detection of the bioactive form. Therefore, this analyte should be tested as simplex assay. An additional datasheet with the preparation instruction will be included in each kit or can be requested by contacting our technical service.

CSF samples

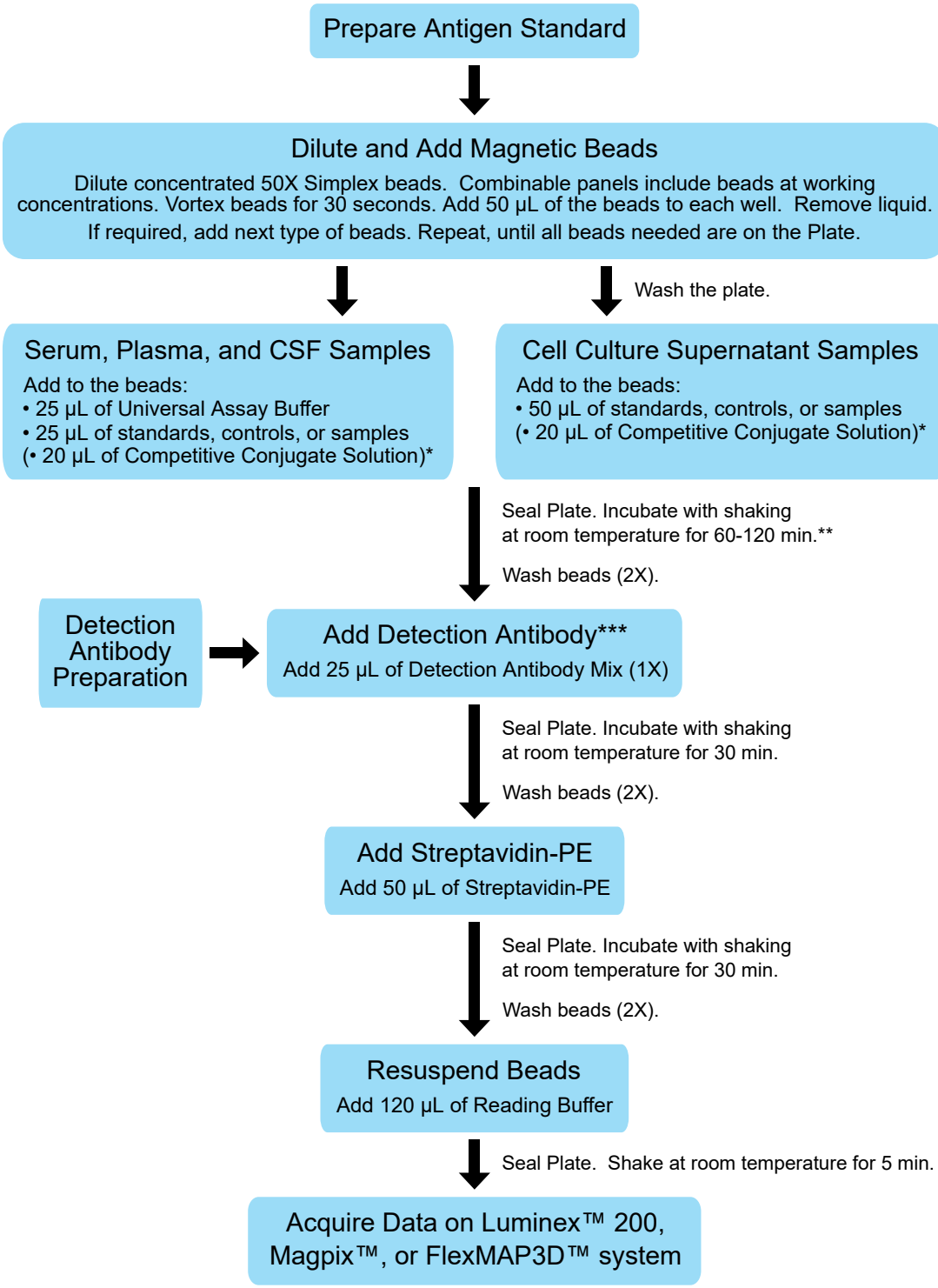
Analytes validated for measuring Cerebrospinal fluid (CSF) samples including recommended dilution factors for analytes with high normal CSF concentration are listed in the table below. When preparing dilution of CSF samples, use Universal Assay Buffer (1X).

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

(continued)

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

Assay protocol overview



* For Competitive Assays, add Competitive Conjugate Solution.

** For assays that require higher sensitivity, 120 min or overnight incubation is recommended.

*** If only using Competitive Assays, this step is obsolete.

Prepare the reagents

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. Store 1X wash buffer at 2°C to 8°C for up to 6 months if needed.

Note: Wash Buffer Concentrate (10X) volume might not be sufficient if using an automated plate washer. For bulk orders use Cat. No. EPX-66666-001.

Prepare 1X universal assay buffer

For analytes with high serum, plasma, or CSF concentration 10X Universal Assay Buffer will be provided. Prepare a 1X working concentration of Universal Assay Buffer by mixing 10 mL of the 10X Universal Assay Buffer with 90 mL ddH_2O.

Prepare 1X competitive conjugate solution for competitive assays

Competitive Conjugate Solution (50X) is included in Competitive Assays. Prepare the Competitive Conjugate Solution (1X) by mixing 50 µL of Competitive Conjugate Solution (50X) with 2,450 µL Universal Assay Buffer (1X).

Prepare 1X simplex beads

IMPORTANT! ProcartaPlex™ simplex kits and/or combinable panels can be mixed for enhanced flexibility. Ensure that the bead regions from your ProcartaPlex™ simplex kits or panels 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.

Simplex kits are provided with concentrated 50X beads. Therefore, dilution of concentrated simplex beads is required.

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

2. Fill up with Wash Buffer (1X) to a final volume of 5 mL. To combine 2 or more different bead vials follow the table below (using a whole plate):

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

(continued)

Number of different simplex bead vials to be mixed	Total volume of mixed bead solution	Volume of wash buffer (1X) to add
5	500 µL	4,500 µL
6	600 µL	4,400 µL

Prepare 1X detection antibody mixture

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

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

Prepare antigen standard

Carefully review the Certificate of Analysis for lot-specific information on the kit components. The majority of kits are supplied with lyophilized multistandards containing 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 preparing antigen standards, the final volume after reconstitution and pooling should be 250 µL. 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, contact our technical support.

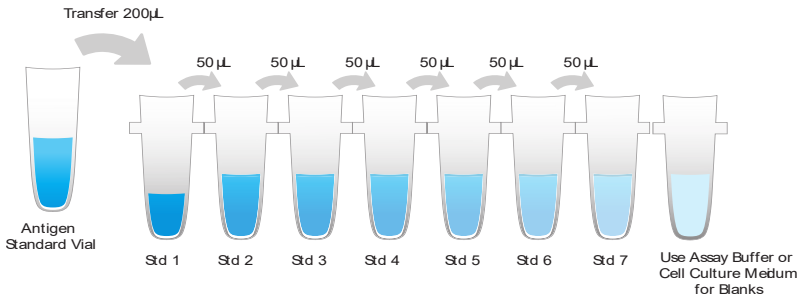
Reconstitution and pooling of standards

1. Centrifuge each different antigen standard vial(s) at 2,000 × *g* for 10 seconds.
2. Add 50 µL of sample type specific buffer into each standard vial. If you want to measure serum, plasma, or CSF samples, use Universal Assay Buffer (1X) to reconstitute the standard; if you want to analyze cell culture supernatant samples, use the cell culture medium that was used to culture the cells to dissolve the standard.
3. Gently vortex the vial(s) for 10 seconds and centrifuge at 2,000 × *g* for 10 seconds to collect contents at the bottom of the vial(s).
4. Incubate on ice for 10 minutes to ensure complete reconstitution.
5. Pool entire contents of each vial into one of the vials and fill up with sample type specific buffer to a total volume of 250 µL.
6. Gently vortex the vial for 10 seconds and centrifuge at 2000 × *g* for 10 seconds to collect contents at the bottom of the vial.

Number 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. Prepare a 4-fold serial dilution of the reconstituted standard(s) using the PCR 8-tube strip provided. Label tubes Std1, Std2, Std3, Std4, Std5, Std6, and Std7.
2. Add 200 µL of the reconstituted antigen standards into the first tube Std1 of the strip.
3. Add 150 µL of sample type specific standard buffer into Std tubes 2–7. Use Universal Assay Buffer for serum, plasma, or CSF samples and cell culture media for culture supernatant samples.
4. Transfer 50 µL of the reconstituted antigen standards from Tube 1 into Tube 2.
5. Mix by pipetting up and down for a total of 10 times.
6. Transfer 50 µL of the mixed standards from Tube 2 into Tube 3.
7. Mix by pipetting up and down for a total of 10 times.
8. Repeat steps 4–7 for Std tubes 4–7.
9. Add 200 µL of Universal Assay Buffer or cell culture medium into tube 8 which serves as a blank. Keep on ice until ready to use.



Assay protocol

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.

   IMPORTANT! If working with Simplex kits only, add 50 µL per well of diluted 1X Simplex beads prepared according to instructions provided (see “Prepare 1X simplex beads” on page 15.

   Combinable panels are provided with magnetic beads at working concentration 1X. Diluted Simplex beads 1X can be added alone or in combination with combinable panels. Note that 50 µL per bead vial per well is required.

   a. Vortex each of the bead vials for 30 seconds and add 5 mL of each 1X bead solution to an appropriate sized tube, if you use a whole plate, otherwise adjust accordingly. After all of the bead vials are added, vortex the tube for another 30 seconds.

b. Pour the bead mix to a disposable reservoir and add the appropriate volume of bead solution to each well of the plate using the table below. Use a multichannel pipette for this step as well as for the steps that follow.

Number of different bead vials to be mixed	Amount added to each well
1	50 µL
2	100 µL
3	150 µL
4	200 µL
5	250 µL
6	300 µL

c. If more than 6 bead vials need to be mixed, proceed to step 3 and repeat steps 2 and 3 until all beads have been added to the plate wells and washed.

3. Wash magnetic beads.
   a. Securely insert the 96-well Flat Bottom Plate into the Hand-Held Magnetic Plate Washer, ensure that the plate is held in place by the tabs, and wait 2 minutes to allow the beads to accumulate on the bottom of each well.

   b. Remove the liquid in the wells by quickly inverting the Hand-Held Magnetic Plate Washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the Hand-Held Magnetic Plate Washer. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.

   c. Add 150 µL of Wash Buffer (1X) into each well and wait 30 seconds to allow the beads to accumulate on the bottom of each well.

   d. Remove the Wash Buffer in the wells by quickly inverting the Hand-Held Magnetic Plate Washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the Hand-Held Magnetic Plate Washer. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.

   e. Remove the 96-well Flat Bottom Plate from the Hand Held Magnetic Plate Washer and proceed to the next step.

4. Add sample type-specific buffer, samples, standards, and blanks, and then incubate.
   a. For serum, plasma, and CSF: Add 25 µL of Universal Assay Buffer (1X) to each well followed by 25 µL of prepared standards or samples into dedicated wells. For cell culture supernatants: Add 50 µL prepared standards or samples into dedicated wells.

   b. For wells designated as blanks: Add an additional 25 µL of Universal Assay Buffer for serum, plasma, or CSF samples. For cell culture supernatant samples, add 50 µL of cell culture medium.

c. *If not working with a Competitive Assay skip this step and PROCEED with step d.*

   For Competitive Assays only: Dilute Competitive Conjugate Solution (50X) as described in Preparation of Reagents. Add 20 µL of the diluted Competitive Conjugate Solution (1X) to each well and proceed with step d.

d. Seal the plate with the provided Plate Seal. Cover the plate with the Black Microplate Lid and shake at 500 rpm for 60–120 minutes at room temperature.

e. Alternatively, the 96-well plate can be incubated overnight. Shake the 96-well plate for 30 minutes at room temperature at 500 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 500 rpm.

IMPORTANT! If only working with Competitive Assays proceed directly to step 7.

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5. Wash the 96-well plate twice following step 3.

6. Add Detection Antibody Mixture and incubate.
   a. Add 25 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 500 rpm.

7. Wash the 96-well plate twice following step 3.

8. Add SAPE and incubate.
   a. Add 50 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 500 rpm.

9. Wash the 96-well plate twice following step 3.

10. Prepare the 96-well plate for analysis on a Luminex™ instrument.
    a. Add 120 µL of Reading Buffer 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 500 rpm.

    c. Remove Plate Seal and run the plate on a Luminex™ 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.

   IMPORTANT! For ProcartaPlex™ getting started guides, technical literature, protocol support tools, and common troubleshooting questions visit thermofisher.com/procartaplexsupport

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

A Troubleshooting

Observation	Probable cause	Recommend solution
Low flow rate	Samples/beads are stuck in flow cell	Remove the 96-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 re-using 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.
	Poor pipetting techniques	Use a multichannel pipettor and careful pipette techniques. Avoid touching pipette tips to sides of the wells when adding Wash Buffer.
Limited dynamic range for BioPlex software users	Instrument calibrated at high PMT settings	Calibrate the instrument using the CAL2 Low RP1 target value.
Low bead count	Volume of bead solution is too low	Add 120 µL Reading Buffer into each well and shake at 500 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 96-well plate in the dark.
	Samples causing the instrument to clog	Remove the 96-well Flat Bottom Plate and perform a wash and rinse to the instrument. Rerun the assay with further dilution of samples

(continued)

Observation	Probable cause	Recommend solution
Low bead count	Probe height is incorrect	Refer to the Luminex™ manual for proper adjustment of the needle height.
	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 500 rpm and shaking for at least 5 minutes before reading.
	Air bubble in the sample loop	Refer to the Luminex™ 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 provided (see "Prepare antigen standard" on page 17.
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.

B Recommended and blank 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
Blank	Blank	8	8	16	16	24	24	32	32	40	40

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

C Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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