

ProcartaPlex™ Multiplex Immunoassay

USER GUIDE

for Simplex Kits and Combinable Panels

Using Magnetic Beads for Serum, Plasma, and Cell Culture Supernatant

Instructions for Mouse and Rat Assays

Publication Number MAN0016936

Revision B.0 (33)



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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. MAN0016936

Revision	Date	Description
B.0 (33)	21 June 2021	Updated table under Dilution of Samples.
A.0 (32)	11 June 2018	New manual.

Limited Use Label Licence No. 660: Label License/Sticker for Luminex Assay Product: Notice to Purchaser: By opening the packaging containing this Assay Product (which contains fluorescently labeled microsphere beads authorized by Luminex Corporation) or using this Assay Product in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return this Assay Product for a full refund prior to using it in any manner.

You, the customer, acquire the right under Luminex Corporation's patent rights, if any, to use this Assay Product or any portion of this Assay Product, including without limitation the microsphere beads contained herein, only with Luminex Corporation's fluorescent analytical test instrumentation marketed under the name Luminex Instrument.

Limited Use Label Licence No. 661: Standard Terms And Conditions For Use of Luminex Assay Product: Note to Purchaser: By opening the packaging containing this assay product or cassette ("Product") or by using such Product in any manner, you are consenting and agreeing to be bound by the following terms and conditions. You are also agreeing that the following terms and conditions constitute a legally valid and binding contract that is enforceable against you. If you do not agree to all of the terms and conditions set forth below, you must promptly return the Product for a full refund prior to using them in any manner.

1. Acceptance - ALL SALES ARE SUBJECT TO AND EXPRESSLY CONDITIONED UPON THE TERMS AND CONDITIONS CONTAINED HEREIN, AND UPON BUYER'S ASSENT THERETO. NO VARIATION OF THESE TERMS AND CONDITIONS SHALL BE BINDING UPON LUMINEX CORPORATION ("LUMINEX") UNLESS AGREED TO IN WRITING AND SIGNED BY AN AUTHORIZED REPRESENTATIVE OF LUMINEX.

For purposes of this agreement, "Seller" shall mean either Luminex, if the Product is purchased directly from Luminex, or a Luminex authorized reseller. Buyer, by accepting the Product shall be deemed to have assented to the terms and conditions set forth herein, notwithstanding any terms contained in any prior or later communications from Buyer and whether or not Seller shall specifically or expressly object to any such terms.

2. Warranties - Notwithstanding Buyer's acceptance thereof, if Product is purchased directly from Luminex, Luminex warrants that the Product shall conform to the quantity and content stated on the label and perform in all material respects consistent with Product specifications accompanying the Product until the expiration date set forth on the Product label. If Product is purchased from a Luminex authorized reseller, any warranty obligations shall be provided in writing directly by such Luminex authorized reseller to Buyer. THIS WARRANTY IS EXCLUSIVE AND LUMINEX MAKES NO OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. Seller's warranties made in connection with this sale shall not be effective if Seller has determined, in its sole discretion, that Buyer has misused the Product in any manner, has failed to use the Product in accordance with industry standards or practices, or has failed to use the Product in accordance with instructions, if any, furnished by Seller. BUYER'S EXCLUSIVE REMEDY WITH RESPECT TO PRODUCT PROVED TO SELLER'S SATISFACTION TO BE DEFECTIVE OR NONCONFORMING SHALL BE REPLACEMENT OF SUCH PRODUCTS WITHOUT CHARGE OR REFUND OF THE PURCHASE PRICE, IN SELLER'S SOLE DISCRETION, UPON THE RETURN OF SUCH PRODUCTS IN ACCORDANCE WITH SELLER'S INSTRUCTIONS. NEITHER SELLER NOR LUMINEX NOR ITS AFFILIATES SHALL IN ANY EVENT BE LIABLE FOR INCIDENTAL, CONSEQUENTIAL OR SPECIAL DAMAGES OF ANY KIND RESULTING FROM ANY USE OR FAILURE OF THE PRODUCT, EVEN IF SELLER OR LUMINEX OR ITS AFFILIATE HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING, WITHOUT LIMITATION, LIABILITY FOR LOSS OF WORK IN PROGRESS, DOWN TIME, LOSS OF REVENUE OR PROFITS, FAILURE TO REALIZE SAVINGS, LOSS OF PRODUCTS OF BUYER OR OTHER USE OR ANY LIABILITY OF BUYER TO A THIRD PARTY ON ACCOUNT OF SUCH LOSS, OR FOR ANY LABOR OR ANY OTHER EXPENSE, DAMAGE OR LOSS OCCASIONED

BY SUCH PRODUCT INCLUDING PERSONAL INJURY OR PROPERTY DAMAGE UNLESS SUCH PERSONAL INJURY OR PROPERTY DAMAGE IS CAUSED BY SELLER'S GROSS NEGLIGENCE.

3. Buyer's Use of Product - Buyer agrees that no rights or licenses under Luminex's patents shall be implied from the sale of the Product, except as expressly provided herein or as specifically agreed to in writing by Luminex, and Buyer does not receive any right under Luminex's patent rights hereunder. Buyer acknowledges and agrees that the Product is sold and licensed only for use with Luminex's instrumentation. In order to maintain the quality of the Product, Buyer may use this Product only once on a single use basis and shall not reuse this Product under any circumstances. Buyer further acknowledges that the Product has not received clearance from the United States Food and Drug Administration or other federal, state or local regulatory agencies and has not been tested by Seller or Luminex for safety or efficacy in food, drug, medical device, cosmetic, commercial or any other use, unless otherwise stated on the Product label or in Seller's technical specifications or material data sheets furnished to Buyer. Buyer expressly represents and warrants to Seller that Buyer will use the Product in accordance with the Product label, if applicable, and will properly test and use any Product in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with the United States Food and Drug Administration and all applicable domestic and international laws and regulations, now and hereinafter enacted.

BUYER HEREBY GRANTS TO LUMINEX A NONEXCLUSIVE, WORLDWIDE, UNRESTRICTED, ROYALTY-FREE, FULLY PAID-UP LICENSE, WITH THE RIGHT TO GRANT AND AUTHORIZE SUBLICENSES, UNDER ANY AND ALL PATENT RIGHTS IN INVENTIONS COMPRISING MODIFICATIONS, EXTENSIONS, OR ENHANCEMENTS MADE BY BUYER TO THE PRODUCT OR TO THE MANUFACTURE OR USE OF THE PRODUCT ("IMPROVEMENT PATENTS"), TO MAKE, HAVE MADE, USE, IMPORT, OFFER FOR SALE OR SELL ANY AND ALL OF THE PRODUCT; EXPLOIT ANY AND ALL METHODS OR PROCESSES; AND OTHERWISE EXPLOIT IMPROVEMENT PATENTS FOR ALL PURPOSES. NOTWITHSTANDING THE FOREGOING, "IMPROVEMENT PATENTS" SPECIFICALLY EXCLUDES PATENT CLAIMS CONCEIVED AND REDUCED TO PRACTICE BY BUYER CONSISTING OF METHODS OF SAMPLE PREPARATION, THE COMPOSITION OF MATTER OF THE SPECIFIC CHEMISTRIES OF THE ASSAYS DEVELOPED BY BUYER AND METHODS OF PERFORMING THE ASSAYS (I.E., THE PROTOCOL FOR THE ASSAY).

Buyer has the responsibility and hereby expressly assumes the risk to verify the hazards and to conduct any further research necessary to learn the hazards involved in using the Product. Buyer also has the duty to warn Buyer's customers, employees, agents, assigns, officers, successors and any auxiliary or third party personnel (such as freight handlers, etc.) of any and all risks involved in using or handling the Product. Buyer agrees to comply with instructions, if any, furnished by Seller or Luminex relating to the use of the Product and to not misuse the Product in any manner. Buyer shall not reverse engineer, decompile, disassemble or modify the Product. Buyer acknowledges that Luminex retains ownership of all patents, trademarks, trade secrets and other proprietary rights relating to or residing in the Product and Buyer receives no rights to such intellectual property rights by virtue of its purchase of Product other than as expressly set forth herein. Buyer shall have no right to use any trademarks owned or licensed to Luminex without the express written permission of Luminex.

4. Buyer's Representations, Release and Indemnity - Buyer represents and warrants that it shall use the Product in accordance with Paragraph 3, "Buyer's Use of Product," and that any such use of the Product will not violate any law, regulation, judicial order or injunction. Buyer agrees to release, discharge, disclaim and renounce any and all claims, demands, actions, causes of action and/or suits in law or equity, now existing or hereafter arising, whether known or unknown, against Seller and Luminex, and their respective officers, directors, employees, agents, successors and assigns (collectively the "Released Parties"), with respect to the use of the Product. Buyer agrees to indemnify and hold harmless the Released Parties from and against any suits, losses, claims, demands, liabilities, costs and expenses (including attorney, accounting, expert witness, and consulting fees) that any of the Released Parties may sustain or incur as a result of any claim against such Released Party based upon negligence, breach of warranty, strict liability in tort, contract or any other theory of law or equity arising out of, directly or indirectly, the use of the Product or by reason of Buyer's failure to perform its obligations contained herein. Buyer shall fully cooperate with the Released Parties in the investigation and determination of the cause of any accident involving the Product which results in personal injury or property damage and shall make available to the Released Parties all statements, reports, recordings and tests made by Buyer or made available to Buyer by others.
5. Patent Disclaimer - Neither Seller nor Luminex warrants that the use or sale of the Product will not infringe the claims of any United States or other patents covering the Product itself or the use thereof in combination with other products or in the operation of any process.

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. All other trademarks are the property of their respective owners.

©2021 Thermo Fisher Scientific Inc. All rights reserved.

Contents

■ ProcartaPlex™ Multiplex Immunoassay	5
Product description	5
How it works	5
Materials provided and storage conditions	6
Precautions and technical hints	7
Required equipment and materials not supplied	7
Sample preparation	7
Plasma sample preparation	8
Serum sample preparation	8
Dilution of samples	8
TGF-β (acidification of samples)	9
Assay protocol overview	10
Preparation of reagents	11
Prepare 1X wash buffer	11
Prepare 1X universal assay buffer	11
Prepare 1X simplex beads	11
Prepare 1X detection antibody mixture	12
Prepare antigen standard	12
Assay protocol	14
Setup of the Luminex™ instruments	16
Analyzing results	16
Troubleshooting	16
Recommended and blank plate layout	19
Customer and technical support	19
Limited product warranty	20



ProcartaPlex™ Multiplex Immunoassay



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](https://www.thermofisher.com/support).

Product description

This user manual is for a ProcartaPlex™ Immunoassay Kit to perform quantitative, multiplexed protein measurements from serum, plasma, 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.

How it works

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 100 protein targets on the Luminex™ 100/200™ and FLEXMAP 3D™ platforms and 50 protein targets on the MAGPIX™ platform.



Materials provided and storage conditions

ProcartaPlex™ Immunoassay Kits contain the components listed below. Refer to 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]	✓		✓
Magnetic Beads, Simplexes (50X) ^[1]	✓		
Magnetic Beads, premixed panels (1X) ^[1]			✓
Streptavidin-PE (SA-PE) (1X) ^[1]		✓	✓
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.



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.

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™ 100/200™ 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. MAGPIX™ instrument does 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.

Required equipment and materials not supplied

- MAGPIX™, Luminex™ 100/200™, FLEXMAP 3D™, or Luminex™-based instrument.
- Glass-distilled or deionized water.
- Adjustable single and multichannel pipettes with disposable tips.
- Multichannel pipette reservoir.
- Beakers, flasks, and cylinders necessary for preparation of reagents.
- Hand-Held Magnetic Plate Washer, vortex mixer and Microtiter™ plate shaker.

Sample preparation

- 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 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 since 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

We recommend to 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 min 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.

Dilution of 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	1,000
Mouse	CRP	1,000
Mouse	ICAM-1	1,000
Mouse	MCP-2 (CCL8)	1,000
Rat	CRP	200,000
Rat	ICAM-1	200
Rat	VCAM-1	200

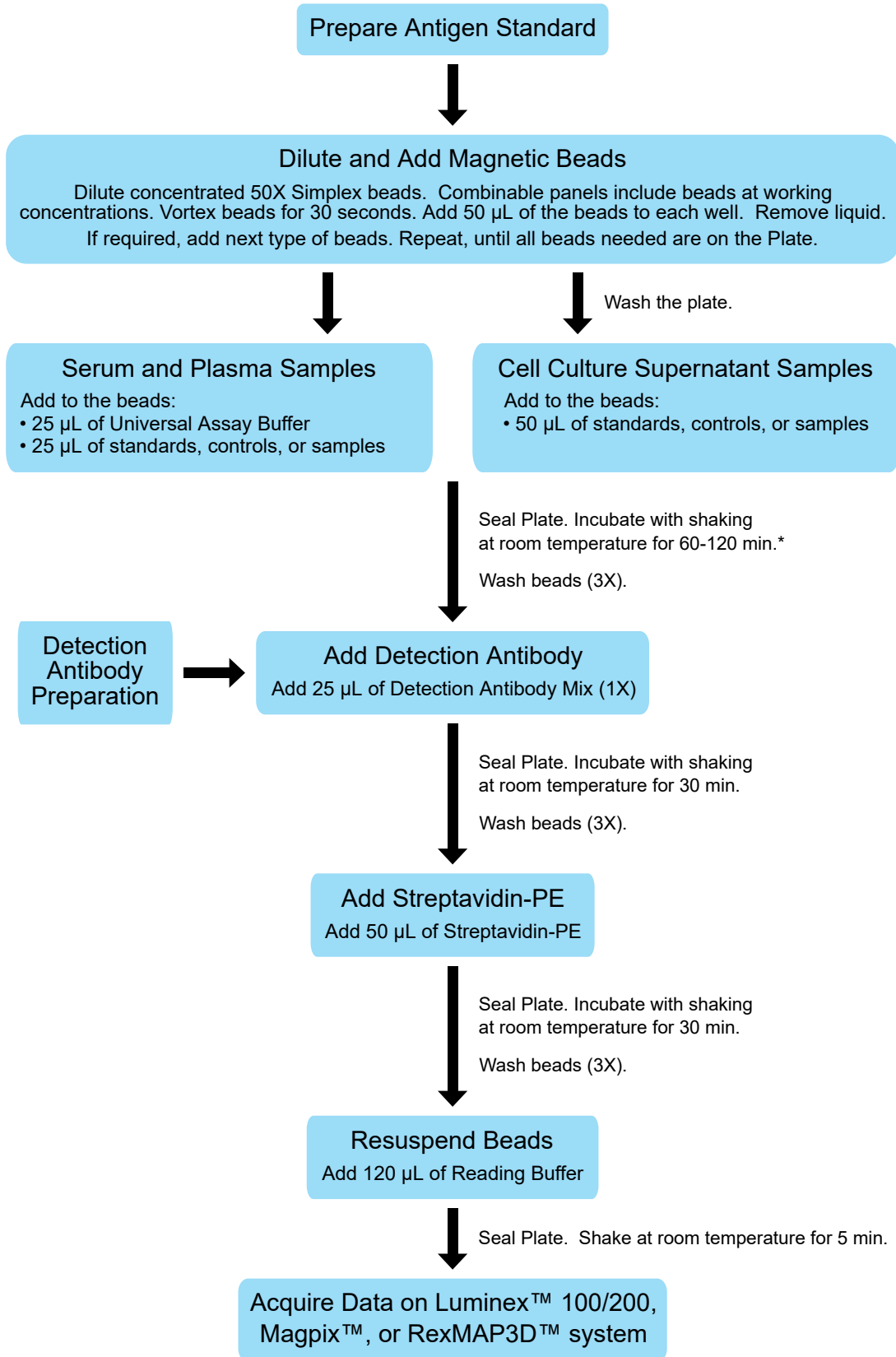


TGF- β (acidification of samples)

TGF- β 1 has to be acid treated for proper detection of the bioactive form. Therefore, this analyte should be tested as simplex assay. An additional datasheet with the preparation instruction will be included in each kit or can be requested by contacting our technical service.



Assay protocol overview





Preparation of 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 ddH₂O. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

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

Prepare 1X universal assay buffer

For analytes with high serum or plasma 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 ddH₂O.

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
5	500 µL	4,500 µL
6	600 µL	4,400 µL



Prepare 1X detection antibody mixture

For simplex and panels, 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 kit-specific antigen standard preparation instructions. 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 5 antigen standard sets contact our technical support.

Reconstitution and pooling of standards

1. Centrifuge each different antigen standard vial(s) at $2,000 \times g$ for 10 sec.
2. Add 50 µL of sample type specific buffer into each standard vial. If you want to measure serum or plasma 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 $2,000 \times g$ 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.

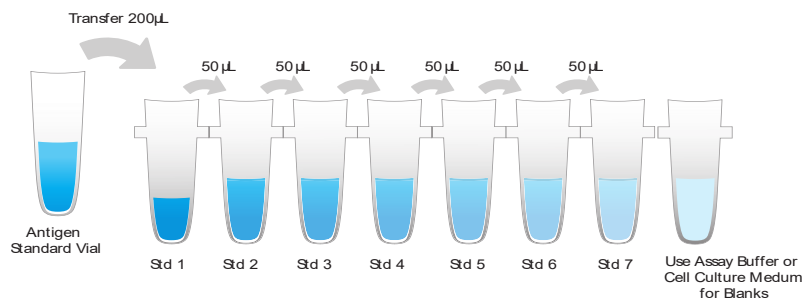


- Gently vortex the vial for 10 seconds and centrifuge at $2000 \times g$ for 10 seconds to collect contents at the bottom of the vial.

Number of standard sets	Reconstitution volume per vial	Pooled volume	Buffer to add	Total volume
1	50 μ L	50 μ L	200 μ L	250 μ L
2	50 μ L	100 μ L	150 μ L	250 μ L
3	50 μ L	150 μ L	100 μ L	250 μ L
4	50 μ L	200 μ L	50 μ L	250 μ L
5	50 μ L	250 μ L	0 μ L	250 μ L

Prepare 4-fold serial dilution

- 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.
- Add 200 μ L of the reconstituted antigen standards into the first tube Std1 of the strip tube.
- Add 150 μ L of sample type specific standard buffer into Std tubes 2–7. Use Universal Assay Buffer for serum or plasma samples and cell culture media for culture supernatant samples.
- Transfer 50 μ L of the reconstituted antigen standards from Tube 1 into Tube 2.
- Mix by pipetting up and down for a total of 10 times.
- Transfer 50 μ L of the mixed standards from Tube 2 into Tube 3.
- Mix by pipetting up and down for a total of 10 times.
- Repeat steps 4–7 for Std tubes 4–7.
- Add 200 μ L 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 11).

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 into 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 below.

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 the 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 the sample type-specific buffer, samples, standards, and blanks and incubate.
 - a. For serum and plasma: Add 25 μ L of Universal Assay Buffer (1X) to each well followed by 25 μ L of prepared standards or samples into dedicated wells. For cell culture supernatants: Add 50 μ L prepared standards or samples into dedicated wells.
 - b. For wells designated as blanks: Add an additional 25 μ L of Universal Assay Buffer for serum or plasma samples. For cell culture supernatant samples, add 50 μ L of cell culture medium.
 - c. 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.
 - d. 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.
 5. Wash the 96-well plate for a total of three washes following step 3.
 6. Add Detection Antibody Mixture and incubate.
 - a. Add 25 μ L 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 for a total of three washes following step 3.
 8. Add SAPE and incubate.
 - a. Add 50 μ L 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 for a total of three washes following step 3.
 10. Prepare the 96-well plate for analysis on a Luminex™ instrument.
 - a. Add 120 μ L 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.



Setup of the Luminex™ instruments

Instrument	Sample size	DD gate	Timeout	Bead event/bead region
Luminex™ 100/200™ FLEXMAP 3D™	50 µL	5,000–25,000	60 seconds	50–100
MAGPIX™	50 µL	N/A	N/A	50–100

Prior to running the assay, ensure that the probe height has been calibrated with 96-well Flat Bottom Plate supplied with the kit. Failure to adjust the probe height can cause damage to the instrument or low bead count. The Luminex™ system allows for calibration of low and high RP1 target values. We recommend RP1 low target value settings for ProcartaPlex™ immunoassays. 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 96-well Flat Bottom Plate can be re-read. Remove the 96-well Flat Bottom Plate from the instrument, insert the 96-well Flat Bottom Plate into the Hand-Held Magnetic Plate Washer, wait 2 minutes, then remove the buffer in the wells by quickly inverting the 96-well Flat Bottom Plate over a sink or waste container. Blot the assembly onto several layers of paper towels to remove any residual solution. Resuspend the beads in 120 µL of Reading Buffer, remove from the Hand-Held Magnetic Plate Washer, seal the 96-well Flat Bottom Plate with a new Plate Seal and Lid, and shake at 500 rpm for 5 minutes at room temperature. The assayed samples may take longer to read because there will be less beads in the well.

Analyzing results

The concentration of the samples can be calculated by plotting the expected concentration of the standards against the 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 (e.g., MAGPIX™, Luminex™ 100/200™, FLEXMAP 3D™). We offer a free and robust analysis software package for data analysis. For download information visit our website or contact our technical support.

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.



(continued)

Observation	Probable cause	Recommend solution
High CVs	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.
	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.
	Instrument needle is partially clogged	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.



(continued)

Observation	Probable cause	Recommend solution
Low signal or sensitivity	Standards not reconstituted and diluted correctly	Prepare fresh antigen standards following the instructions in the "Prepare antigen standard" on page 12.
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 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												

Customer and technical support

Visit [thermofisher.com/support](https://www.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/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.

