invitrogen

ProcartaPlex™ Multiplex Immunoassay

USER GUIDE

Instructions for Platinum™ Human Simplex Assays

Using Magnetic Beads for Serum and Plasma (EDTA, Citrate) Samples

Publication Number MAN0016942

Revision A.0 (30)

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Manufacturer: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES 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.

Limited Use Label License No. 358: Research Use Only: Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@thermofisher.com** or Licensing and Commercial Supply, Thermo Fisher Scientific, 5823 Newton Drive, Carlsbad, CA, 92008, United States.

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.

Contents

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**.

Product use

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.

Overcome matrix effects

The components in complex biological matrices such as serum and plasma may cause so called matrix effects which can impact the readout of many cytokines (low spike recovery and dilution linearity).Thermo Fisher Scientific developed matrix type specific sample diluents which assure high performance specifications comparable to those of traditional ELISA assys. The newly developed surrogate matrices for dilution of serum or plasma samples included in Platinum™ ProcartaPlex™ kits give spike and dilution-recovery results in the range of 70-130%.

How it works

ProcartaPlex™ Immunoassays incorporate magnetic microsphere technology licensed from the Luminex™ Corporation to enable the simultaneous detection and quantitation of multiple protein targets in diverse matrices. The platform allows the simultaneous detection from a single sample of up to 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 Simplex and Basic 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
Antigen Standards, premixed		√
Detection Antibody, premixed (50X)[1]	√	
Antibody Magnetic Beads, premixed (50X) [1]	√	
High Control		√
Low Control		√
Streptavidin-PE (SA-PE) (1X)[1]		√
Wash Buffer Concentrate (10X)[1]		√
Serum Assay Diluent (1X)[1]		√[2]
Plasma Assay Diluent (1X)[1]		√ [2]
Detection Antibody Diluent[1]		√
Reading Buffer[1]		√
PCR 8-Tube Strip		√
96-Well Flat Bottom Plate		√
Black Microplate Lid		√
Plate Seals		√

[1] Contains sodium azide. See WARNING.

[2] Depending on the sample type used Assay Diluent for Serum or Plasma will be provided

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, buildup 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 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 doesn't 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 multi-channel 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.
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.

Note: Only EDTA and Citrate Plasma Samples have been tested and validated with this kit.

Serum sample preparation

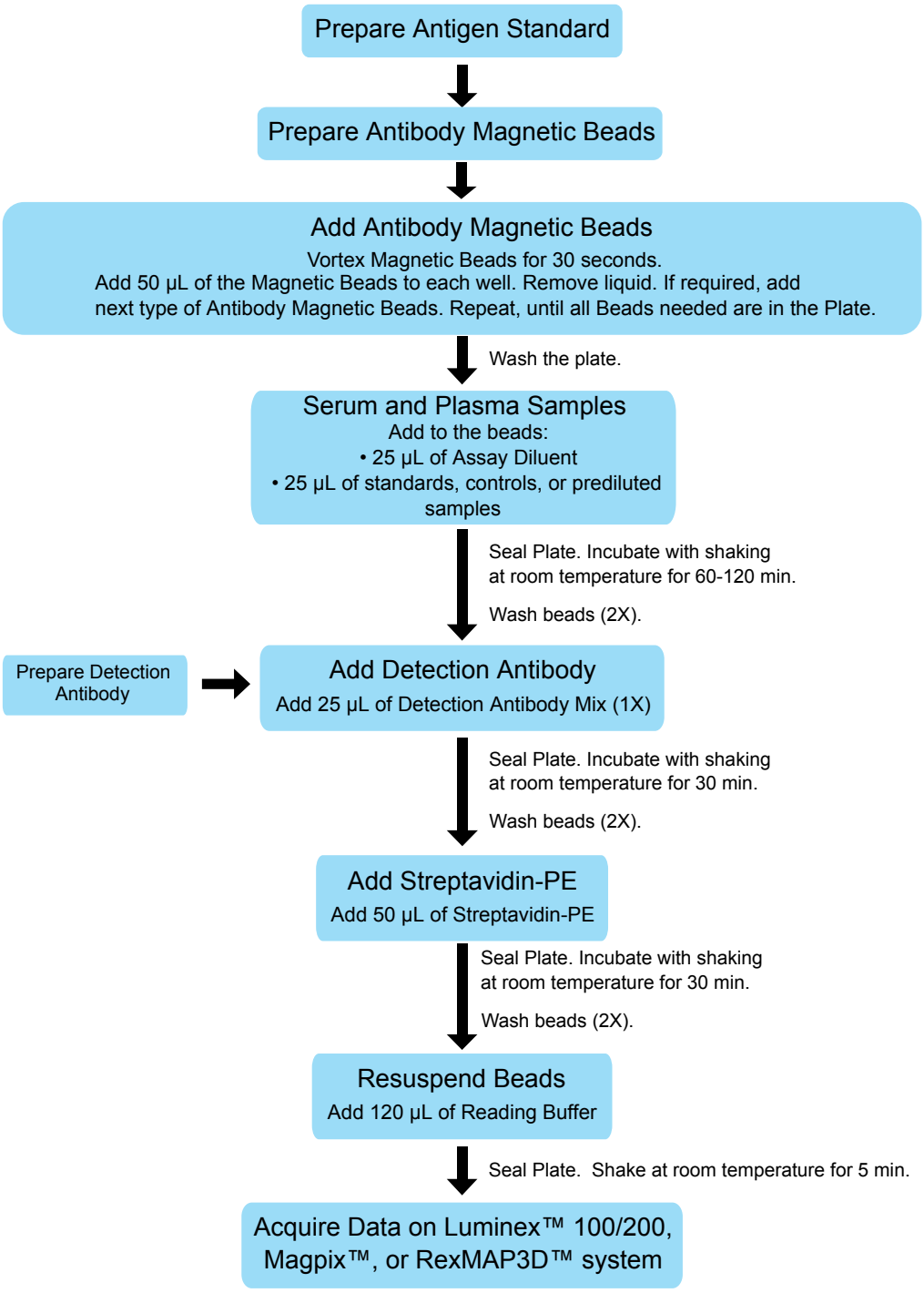
Spin down serum samples at 1,000 × *g* for 10 minutes at 20–25°C before running the assay.

1. Allow blood to clot for 20–30 minutes at 20–25°C.
2. Centrifuge at 1,000 × *g* for 10 minutes at 20–25°C.
3. Collect the serum fraction. (Alternatively, use any standard serum separator tube following the manufacturer's instructions.)
4. Use immediately or aliquot and store at –80°C.

Dilution of samples

Dilute the samples 4-fold in appropriate assay diluent (1X) (e.g., 20 µL of sample into 60 µL of diluent).

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 ddH_2O. 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 antigen standard and controls

Platinum™ ProcartaPlex™ Immunoassay Kits are supplied with lyophilized multi-standard and controls containing a mix of multiple proteins. Each kit is shipped with two identical vials of each premixed antigen standard and high/low control set from the same lot to permit the user to run the assay twice if running a partial plate.

Note: After usage remaining standards and controls cannot be stored and have to be discarded.

Reconstitution of standards and controls

1. Centrifuge each different antigen standard set vial(s) at 2,000 x *g* for 10 seconds.
2. Add 250 µL of appropriate assay diluent into each control vial. Add 50 µL of appropriate assay diluent into each different standard vial.
3. Gently vortex all standard and controls the vials for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial(s).
4. Incubate on ice for 10 min to ensure complete reconstitution.
5. **Only for the standards.** Pool entire contents of each different standard vial into one of the vials and add appropriate assay diluent to quantity sufficient (q.s) to 250 µL (see table below for example).

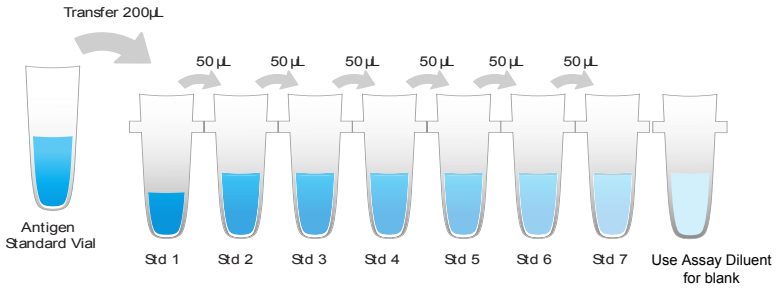
# of Standard sets	Reconstitution volume per vial	Pooled volume	Buffer to add	Total volume
1	50 µL	50 µL	200 µL	250 µL
2	50 µL	100 µL	150 µL	250 µL

Note: After reconstitution controls are ready to be used for the Assay Protocol step 4.

Prepare 4-fold serial dilution

1. Refer to Certificate of Analysis for the value of each premixed standard with assigning S1 values for each analyte for the current lot.
2. 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.
3. Add 200 µL of the reconstituted antigen standard into the first tube of the strip and label as Standard 1 (Std1).
4. Add 150 µL of appropriate assay diluent into Std tubes 2–7.

5. Transfer 50 µL of the reconstituted antigen standard from Tube 1 into Tube 2.
6. Mix by pipetting up and down for a total of 10 times.
7. Change the pipette tip and transfer 50 µL of the mixed standards from Tube 2 into Tube 3.
8. Mix by pipetting up and down for a total of 10 times.
9. Repeat steps 5–8 for Std tubes 4–7.
10. Add 200 µL of appropriate assay diluent into tube 8, which serves as a blank. Keep on ice until ready to use.



Expected values of controls

- Control High: S2-S3
- Control Low: S5-S6

Note: All control ranges were evaluated in appropriate assay diluent and 2 hours incubation at room temperature.

Antibody magnetic beads (1X)

Antibody Magnetic Beads in Platinum Simplex Kits are provided as 50X concentrate. If you want to combine Simplex Kits 120 µL of each 50X concentrated Antibody Magnetic Beads must be added to the mixing bottle and volume brought to 6 mL. Table below are an example for 96 and 48-wells.

Table 1 Example for 96-wells

Number of Vials of Antibody Magnetic Beads	Total volume of mixed Antibody Magnetic Beads	Volume of Wash Buffer (1X) to add
1	120 µL	5880 µL
2	240 µL	5760 µL
3	360 µL	5640 µL
4	480 µL	5520 µL
5	600 µL	5400 µL
6	720 µL	5280 µL

Table 2 Example for 48-wells

Number of Vials of Antibody Magnetic Beads	Total volume of mixed Antibody Magnetic Beads	Volume of Wash Buffer (1X) to add
1	60 µL	2940 µL
2	120 µL	2880 µL
3	180 µL	2820 µL
4	240 µL	2760 µL
5	300 µL	2700 µL
6	360 µL	2640 µL

Prepare 1X detection antibody mixture

For simplex kits detection antibody is provided at 50X concentration. If you want to combine simplex kits add 60 µL of each different detection antibody concentrate to the mixing bottle and bring volume to a total of 3 mL.Tables below are an example for 48 and 96-wells.

Table 3 Example for using 96-wells

Number of vials of Detection Antibody	Total volume of mixed Detection Antibody	Volume of Detection Antibody Diluent to add
1	60 µL	2940 µL
2	120 µL	2880 µL
3	180 µL	2820 µL
4	240 µL	2760 µL

Table 4 Example for using 48-wells

Number of vials of Detection Antibody	Total volume of mixed Detection Antibody	Volume of Detection Antibody Diluent to add
1	30 µL	1470 µL
2	60 µL	1440 µL
3	90 µL	1410 µL
4	120 µL	1380 µL

Assay protocol

1. Define the plate map.

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

2. Add Antibody Magnetic Beads to the plate.
   a. Vortex the Antibody Magnetic Beads (1X) vial for 30 seconds.

   b. Add 50 µL of the Antibody Magnetic Beads solution to each well of the plate. Use a multichannel pipette for this step as well as for the steps below.

3. Wash Antibody 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 appropriate assay diluent, prediluted samples, standards, controls and blanks and incubate.
   a. Add 25 µL of appropriate assay diluent to each well followed by 25 µL of prepared standards, controls or prediluted samples into dedicated wells.

   b. For wells designated as blanks: Add an additional 25 µL of appropriate assay diluent for serum or plasma samples.

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

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

6. Add 1X Detection Antibody Mixture and incubate.
   a. Add 25 µL of Detection Antibody 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.

Performance characteristics

Spike and dilution recovery

Similar to our coated ELISA, Platinum™ ProcartaPlex™ Immunoassays have to pass more than 30 qualification criteria to guarantiee reproducible high performance of the kits. One release criteria of Platinum ProcartaPlex kits is that both spike and dilution recovery have to be 100%+/-30% in serum, citrate and EDTA plasma.

Table 5 Overall mean spike and dilution recovery

Mean recoveries for each matrix were calculated across the complete portfolio of ProcartaPlex™. Platinum™ assays target specification of 100% +/- 30% are fulfilled, also regarding min/max values. The spike and dilution recovery was evaluated in a minimum of 5 individual donor samples per matrix. Spikes with recombinant proteins were performed using 3 different known concentrations (high, medium and low), covering the whole range of the assay. For dilution recovery (spiked) samples were prediluted 1:4 and then diluted in 4-fold serial dilution from 1:16 to 1:256. Recovery values may vary within different sample collectives. Therefore, the values provided above have to be seen as example values.

Analyte	Spike Recovery (%)			Dilution Recovery (%)		
	Serum	Plasma		Serum	Plasma	
		Citrate	EDTA		Citrate	EDTA
BDNF	82	85	86	95	106	102
Eotaxin	105	94	88	82	90	78
GM-CSF	90	94	89	110	84	84
GRO alpha	108	107	111	90	87	84
HGF	92	113	113	97	100	95
IFN alpha	99	88	113	103	91	92
IFN gamma	77	100	102	92	94	91
IL-10	79	91	91	104	90	89
IL-12p70	102	94	90	96	99	99
IL-13	98	97	94	90	93	90
IL-15	103	96	84	101	80	84
IL-16	84	95	91	90	87	79
IL-17A	81	79	78	103	88	87
IL-1 alpha	108	97	92	113	100	94
IL-1 beta	102	93	93	95	100	95
IL-1RA	111	103	90	108	97	100
IL-2	102	82	87	84	92	92
IL-20	94	95	99	95	93	88
IL-21	98	83	85	97	81	83
IL-4	97	99	96	90	96	90
IL-5	016	85	85	93	104	91
IL-6	84	87	86	95	94	97
IL-7	110	103	102	92	97	93
IL-8	85	95	92	94	90	89
IL-9	92	95	86	106	87	89
IP-10	99	97	86	87	95	96
LIF	98	110	100	95	99	94
MCP-2	118	97	95	99	94	97

Analyte	Spike Recovery (%)			Dilution Recovery (%)		
	Serum	Plasma		Serum	Plasma	
		Citrate	EDTA		Citrate	EDTA
MIP-1 alpha	86	86	76	95	96	86
MIP-1 beta	87	95	89	91	95	82
OPG	85	102	105	92	105	94
PFGF-BB	88	96	87	101	103	101
PECAM-1	89	121	117	88	90	82
P-Selectin	83	105	96	89	97	99
RANTES	99	84	85	90	99	93
SCF	103	94	94	98	92	91
TNF-RII	84	79	93	94	97	102
TNF alpha	99	111	112	104	75	81
tpA	77	103	106	91	101	99
TSLP	94	103	102	99	88	92
VEGF-A	87	93	86	94	93	92
VEGF-D	88	95	83	105	90	86

Setup of the 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 since 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 reusing the Plate Seal	Use a new Plate Seal for each incubation step.
	Incomplete washing	After adding the standards and samples, it is very important that any excess standards are removed during the wash step.
	Contamination from contents from adjacent wells	Avoid splashing the Wash Buffer during wash steps into adjacent wells.
	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.

Observation	Probable cause	Recommend solution
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.
	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 in "Prepare antigen standard and controls" on page 10
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	7	7	15	15	23	23	31	31
2	2	2	2	8	8	16	16	24	24	32	32
3	3	3	3	9	9	17	17	25	25	33	33
4	4	4	4	10	10	18	18	26	26	34	34
5	5	5	5	11	11	19	19	27	27	35	35
6	6	6	6	12	12	20	20	28	28	36	36
7	7	Control Low	Control Low	13	13	21	21	29	29	37	37
Blank	Blank	Control High	Control High	14	14	22	22	30	30	38	38

	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** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - 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 found on Life Technologies' website 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**.