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Platinum ProcartaPlex™ Human Panel 1 42-Plex

USER GUIDE

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ThermoFisher SCIENTIFIC

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

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Revision	Date	Description
A.0 (30)	15 February 2021	New manual

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Platinum ProcartaPlex™ Human Panel 1 42-Plex

Introduction

The Platinum ProcartaPlex™ Human Panel 1 42-Plex has been optimized for detection of multiple analytes from serum and plasma. This panel assures high performance specifications (e.g., spike and dilution recovery results of 70–130%) by using matrix type specific diluents for plasma and serum that help to overcome matrix effects.

The panel is provided in a ready-to-use format with individual vials of 1X capture and detection reagents that require less pipetting and experimental setup. These reagents are not combinable with simplexes or other panels.

ProcartaPlex™ preconfigured panels are extensively tested for analyte combinability, interference and cross-reactivity to provide the highest level of validation and precision. All ProcartaPlex™ panels are supplied with the necessary reagents to perform the assay.

Analytes		
BDNF	Eotaxin	GM-CSF
Gro alpha	HGF	IFN-alpha
IFN-gamma	IL-1 alpha	IL-1 beta
IL-1RA	IL-2	IL-4
IL-5	IL-6	IL-7
IL-8	IL-9	IL-10
IL-12p70	IL-13	IL-15
IL-16	IL-17A	IL-20
IL-21	IP-10	LIF
MCP-2	MIP-1 alpha	MIP-1 beta
OPG	PDGF-BB	PECAM-1
P-Selectin	RANTES	SCF

(continued)

Analytes		
TNF alpha	TNF-R2	tPA
TSLP	VEGF-A	VEGF-D

Contents and storage

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

Contents	Amount
Platinum Standard Mix (lyophilized)	2 each
Platinum High Control	2 each
Platinum Low Control	2 each
Biotinylated Detection Antibody Mix (1X)	1 x 3.5 mL
Capture Bead Mix (1X)	1 x 5 mL
Streptavidin-PE (SA-PE) (1X)	1 x 5 mL
Wash Buffer (10X)	1 x 25 mL
Reading Buffer (1X)	1 x 40 mL
Serum Assay Diluent	1 x 10 mL
Plasma Assay Diluent	1 x 10 mL
8-Tube Strip	2 each
Flat Bottom 96-well Plate, black	1 each
Microplate Lid	1 each
Plate Seals	8 each

Retain the lot-specific Certificate of Analysis that contains the product expiration date. The Certificate of Analysis also contains important information such as bead number, analyte names and highest standard concentration required for the assay setup on the xMAP instrument.

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Required materials not supplied

- xMAP™ instrument
- Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000)
- Deionized water
- Vortex mixer (e.g., Cat. No. 88882010)
- Microcentrifuge
- Adjustable single and multichannel pipettes with disposable tips and low volume reservoirs (e.g., Cat. No. 95128093)
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Orbital microplate shaker with at least 1.5 mm or 0.059 inch orbit diameter capable of maintaining a speed of 600 ± 50 rpm (e.g., Cat. No. 88882006)

Note: The use of rockers or large orbit shakers may cause adverse results.

Precautions and technical hints

1. Thoroughly read this User Guide and Certificate of Analysis prior to using the kit.
2. All chemicals should be considered potentially hazardous.
3. To avoid cross-contamination, do not invert the assay plate during the assay or allow contents from one well to mix with another well.
4. Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
5. Ensure that the xMAP™ instrument has been properly calibrated and set up prior to preparing and running the assay.

Workflow

Assay protocol

Prepare antigen standard

Add capture beads

1. Vortex capture beads for 30 sec. Add 50 µL of the capture beads to each well.
2. Remove liquid.

Note: Wash the plate after adding the beads.

Add samples, controls, and standards

1. Add the following according to sample type
   -**For serum and plasma samples:** Add 25 µL of appropriate assay diluent, then add 25 µL of standards, controls, or prediluted samples. For background wells, add 50 µL of assay diluent.
2. Seal the plate and incubate with shaking at room temp for 2 hr.
3. Wash plate twice.

Add detection antibody

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

Add Streptavidin-PE

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

Resuspend beads

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

Acquire data on xMAP™system

2 Methods

Sample preparation

Thaw frozen serum and plasma samples on ice and mix well by vortexing. Centrifuge at 10,000 × *g* for 5–10 minutes to pellet out particulates. Avoid multiple freeze/thaw cycles. If samples are high in lipid content, centrifuge at 10,000 × *g* for 10 minutes and transfer contents to a new tube.

Plasma sample preparation

Note: Only EDTA and sodium citrate plasma samples have been tested and validated with this kit.

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 store aliquots at –80°C.

Serum sample preparation

1. Allow blood to clot for 20–30 minutes at 20–25°C.
2. Centrifuge at 1,000 × *g* for 10 minutes at 20–25°C.
3. Collect the serum fraction. Alternatively, a serum separator tube can be used following the manufacturer's instructions.
4. Use immediately or store aliquots at –80°C. Avoid multiple freeze/thaw cycles.

Dilution of plasma and serum samples

Dilute the samples 4-fold in the appropriate Assay Diluent (1X) according to the following table:

Tube	Sample volume	Assay diluent volume
Dilution 1 (1:4)	20 µL	60 µL

Preparation of reagents

Before starting with the assay protocol, define the plate map. Mark the standard, sample and background wells using the plate map found in Appendix A, "Recommended plate layout" to determine the number of wells used.

Prepare 1X Wash Buffer

Bring the Wash Buffer Concentrate (10X) to room temperature and vortex for 15 seconds. Mix 20 mL of the Wash Buffer Concentrate (10X) with 180 mL ddH_2O. Mix gently to avoid foaming. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

Note: Additional Wash Buffer Concentrate (200 mL, Cat. No. EPX-66666-001) can be purchased separately for automated plate washers.

Prepare Standard Mix, High Control, and Low Control

This kit is supplied with one lyophilized Standard Mix for generation of standard curves and High and Low Controls. Two vials of each Standard Mix, High Control, and Low Control are provided to permit the user to run the assay twice if running a partial plate. For experiments measuring serum or plasma samples, use appropriate assay diluent as the diluent to reconstitute and dilute the standard and controls.

Note: Change pipette tips after each dilution step and avoid air bubbles.

1. Centrifuge the standard mix stock vial and both control vials at 2,000 x *g* for 10 seconds.
2. Add 250 µL of diluent to the standard and control vials.
3. Vortex the vials at high speed for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vials.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.

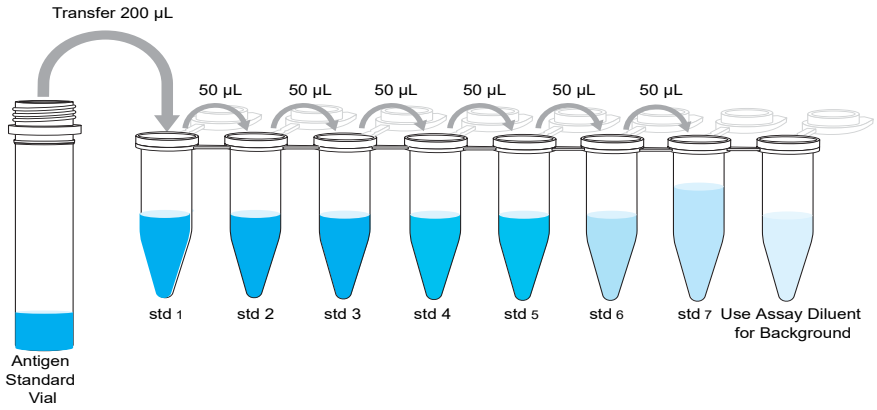
Note: After reconstitution, the controls are ready to be used in the assay protocol. The standard will need further dilution as described in the following section.

Prepare 4-fold serial dilution

1. Label the tubes in the 8-Tube Strip: Std1, Std2, Std3, Std4, Std5, Std6 and Std7.
2. Add 200 µL of the reconstituted standard mix into Std1 tube.
3. Add 150 µL of diluent into Std2–Std7 tubes.
4. Transfer 50 µL from Std1 tube into Std2 tube.
5. Mix by pipetting up and down 10 times.
6. Transfer 50 µL of the mixed standards from Std2 tube into Std3 tube using new pipette tip.
7. Mix by pipetting up and down 10 times.
8. Repeat steps 4–7 for tubes Std4–Std7, changing pipette tips between dilution steps.
9. Add 150 µL of diluent to the last tube of the 8-Tube Strip to serve as a background.

10. Keep tubes on ice until ready to use.

Note: Use the reconstituted standard and controls immediately. The reconstituted standard and controls cannot be stored. Discard unopened standard and control vials if the entire plate was used in a single experiment.



Expected values of controls

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

Note: All control ranges have been evaluated in both Serum and Plasma Assay Diluent and 2 hours incubation at room temperature.

Assay protocol

1. Add Capture Bead Mix to the plate.
   a. Vortex the 1X Capture Bead Mix vial for 30 seconds at high speed.
   b. Using a multichannel pipette, add 50 µL of the Capture Bead Mix to each well of the plate.
2. Wash beads using a Hand-Held Magnetic Plate Washer.

Note: To avoid loss of beads, secure the plate using the clamps on both sides of the Hand-Held Magnetic Plate Washer during this procedure.

Note: This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000). Other washers should be validated by the end user.

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   a. Place the plate on the Hand-Held Magnetic Plate Washer and wait 2 minutes to allow the beads to settle on the bottom of each well.
   b. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
   c. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
   d. Add 150 µL of 1X Wash Buffer into each well and wait 30 seconds.
   e. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
   f. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
   g. Remove the plate from the magnet and proceed to step 3.
3. Add samples, controls, and standards to the plate.
   a. **Serum and plasma**: Add 25 µL of appropriate assay diluent to each well followed by 25 µL of prepared standards, controls, or prediluted samples as defined on the plate layout. Add an additional 25 µL of appropriate assay diluent to the wells designated as backgrounds.
   b. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm for 2 hours at room temperature.

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

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4. Remove and discard the Plate Seal. Wash the plate following the steps below.
   a. Place the plate on the Hand-Held Magnetic Plate Washer and wait 2 minutes to allow particles to settle on the bottom of each well.

b. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.

c. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.

d. Add 150 µL of 1X Wash Buffer into each well and wait 30 seconds.

e. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.

f. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.

g. Repeat steps 4d-4f once for a total of two washes.

h. Remove the plate from the magnet and proceed to the next step.

5. Add Biotinylated detection Antibody Mix to the plate.
   a. Using a multichannel pipette, add 25 µL of the detection antibody solution to each well of the plate. Gently tap the plate to evenly distribute the solution in the wells.

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

   b. Seal the plate using a new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.

6. Wash the plate following step 4.

7. Add Streptavidin-PE (SA-PE) to the plate.
   a. Add 50 µL of SA-PE solution to each well.

   b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.

8. Wash the plate following step 4.

9. Prepare the plate for analysis on a xMAP™ instrument.
   a. Add 120 µL of reading buffer into each well.

   b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 5 minutes at room temperature.

10. Remove the Plate Seal and run the plate on a xMAP™ instrument.

Performance characteristic: Spike and dilution recovery

Similar to our coated ELISA kits, Platinum ProcartaPlex™ assays 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.

Analytes	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	106	85	85	93	104	91
IL-6	84	87	86	95	94	97
IL-7	110	103	102	92	97	93

(continued)

Analytes	Spike Recovery (%)			Dilution Recovery (%)		
	Serum	Plasma		Serum	Plasma	
		Citrate	EDTA		CItrate	EDTA
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
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
PDGF-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-R2	84	79	93	94	97	102
TNF alpha	99	111	112	104	75	81
tPA	77	103	106	91	101	99
TSLP	94	102	102	99	88	92
VEGF-A	87	93	86	94	93	92
VEGF-D	88	95	83	105	90	86

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.

Instrument settings

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

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

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

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

Analyze results

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

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

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

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

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

   Note: The samples have been diluted 1:4, which must be accounted for in the software analysis.

   IMPORTANT! For ProcartaPlex™ getting started guides, technical literature, protocol support tools, and common troubleshooting questions visit thermofisher.com/procartaplexsupport
   For more complete troubleshooting questions and answers, visit our FAQ database at thermofisher.com/procartaplexfaqs

A Recommended 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	CH[1]	CH	13	13	21	21	29	29	37	37
Bkgd[2]	Bkgd	CL[3]	CL	14	14	22	22	30	30	38	38

[1] Control High
[2] Background
[3] Control Low

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