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ProcartaPlex™ Mouse Immune Monitoring Panel 384-well (Lyophilized Beads) 48-Plex

USER GUIDE

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ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. MAN0024978

Revision	Date	Description
A.0 (30)	03 May 2021	New manual.

Contents

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Introduction

The ProcartaPlex™ Mouse Immune Monitoring Panel 384-well (Lyophilized Beads) 48-Plex is provided with pre-dispensed capture beads on the plate eliminating the bead dispensation step in the assay workflow minimizing bead dispensation errors. The panel has been optimized for detection of multiple analytes from serum, plasma, and cell culture supernatants and is provided in a ready-to-use format with individual vials of 1X detection reagents that require less pipetting and experimental setup. These reagents are not combinable with simplexes or other panels.

Analytes		
BAFF	Betacellulin (BTC)	ENA-78 (CXCL5)
Eotaxin (CCL11)	G-CSF (CSF-3)	GM-CSF
Gro alpha (CXCL1)	IFN alpha	IFN gamma
IL-1 alpha	IL-1 beta	IL-2
IL-2R	IL-3	IL-4
IL-5	IL-6	IL-7
IL-7R alpha	IL-9	IL-10
IL-12p70	IL-13	IL-15/IL-15R
IL-17A (CTLA-8)	IL-18	IL-19
IL-22	IL-23	IL-25 (IL-17E)
IL-27	IL-28	IL-31
IL-33	IL-33R (ST2)	IP-10 (CXCL10)
Leptin	LIF	M-CSF
MCP-1 (CCL2)	MCP-3 (CCL7)	MIP-1 alpha (CCL3)
MIP-1 beta (CCL4)	MIP-2	RANKL
RANTES (CCL5)	TNF alpha	VEGF-A

Contents and storage

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

Contents	Amount
Standard Mix D (lyophilized)	1 each
Control High D	1 each
Control Low D	1 each
Biotinylated Detection Antibody Mix (1X)	1 x 3.5 mL
384-well Plate with lyophilized Capture Bead Mix (1X)	1 each
Streptavidin-PE (SA-PE) (1X)	1 x 5 mL
Wash Buffer (10X)	1 x 100 mL
Reading Buffer (1X)	1 x 40 mL
Universal Assay Buffer (1X)	1 x 10 mL
8-Tube Strip	2 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 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

IMPORTANT! Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000) cannot be used with 384-well plates.

- FLEXMAP 3D™ or INTELLIFLEX™ instrument
- Deionized water
- Fresh cell culture medium for running cell culture supernatant samples
- Vortex mixer (e.g., Cat. No. 88882010)

- Microcentrifuge
- Adjustable single and multichannel pipettes with disposable tips and low volume reservoirs (e.g., Cat. No. 95128093)
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Automated Plate Washer suited for the washing of 384-well plates.This assay was assessed by using the Biotek EL406 using the ring magnet (Biotek Cat. No. 7102215). Every other washer needs to be adjusted to the 384-well mircroplates
- Orbital microplate shaker with at least 1.5-mm or 0.059-inch orbit diameter capable of maintaining a speed of 1,400 ± 50 rpm

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

Precautions and technical hints

IMPORTANT! The 384-well plate with the lyophilized beads needs to be stored in the aluminum pouch. The aluminum pouch should only be opened directly before use. Carefully remove the plate from the pouch. After the plate is removed from the pouch, it should be held in an upright position.

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. For FLEXMAP 3D™ or INTELLIFLEX™ instruments, initiate the startup protocol to warm up the lasers for at least 30 minutes. Ensure that the instrument has been properly calibrated and set up prior to preparing and running the assay.

Workflow

Assay protocol

Prepare antigen standard

Dissolve lyophilized capture beads

1. Pipette 75 µL of Wash Buffer to each well and allow to sit for 10 min.
2. Wash plate once after incubation.

Add samples, controls, and standards

1. Add the following according to sample type

   -**For serum and plasma samples:** Add 6.3 µL of Universal Assay Buffer, then add 6.3 µL of standards, controls, or samples. For background wells, add 12.5 µL of 1X UAB.

   -**For cell culture supernatant samples:** Add 12.5 µL of standards, controls, or samples. For background wells, add 12.5 µL of cell culture medium.
2. Seal the plate and incubate with shaking at room temp for 2 hr.
3. Wash plate three timese.

Add detection antibody

1. Add 6.3 µL of Detection Antibody Mix (1X).
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate three times.

Add Streptavidin-PE

1. Add 12.5 µL of Streptavidin-PE.
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate three times.

Resuspend beads

1. Add 50 µL of Reading Buffer.
2. Seal the plate and shake at room temp for 5 min.

Acquire data on FLEXMAP 3D™ or INTELLIFLEX™ instrument

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

1. Collect samples in sodium citrate or EDTA tubes. If using heparin as an anticoagulant, no more than 10 IU of heparin per mL of blood collected should be used to prevent assay interference that can result in a false positive signal.
2. Centrifuge samples at 1,000 × *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.

Cell culture supernatant preparation

1. Centrifuge samples at 1,400 rpm for 10 minutes at 4°C to remove particulates.
2. Aliquot the clarified medium into clean polypropylene microcentrifuge tubes.
3. Use immediately or store aliquots at –80°C. Avoid multiple freeze/thaw cycles.

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 100 mL of the Wash Buffer Concentrate (10X) with 900 mL ddH_2O. Mix gently to avoid foaming. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

Note: Wash Buffer Concentrate volume in this kit is calculated for the use of the Biotek EL406 automated plate washer. Additional Wash Buffer Concentrate (200 mL, Cat. No. EPX-66666-001) can be purchased separately.

Prepare Standard Mix

This kit is supplied with one lyophilized Standard Mix for generation of standard curves. For experiments measuring serum or plasma samples, use 1X UAB as the diluent to reconstitute and dilute the standard. For experiments measuring cell culture supernatant samples, use fresh cell culture medium as the diluent.

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

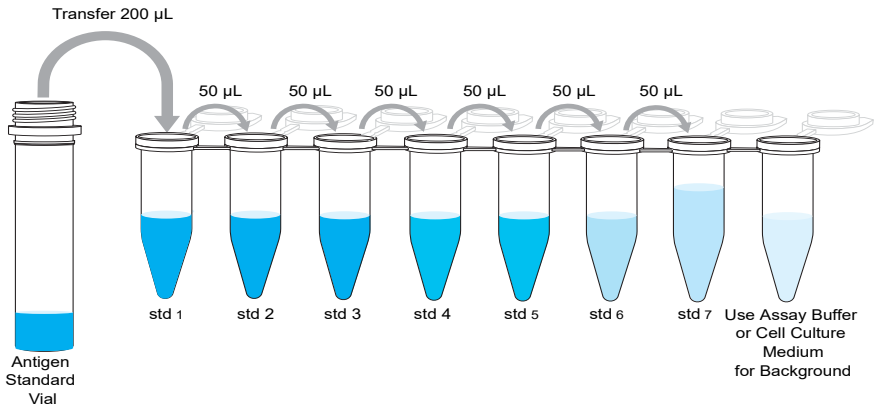
1. Centrifuge the standard mix stock vial at 2,000 x *g* for 10 seconds.
2. Add 250 µL of diluent to the stock vial.
3. Vortex the vial at high speed for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial.
4. Incubate on ice for 10 minutes to ensure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

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 immediately. The reconstituted standard cannot be stored.



Prepare High and Low Control

This kit is supplied with one High and one Low Control. For experiments measuring serum or plasma samples, use 1X UAB as the diluent to reconstitute and dilute the controls. For experiments measuring cell culture supernatant samples, use fresh cell culture medium as the diluent.

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

1. Centrifuge each Control stock vial at 2,000 x *g* for 10 seconds.
2. Add 250 µL of diluent to each Control stock vial.
3. Vortex the vials at high speed for 30 seconds and centrifuge at 2,000 × *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, controls are ready to be used for the assay protocol. Use the reconstituted controls immediately. The reconstituted controls cannot be stored.

Expected values of controls

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

Note: All control ranges were evaluated in Universal Assay Buffer and 2 hours incubation at room temperature.

Assay protocol

The 384-well plate with pre-dispensed, lyophilized beads needs to be used at once in a single assay run.

1. Prepare the 384-well plate with pre-dispensed, lyophilized beads.
   a. Add 75 µL of Wash Buffer to each well of the plate to reconstitute the lyophilized bead mix.
   b. Let the Wash Buffer sit for 10 min with no shaking.
      (Optional): Use an automated plate washer to pipette the Wash Buffer into the wells.
2. Wash beads using an Automated Plate Washer.

   Note: Be sure to create two wash protocols for the Automated Plate Washer: 1st protocol for one wash; 2nd protocol for three washes.

   a. Securely place the 384-well Flat Bottom plate on the Automated Plate Washer.
   b. Wash the beads once with the Automated Plate Washer. The washer should be programmed in a way that the beads settle for 2 min on the magnet before the buffer is removed. Between each washing step should be a 60-sec pause. For more details, see “Settings of the Biotek EL406 plate washer” on page 13.
   c. Remove the 384-well Flat Bottom Plate from the Plate Washer and proceed to the next step.
3. Add samples, controls, and standard to the plate.

   IMPORTANT! Gently touch the bottom of the well to dispense the solution for this and all other steps below.

   a. **Serum and plasma**: Add 6.3 µL of 1X UAB to each well followed by 6.3 µL of prepared standard, controls, or samples as defined on the plate layout. Add an additional 6.3 µL of 1X UAB to the wells designated as backgrounds.
   b. **Cell culture supernatants**: Add 12.5 µL of prepared standard, controls, or samples as defined on the plate layout. Add 12.5 µL of cell culture medium to the wells designated as backgrounds.
   c. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 1,400 ± 50 rpm for 2 hours at room temperature.

      Note: ProcartaPlex™ Quality Control assay validation is always performed at a 2-hr incubation at room temperature. For those wishing to perform the assay over two days, the 384-well plate can be incubated overnight. Shake the 384-well plate for 30 min at room temperature at 1,400 ± 50 rpm, then transfer the plate to 4°C and store on a level surface. After overnight incubation, shake the plate for an additional 30 min at room temperature at 1,400 ± 50 rpm.
4. Wash the 384-well plate three times following the procedure in the previous step 2.

5. Add Biotinylated Detection Antibody Mix to the plate.
   a. Using a multichannel pipette, add 6.3 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 1,400 ± 50 rpm for 30 minutes at room temperature.

6. Wash the 384-well plate three times following the procedure in the previous step 2.

7. Add Streptavidin-PE (SA-PE) to the plate.
   a. Add 12.5 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 1,400 ± 50 rpm for 30 minutes at room temperature.

8. Wash the 384-well plate three times following the procedure in the previous step 2.

9. Prepare the plate for analysis on a FLEXMAP™ or INTELLIFLEX™ instrument.
   a. Add 50 µL of reading buffer into each well.

   b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 1,400 ± 50 rpm for 5 minutes at room temperature.

10. Remove the Plate Seal and run the plate on a FLEXMAP™ or INTELLIFLEX™ instrument.

Settings of the Biotek EL406 plate washer

The following table outlines settings for the Biotek EL406 microplate washer using the ring magnet (Biotek Cat. No. 7102215).

Step	Description
Step 1: Shake/Soak	Shake: No
	Soak: YES 120 seconds (=before wash step begins)
	Home carrier: YES
Step 2: Washer wash[1]	**Cycles** Number of cycles for bead wash: 1 Number of cycles for 384-well plate wash: 3
	Aspiration • TOP • Travel rate: 6 CW (14.7 mm/sec) • Asp Delay: 0 sec • Asp x-position: 0 (center of well) • Asp y-position: 0 (center of well) • Asp height: 30 (3.811 mm) * • Secondary Asp: NO)
	Dispense • Rate: 09 • Volume: 50 µL/well • Vacuum Delay: 0 • (Buffer A) • Disp x-position: 0 (center of well) • Disp y-position: 0 (center of well) • Disp height: 120 (15.245mm)
	OPTS Midcycle • Wash Soak: YES 60 sec (=in between the two wash cycles) • Home carrier: YES Post Final Asp: YES (same settings as above) (=aspiration as last step of program)

[1] To test the setup of the washer, run the program with wash buffer. Then, using a pipette, manually check the residual volume of 10–15 wells in random positions. The residual volume in the wells should not exceed 6 µL.

Instrument settings

Instrument	Bead type	Acquisition volume	Reporter Gain	DD gate	Timeout	Min. bead count
FLEXMAP 3D™	MagPlex™	40 µL	Enhanced PMT	7,500–25,000	60 sec	50
INTELLIFLEX™	MagPlex™	40 µL	High PMT FLEXMAP 3D™	4,000–13,000	60 sec	50

Prior to running the assay, ensure that the probe height has been calibrated with a 384-well Flat Bottom Plate supplied with the kit. Failure to adjust the probe height can cause damage to the instrument or low bead count. 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 384-well Flat Bottom Plate can be re-read. Remove the 384-well Flat Bottom Plate from the instrument, insert the 384-well Flat Bottom Plate into the automated plate washer. Let the beads settle for 2 minutes on the magnet and then aspirate the fluid. Resuspend the beads in 50 µL of Reading Buffer, seal the 384-well Flat Bottom Plate with a new Plate Seal and Lid and shake at 1,400 ± 50 rpm for 5 minutes at room temperature. The assayed samples may take longer to read since there will be less beads in the well.

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. 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.

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: 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

Recommended plate layout

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	S1	S1	Con H[1]	Con H	23	23	39	39	55	55	71	71	87	87	103	103	119	119	135	135	151	151	167	167
B	S2	S2	Con L[2]	Con L	24	24	40	40	56	56	72	72	88	88	104	104	120	120	136	136	152	152	168	168
C	S3	S3	9	9	25	25	41	41	57	57	73	73	89	89	105	105	121	121	137	137	153	153	169	169
D	S4	S4	10	10	26	26	42	42	58	58	74	74	90	90	106	106	122	122	138	138	154	154	170	170
E	S5	S5	11	11	27	27	43	43	59	59	75	75	91	91	107	107	123	123	139	139	155	155	171	171
F	S6	S6	12	12	28	28	44	44	60	60	76	76	92	92	108	108	124	124	140	140	156	156	172	172
G	S7	S7	13	13	29	29	45	45	61	61	77	77	93	93	109	109	125	125	141	141	157	157	173	173
H	Bkg [3]	Bkg	14	14	30	30	46	46	62	62	78	78	94	94	110	110	126	126	142	142	158	158	174	174
I	1	1	15	15	31	31	47	47	63	63	79	79	95	95	111	111	127	127	143	143	159	159	175	175
J	2	2	16	16	32	32	48	48	64	64	80	80	96	96	112	112	128	128	144	144	160	160	176	176
K	3	3	17	17	33	33	49	49	65	65	81	81	97	97	113	113	129	129	145	145	161	161	177	177
L	4	4	18	18	34	34	50	50	66	66	82	82	98	98	114	114	130	130	146	146	162	162	178	178
M	5	5	19	19	35	35	51	51	67	67	83	83	99	99	115	115	131	131	147	147	163	163	179	179
N	6	6	20	20	36	36	52	52	68	68	84	84	100	100	116	116	132	132	148	148	164	164	180	180
O	7	7	21	21	37	37	53	53	69	69	85	85	101	101	117	117	133	133	149	149	165	165	181	181
P	8	8	22	22	38	38	54	54	70	70	86	86	102	102	118	118	134	134	150	150	166	166	182	182

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

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
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