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ProcartaPlex™ Automation Setup Kit

USER GUIDE

for 384-well Assays

Catalog Number EPX-SETUP-384

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Revision B.0

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Revision history: Pub. No. MAN0025568

Revision	Date	Description
B.0	21 March 2022	• A few minor edits were made to the publication. • The product SKU was added to the contents and storage table.
A.0	14 July 2021	Baseline for this revision history.

Contents

1 ProcartaPlex™ Automation Setup Kit

Product description

The ProcartaPlex™ Automation Setup Kit is used to adjust an automated plate washer, as needed, for a ProcartaPlex™ 384-well immunoassay wash without any performance or bead loss issues. The ProcartaPlex™ Automation Setup Kit can also be used for setting up and validating other automation equipment for ProcartaPlex™ 384-well assays, such as liquid handlers.

For the most current version of user documentation, visit our website **http://www.thermofisher.com**.

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.

Table 1 ProcartaPlex™ Automation Setup Kit, 6 tests (Cat. No. EPX-SETUP-384)

Contents	Amount
Automation Setup Beads (1X) (bead regions 20, 30, and 55)	1 x 4.5 mL
Wash Buffer (10X)[1]	1 x 200 mL
Reading Buffer (1X)	1 x 40 mL
384-well Flat Bottom Plate, black	6 plates
Plate Seals	6 seals

[1] Contains sodium azide. See CAUTION.

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

- FLEXMAP 3D™ or INTELLIFLEX™ instrument
- Glass-distilled or deionized water
- Adjustable single and multichannel pipettes with disposable tips
- Multichannel pipette reservoir
- Tubes, beakers, flasks, and cylinders for preparation of reagents
- Vortex mixer
- Orbital microplate shaker with at least 1.5 mm or 0.059 inch orbital diameter capable of maintaining a speed of 1,400 ± 50 rpm
- Automated plate washer suited for washing 384-well plates

Note: The ProcartaPlex™ Multiplex Immunoassays (384-well) were assessed using the BioTek™ EL406 plate washer and 384-well ring magnet (BioTek™ Part No. 7102215). See “Settings for the BioTek™ EL406 plate washer” on page 11 for the correct settings for the BioTek™ EL406 plate washer.

Settings for other automated magnetic plate washers need to be adjusted to the ProcartaPlex™ 384-well plates.

Workflow

Assay protocol

Prepare the 1X Wash Buffer

Mix 200 mL of Wash Buffer (10x) with 1,800 mL ddH_2O.

Prepare serial dilution of the Automation Setup Beads

1. Vortex the Automation Setup Beads (1X), then add 750 µL to tube 1.
2. Add 150 µL of Reading Buffer to tubes 2–5.
3. Vortex, then transfer 600 µL from tube 1 to tube 2. Vortex again.
   Repeat this step for the remaining tubes in the series.

Define the plate map

Mark the bead dilution, wash buffer, and residual volume wells using the recommended plate layout.

Add bead dilutions and 1X Wash Buffer to the plate

1. Add 25 µL of each bead dilution to the designated wells of the plate.
2. Add 25 µL of the 1X Wash Buffer to the remaining wells of the plate.

Wash the plate, then determine residual volume

1. Wash the plate 7 times using the washer settings recommended for your magnetic plate washer.
2. Determine the residual volume in 10–15 of the designated wells of the plate.

Add Reading Buffer, then run the plate

1. Add 50 µL of Reading Buffer to each well containing beads.
2. Seal the plate and shake at room temperature for 5 minutes.
3. Run the plate on a FLEXMAP 3D™ or INTELLIFLEX™ instrument.

Analyze the results

Export the CSV file containing the bead counts from each well.

2 Methods

Procedural guidelines

- Thoroughly read this user guide.
- For FLEXMAP 3D™ and INTELLIFLEX™ instruments, initiate the startup protocol to warm up the lasers for at least 30 minutes.
  - Ensure that the Luminex™ instrument is calibrated to the manufacturer's instructions.
- Do not invert the 384-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.

Prepare the 1X Wash Buffer

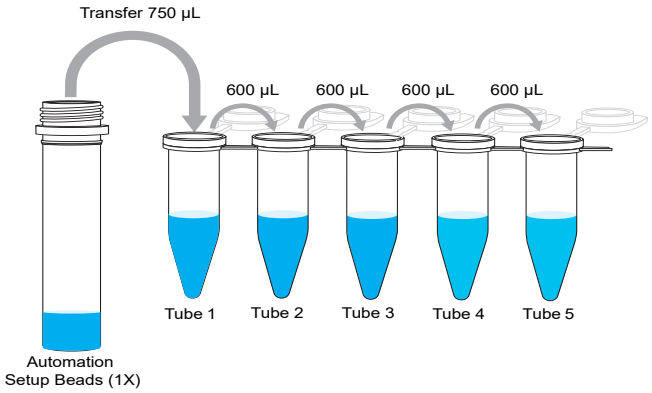
1. Bring the Wash Buffer (10X) to room temperature, then vortex for 15 seconds.
2. Mix 200 mL of the Wash Buffer (10X) with 1,800 mL ddH_2O.
   Mix gently to avoid foaming.

1X Wash Buffer can be stored at 2°C–8°C for up to 6 months.

Prepare serial dilution of the Automation Setup Beads

1. Label 5 tubes: 1, 2, 3, 4, and 5.
2. Vortex the Automation Setup Beads (1X) for 30 seconds, then add 750 µL to tube 1.
3. Add 150 µL of Reading Buffer to tubes 2–5.
4. Vortex, then transfer 600 µL from tube 1 to tube 2. Vortex again for 30 seconds.
5. Transfer 600 µL of the mixed beads from tube 2 to tube 3, then vortex for 30 seconds.

6. Repeat step 5 for tubes 4 and 5.



Define the plate map

Mark the bead dilution, wash buffer, and residual volume wells using the recommended plate layout on page 14 of this manual.

Perform the assay

Add bead dilutions and 1X Wash Buffer to the plate

1. Add 25 µL of each bead dilution to the plate in triplicate (see "Recommended plate layout" on page 14).
2. Add 25 µL of 1X Wash Buffer to the remaining wells of the plate.

Wash the plate, then determine residual volume

1. Wash the plate 7 times using the washer settings recommended for your specific magnetic bead washer.

Note: See "Settings for the BioTek™ EL406 plate washer" on page 11 for the wash settings of the BioTek™ EL406 microplate washer using the ring magnet.

- For the BioTek™ EL406 plate washer, use 1x1 wash and 3x2 wash to get to a total of 7 washes.
- Test and optimize the recommended settings for your magnetic plate washer using the instructions in this protocol. The most critical parameters to optimize for are:
  - Bead loss
  - Residual volume

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2. Remove the plate from the automated plate washer, then determine the residual volume in 10–15 wells of the plate using a single channel pipette (see “Recommended plate layout” on page 14 for the wells to test for residual volume).
   - Only use wells that contain wash buffer, not the wells that contain beads.
   - Residual volume in the wells should be less than 6 µL.

Add Reading Buffer, then run the plate

1. Add 50 µL of Reading Buffer to each well containing beads.
2. Seal the plate with a new Plate Seal, then incubate at room temperature for 5 minutes on a plate shaker set at 1,400 ± 50 rpm.
3. Remove the Plate Seal and run the plate on a FLEXMAP 3D™ or INTELLIFLEX™ instrument (see “Set up the instrument” on page 9).

Note: Prior to running the instrument, initiate the startup protocol to warm up the lasers for at least 30 minutes.

Set up the instrument

Select bead regions 20, 30, and 55 for the measurement of the ProcartaPlex™ Automation Setup Kit.

Instrument	Acquisition volume	Timeout (optional)	Bead type	DD gate	Min. bead count
FLEXMAP 3D™	40 µL	60 seconds	MagPlex™	7,500–20,000	50
INTELLIFLEX™	40 µL	40 seconds	MagPlex™	7,000–17,000	50

Note: Prior to running the assay, ensure that the probe height has been calibrated with the 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. For details on how to adjust the probe height, see the FLEXMAP 3D™ or INTELLIFLEX™ user manual.

Analyze the results

In xPONENT, export the CSV file which contains the bead count for every well. The bead counts for the first three bead dilutions should be ≥50 for every well and for every bead region/ID.

Note: The bead dilutions from tubes 4 and 5 may show bead counts below 50.

Troubleshooting

Troubleshooting

Observation	Probable cause	Recommended solution
Residual volume in wells is >6 µL	Comb height was set incorrectly	Lower the comb height of the washer.
All bead wells show bead counts <50	Beads were diluted incorrectly	Dilute the beads. The supplied stock serves as the first dilution.
	Comb was positioned incorrectly	Adjust the comb height or position.
A few wells of the first three bead dilutions show bead counts <50	Beads were not vortexed enough	Vortex beads thoroughly when repeating the experiment.
	Washer was not set up correctly	Change the plate washer settings.

B

Plate washer settings

Settings for the BioTek™ EL406 plate washer

The following table outlines settings for the BioTek™ EL406 microplate washer using the ring magnet (BioTek™ Part 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	**Cycles** Number of cycles for bead wash: 1 Number of cycles for 384-well plate wash: 2
	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)

(continued)

Step	Description
Step 2: Washer wash	**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)

Recommended plate layout

Recommended plate layout

x = determine residual volume

	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	Tube 1 beads				x																			
B	Tube 2 beads					x																		
C	Tube 3 beads					x																		
D	Tube 4 beads						x			x														
E	Tube 5 beads										x													
F																								
G												x												
H																	x							
I																		x						
J																								
K																			x					
L																								
M																						x		
N																							x	
O																							x	
P																								x