

# Maxpar Direct Immune Profiling Assay in PBMC

**IMPORTANT** Before using this quick reference, read and understand the detailed instructions and safety guidelines in the Maxpar® Direct™ Immune Profiling Assay™ Cell Staining and Data Acquisition User Guide (400286).

## Workflow Overview

Workflow Step	Time (approximate)						
<b>Day 1: Cell Staining (3x10<sup>6</sup> PBMC per tube)</b>							
<b>1 Prepare sample.</b> Aliquot, count, and determine PBMC viability.	Variable						
<b>2 FcR-block, stain cells, and fix cells.</b> Surface marker staining, Cell-ID™ live/dead intercalator-103Rh staining, and fresh fix.	1 hr 20 min						
<b>3 Stain cells with Cell-ID™ Intercalator-Ir.*</b>	10 min (hands-on) Incubate overnight.						
<b>Day 2: Data Acquisition</b>							
<b>1 Set up the instrument.†</b> <ul style="list-style-type: none"> <li>Helios™—Install WB Injector, warm up, tune, perform bead sensitivity test, and condition the instrument with Maxpar Cell Acquisition Solution (CAS). • 1 hr 35 min</li> <li>CyTOF® XT—Check nebulizer spray, load beads, warm up and tune. • 55 min</li> </ul>							
<b>2 Wash and count cells.</b>	25 min (variable)						
<b>3 Acquire data.†</b> Helios or CyTOF XT™ system, Maxpar Direct Immune Profiling Assay template, and CyTOF Software: <table border="0" style="width: 100%;"> <tr> <td></td> <td style="text-align: right;">(per sample):</td> </tr> <tr> <td>• Helios—v6.7.1016 (or later)</td> <td>• 20–25 min‡</td> </tr> <tr> <td>• CyTOF XT—v8.0 (or later)</td> <td>• 35–45 min‡</td> </tr> </table>		(per sample):	• Helios—v6.7.1016 (or later)	• 20–25 min‡	• CyTOF XT—v8.0 (or later)	• 35–45 min‡	
	(per sample):						
• Helios—v6.7.1016 (or later)	• 20–25 min‡						
• CyTOF XT—v8.0 (or later)	• 35–45 min‡						
<b>4 Perform post-run instrument maintenance.†</b> <ul style="list-style-type: none"> <li>Helios—Shut down system, remove injector, and clean parts. • 1 hr</li> <li>CyTOF XT—Shut down system and clean parts. • automated</li> </ul>							
<b>5 Normalize data.</b> CyTOF Software: <table border="0" style="width: 100%;"> <tr> <td></td> <td style="text-align: right;">(variable):</td> </tr> <tr> <td>• Helios—v6.7.1016 (or later)</td> <td>• 3–5 min/sample</td> </tr> <tr> <td>• CyTOF XT—v8.0 (or later)</td> <td>• automated</td> </tr> </table>		(variable):	• Helios—v6.7.1016 (or later)	• 3–5 min/sample	• CyTOF XT—v8.0 (or later)	• automated	
	(variable):						
• Helios—v6.7.1016 (or later)	• 3–5 min/sample						
• CyTOF XT—v8.0 (or later)	• automated						
<b>6 Analyze normalized data.</b>	Variable						

\* Potential stopping point

† Instrument operator: See Appendix A or B in the Maxpar Direct Immune Profiling Assay User Guide (400286) for information. We recommend that you read and understand the procedures before using the assay and before transferring this information to those responsible for instrument operation.

‡ Includes a wash step prior to sample acquisition.

## Day 1: Cell Staining

### Prepare the Reagents

**IMPORTANT** To ensure reliable results:

- Retrieve, mix, and centrifuge reagents as directed.
- Use frozen aliquots of Cell-ID Intercalator-Ir (S00093)
- Open a single-use Pierce™ 16% Formaldehyde (FA) ampule and prepare the 1.6% FA solution in Maxpar PBS (S00125) immediately before use in the fixation process.

### PBMC Preparation

- 1 Prepare PBMC from frozen PBMC aliquots using your preferred method to minimize environmental and experimental contaminants, making sure to lyse and remove red blood cells (RBC) to ensure maximum PBMC recovery.
- 2 Count cells and determine the cell viability of each sample. For best results, we recommend using samples with ≥80% cell viability and minimal to no RBC contamination.
- 3 Centrifuge each sample at 300 x g for 5 min, carefully aspirate supernatant, and resuspend in residual volume by gently vortexing.
- 4 Wash cells by adding 5 mL of Maxpar Cell Staining Buffer [CSB (201068)].
- 5 Centrifuge each sample at 300 x g for 5 min, carefully aspirate supernatant, and resuspend in residual volume by gently vortexing.

### FcR-Block Cells

- 1 Resuspend cells in CSB to a final concentration of 6 x 10<sup>7</sup> cells/mL.
- 2 Aliquot 50 µL (3 x 10<sup>6</sup>) of cells into a 1.5 mL tube for FcR-blocking.
- 3 Add 5 µL of Human TruStain FcX™ to each tube. Gently vortex to mix.
- 4 Incubate the tubes at room temperature for 10 min.
- 5 Continue to Antibody Staining without washing the cells.

## Antibody Staining

- 1 Add 215  $\mu\text{L}$  of CSB to each tube of PBMC, for a final volume of 270  $\mu\text{L}$ .
- 2 Transfer the 270  $\mu\text{L}$  ( $3 \times 10^6$  cells) of the FcR-blocked PBMC directly into a 5 mL tube containing the dry antibody pellet. Final volume of the resuspended dry antibody pellet and PBMC is approximately 300  $\mu\text{L}$ .  
**IMPORTANT** Open the Maxpar Direct Immune Profiling Assay foil packet (S00124) no more than 1 hr before use. Open cap only when ready to add sample.
- 3 Gently vortex to mix each tube and incubate at room temperature for 30 min.
- 4 Wash cells by adding 3 mL of CSB to each tube, gently vortex, and centrifuge at 300  $\times g$  for 5 min.
- 5 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- 6 Repeat Steps 4–5 for a total of 2 washes.
- 7 Proceed to Fix Cells.

## Fix Cells

- 1 Prepare a fresh 1.6% FA solution from the 16% formaldehyde stock ampule. Use a 1 mL Norm-Ject® latex-free syringe and compatible 0.1  $\mu\text{m}$  syringe filter to filter the stock formaldehyde, and then dilute 1 part of filtered stock formaldehyde with 9 parts Maxpar PBS.
- 2 Gently vortex to resuspend cells in residual volume.  
**IMPORTANT** It is essential to thoroughly disrupt the pellet by vortexing before adding the fresh fixative.
- 3 Add 1 mL of the 1.6% FA solution to each tube (containing  $3 \times 10^6$  cells in suspension) and gently vortex to mix well.
- 4 Incubate cells at room temperature for 10 min.
- 5 Centrifuge cells at 800  $\times g$  for 5 min.
- 6 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.

## Stain Cells with Cell-ID Intercalator-Ir

- 1 Prepare 1 mL of intercalation solution for each sample by adding Cell-ID Intercalator-Ir into Maxpar Fix and Perm Buffer (S00092) to a final concentration of 125 nM (a 1,000X dilution of the 125  $\mu\text{M}$  stock solution) and vortex to mix.
- 2 Add 1 mL of intercalation solution to each tube and gently vortex. Ensure that cells are well resuspended.
- 3 Incubate the samples at 2–8 °C overnight.  
**STOPPING POINT** Samples can be stored in intercalation solution at 2–8 °C for up to 48 hr before data acquisition.

## Day 2: Data Acquisition

### Set Up the Instrument

Make sure the Helios or CyTOF XT system is warmed up, successfully tuned, and ready to acquire data before proceeding to wash and count cells stained with Intercalator-Ir. Cells should be run on the same day they are washed from intercalation solution.

**IMPORTANT** Before you begin:

- Before starting the instrument, ensure that you are using the correct version of CyTOF Software for Maxpar Direct Immune Profiling Assay.
  - Helios—v6.7.1016 (108520) or later
  - CyTOF XT—v8.0 (130069) or later
- Samples stained with the Maxpar Direct Immune Profiling Assay antibody panel must be run using the **Maxpar Direct Immune Profiling Assay.tem** file or a template derived from it for analysis in Maxpar Pathsetter™ software (401018).

### Wash and Count Cells

- 1 Once the instrument tuning has passed, centrifuge tubes containing cells in intercalation solution at 800  $\times g$  for 5 min.
- 2 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- 3 Wash cells by adding 2 mL of CSB to each tube and gently vortex to mix. Centrifuge tubes at 800  $\times g$  for 5 min.
- 4 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- 5 Repeat wash steps 3–4 once for a total of 2 washes with CSB.
- 6 Wash cells by adding 2 mL of Maxpar Cell Acquisition Solution [CAS (201240), Helios only] or Maxpar Cell Acquisition Solution Plus for CyTOF XT [CAS Plus (201244), CyTOF XT only] to each tube and gently vortex. Centrifuge tubes at 800  $\times g$  for 5 min.
- 7 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- 8 Add 2 mL of CAS (Helios only) or CAS Plus (CyTOF XT only) to each tube and gently vortex.
- 9 (CyTOF XT only) Filter cells through 35  $\mu\text{m}$  cell strainer cap tubes into a new 5 mL polypropylene tube.
- 10 Reserve a small volume (approximately 10  $\mu\text{L}$ ) from each tube to count cells.
- 11 Centrifuge tubes at 800  $\times g$  for 5 min. While tubes are in the centrifuge, go to Step 12.
- 12 Count cells in the reserved volume from each tube. Make sure to note the cell count for each tube.

- **13** When centrifuging is complete, carefully aspirate (for CyTOF XT, leave approximately 100 µL residual volume in the tube) and discard supernatant.
- **14** Leave cells pelleted at 2–8 °C until ready to run.

### Acquire Cells for Analysis

**IMPORTANT** Run cells on the same day they are washed from intercalation solution.

### Acquire Cells on Helios

- **1** Shake the 1X EQ™ Four Element Calibration Beads [EQ4 beads (201078)] vigorously to resuspend.
- **2** Prepare enough volume of 0.1X EQ4 beads by diluting 1 part beads to 9 parts CAS to completely resuspend cells to the maximum recommended cell concentration of  $1 \times 10^6$  cells/mL.
- **3** Immediately before data acquisition, completely resuspend cells to the maximum recommended cell concentration of  $1 \times 10^6$  cells/mL.
- **4** Filter the cells through a 35 µm cell strainer into a new 5 mL polypropylene tube.
- **5** Acquire at least 300,000 events using the Maxpar Direct Immune Profiling Assay acquisition template.
- **6** Perform post-run instrument maintenance.
- **7** Normalize FCS files with CyTOF Software v6.7.1016 or later.
- **8** Transfer the normalized FCS files from their saved folder to a location for further analysis.
- **9** Import the normalized FCS files into Maxpar Pathsetter software for automated analysis.

### Acquire Cells on CyTOF XT

**IMPORTANT** Immediately before data acquisition, the instrument operator should ensure that a sufficient amount of 1X EQ Four Element Calibration Beads [EQ4 beads (201078)] is in Location 15 of the carousel and CAS Plus is in the reservoir bottle for sample resuspension and pre-wash cycles.

- **1** Set up the acquisition protocol using the Maxpar Direct Immune Profiling Assay acquisition template with **EQ4-MDIPA** beads detection selected. In the acquisition protocol:
  - a Select **Pellet** format. By default, **Add Beads** is checked for pelleted samples.
  - b Select **Events** in the Stopping Parameter column, and enter **300** in the Events (x 1000) field to acquire 300,000 events
  - c Enter the resuspension volume of each sample in the **Volume (µL)** column.
  - d Select **Light** from the Pre-wash Cycle column.
- **2** Retrieve the tubes containing the pelleted samples from 2–8 °C.
- **3** Place the samples into the carousel, and ensure that the sample tube matches its corresponding **Location** designated in the Acquisition Protocol batch tab.
- **4** In the acquisition protocol, click **Record** to start acquisition.
- **5** Perform post-run instrument maintenance.
- **6** Transfer the automatically normalized FCS files with the suffix **\_Processed** after the Sample Name from their saved folder to a location for further analysis.
- **7** Import the normalized FCS files into Maxpar Pathsetter software for automated analysis.

### Safety

Use standard laboratory safety protocols. Read and understand the safety data sheets (SDSs) before handling chemicals. To obtain SDSs, go to [fluidigm.com/sds](https://fluidigm.com/sds) and search for the SDS using either the product name or the part or catalog number.

**For technical support visit [fluidigm.com/support](https://fluidigm.com/support). For general support visit [fluidigm.com/support](https://fluidigm.com/support).**

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | [techsupport@fluidigm.com](mailto:techsupport@fluidigm.com) Latin America +1 650 266 6100 | [techsupportlatam@fluidigm.com](mailto:techsupportlatam@fluidigm.com)  
 Europe/Middle East/Africa/Russia +33 1 60 92 42 40 | [eu.support@fluidigm.com](mailto:eu.support@fluidigm.com) China (excluding Hong Kong) +86 21 3255 8368 | [techsupportchina@fluidigm.com](mailto:techsupportchina@fluidigm.com)  
 Japan +81 3 3662 2150 | [techsupportjapan@fluidigm.com](mailto:techsupportjapan@fluidigm.com) All other Asian countries/India/Australia +1 650 266 6100 | [techsupportasia@fluidigm.com](mailto:techsupportasia@fluidigm.com)

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

Information in this publication is subject to change without notice. **Patent and license information:** [fluidigm.com/legal/notices](https://fluidigm.com/legal/notices). **Limited Use Label License:** The purchase of this Fluidigm Instrument and/or Consumable product conveys to the purchaser the limited, nontransferable right to use with only Fluidigm Consumables and/or Instruments respectively except as approved in writing by Fluidigm. **Trademarks:** Fluidigm, the Fluidigm logo, Cell-ID, CyTOF, CyTOF XT, Direct, EQ, Helios, Immune Profiling Assay, Maxpar, and Pathsetter are trademarks and/or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners. © 2021 Fluidigm Corporation. All rights reserved. 07/2021