

# Maxpar Direct Immune Profiling Assay in Whole Blood

**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 (PN 400286).

## Workflow Overview

### Day 1: Cell Staining

Step	Time
<b>1 Prepare reagents.</b> Heparin blocking solution	20 min
<b>2 Prepare sample.</b> Heparin blocking	Variable
<b>3 Stain, lyse red blood cells (RBCs), and fix cells.</b> Surface marker and live/dead intercalator-103Rh staining	80 min
<b>4 Stain cells with Cell-ID™ Intercalator-Ir.*</b>	10 min (hands-on) Incubate overnight.

### Day 2: Data Acquisition

Step	Time
<b>1 Set up Helios.†</b> Install Helios™ WB Injector, warm up, tune, perform bead sensitivity test, and condition the instrument with Maxpar Cell Acquisition Solution (CAS).	~105 min
<b>2 Wash and count cells.</b>	25 min (variable)
<b>3 Acquire data.†</b> <b>Maxpar Direct Immune Profiling Assay</b> template CyTOF® Software v6.7.1016 (or higher) Helios system	10–15 min per sample
<b>4 Perform post-run instrument maintenance.†</b> Shut down system, remove injector, and clean parts.	1 hr 25 min
<b>5 Normalize data.</b> CyTOF Software v6.7.1016 (or higher)	20 min (variable)
<b>6 Analyze normalized data.</b>	Variable

\* Potential stopping point

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

## Day 1: Cell Staining

### Prepare the Reagents

**IMPORTANT** To ensure reliable results:

- Retrieve, mix, and centrifuge reagents as directed.
- Use frozen aliquots of Intercalator-Ir.
- Open a single-use Pierce™ 16% Formaldehyde (FA) ampule and prepare the 1.6% FA solution in Maxpar PBS immediately before use in the fixation process.
- Prepare the 10 KU/mL heparin solution by adding 1 mL of Maxpar PBS solution to 10 KU of sodium heparin salt. This solution can be stored at 2–8 °C while you prepare samples for blocking.
- Retrieve Cal-Lyse™ lysing solution. Aliquot enough solution to test while protecting the solution from light.

### Antibody Staining

**IMPORTANT** Whole blood samples should be collected in sodium heparinized tubes [BD Vacutainer® glass blood collection tubes with sodium heparin (Cat. No. 366480)].

- **1** Add 10 µL of 10 KU/mL heparin solution per 1 mL of whole blood for a final concentration of 100 U/mL. Heparin block the volume of whole blood that will be used for staining. This step reduces nonspecific binding of antibodies.
- **2** Gently vortex to mix each tube and incubate the tubes for 20 min at room temperature.
- **3** Aliquot 270 µL of heparin-blocked whole blood into one 5 mL tube containing the dry antibody pellet. The final volume of the Ab pellet and blood is 300 µL.  
**NOTE** 270 µL of whole blood is required per sample for efficient staining. If using less than 270 µL of blood, add Maxpar Cell Staining Buffer (CSB) to reach a total volume of 270 µL.  
**NOTE** Open assay foil packet no more than 1 hr before use. Open cap only when ready to add sample.
- **4** Gently vortex to mix each tube.
- **5** Incubate tube for 30 min at room temperature.

### Red Blood Cell Lysis

- **1** Immediately after staining is complete, add 250 µL of Cal-Lyse lysing solution to each tube.
- **2** Gently vortex to mix each tube and incubate the tubes in the dark for 10 min at room temperature.
- **3** Add 3 mL of Maxpar Water to each tube.

- 4 Gently vortex to mix each tube and incubate for 10 min at room temperature.  
**IMPORTANT** The cell suspension should be translucent after the 10 min incubation. If the cell suspension is not translucent, gently vortex the sample again and incubate it for an additional 5 min at room temperature in the dark.
- 5 Centrifuge the tubes at 300 x *g* for 5 min and carefully aspirate the supernatant.
- 6 Wash cells by adding 3 mL of CSB to each tube and gently vortex to resuspend the cells.
- 7 Centrifuge the tubes at 300 x *g* for 5 min and carefully aspirate the supernatant.
- 8 Repeat wash steps 6–7 twice for a total of 3 washes.
- 9 Visually inspect the cell pellet and the cell supernatant after each wash.  
**NOTE** If the cell pellet is still mostly red after 3 washes with CSB, wash the pellet once with Maxpar Water and once more with CSB.
- 10 Gently vortex to resuspend cells in the residual volume.

## Fix Cells

- 1 Prepare a fresh 1.6% FA solution from the 16% formaldehyde stock ampule. Use a 1 mL Norm-Ject® rubber-free syringe and compatible 0.1 µm syringe filter to filter the stock formaldehyde, and then dilute 1 part of filtered stock formaldehyde with 9 parts Maxpar PBS.  
**NOTE** For example, to prepare the 1.6% FA solution for one sample, add 100 µL of filtered 16% stock formaldehyde to 900 µL of PBS. Include 10% volume overage for multiple samples.
- 2 Gently vortex to resuspend cells in residual volume.
- 3 Add 1 mL of the 1.6% FA solution to each tube and gently vortex to mix.
- 4 Incubate for 10 min at room temperature.
- 5 Centrifuge the tubes at 800 x *g* for 5 min.  
**NOTE** The increased centrifuge speed after cell fixation results in greater cell recovery.
- 6 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in the residual volume.

## Stain Cells with Intercalator-Ir



**DANGER** Maxpar Fix and Perm Buffer contains formaldehyde. Read the safety data sheet (see [Safety](#)).

- 1 Prepare 1 mL of intercalation solution for each sample by adding Cell-ID Intercalator-Ir into Maxpar Fix and Perm Buffer to a final concentration of 125 nM (a 1,000X dilution of the 125 µM stock solution) and vortex to mix.  
**NOTE** For example, to prepare intercalation solution for one sample, add 1 µL of 125 µM Intercalator-Ir to 1 mL of Fix and Perm Buffer. Include 10% volume overage for multiple samples.
- 2 Add 1 mL of the intercalation solution to each tube and gently vortex.
- 3 Incubate at 2–8 °C overnight.  
**STOPPING POINT** Samples can be stored in intercalation solution for up to 48 hr before data acquisition.

## Day 2: Data Acquisition

### Set Up the Instrument

Make sure the Helios system is 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 starting Helios, ensure that you are using CyTOF Software v6.7.1016 (or higher) for Maxpar Direct Immune Profiling Assay.

**IMPORTANT** Samples stained with the Maxpar Direct Immune Profiling Assay antibody panel must be run using the **Maxpar Direct Immune Profiling Assay.tem** file for analysis in Maxpar Pathsetter™ software.

**NOTE** Even if you do not plan to operate the instrument, we recommend that you read and understand the procedures in the Helios User Guide (PN 400250) before using the kit and before transferring this information to those responsible for instrument operation.

### Wash and Count Cells

- 1 Centrifuge tubes containing cells in intercalation solution at 800 x *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. Centrifuge tubes at 800 x *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 two washes with CSB.
- 6 Wash cells by adding 2 mL of Maxpar Cell Acquisition Solution (CAS) to each tube and gently vortex. Centrifuge tubes at 800 x *g* for 5 min.

- 7 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- 8 Add 2 mL of CAS to each tube and gently vortex. Reserve a small volume (approximately 10 µL) from each tube to count cells. Centrifuge tubes at 800 x g for 5 min. While tubes are in the centrifuge, go to Step 9.
- 9 Count cells in the reserved volume from each tube. Make sure to note the cell count for each tube.  
**NOTE** Cell loss during wash steps leads to a lower cell concentration than the initial count before staining.
- 10 When centrifuging is complete, carefully aspirate and discard supernatant.
- 11 Leave cells pelleted at 2–8 °C until ready to run on Helios.
- 1 Shake the 1X EQ beads vigorously to resuspend.
- 2 Prepare a sufficient volume of 0.1X EQ beads by diluting 1 part beads to 9 parts CAS.
- 3 Immediately before data acquisition, completely resuspend cells to the maximum recommended cell concentration of 1 x 10<sup>6</sup> cells/mL.
- 4 Filter the cells through a 35 µm cell strainer.
- 5 Acquire at least 400,000 events using the Maxpar Direct Immune Profiling Assay acquisition template.
- 6 Normalize FCS files with CyTOF Software v6.7.1016 or higher.
- 7 Import the normalized FCS files into Maxpar Pathsetter software for analysis.

## Acquire Cells for Analysis

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

**NOTE** Immediately before data acquisition, the instrument operator should resuspend the samples to the maximum recommended cell concentration of 1.0 x 10<sup>6</sup> cells/mL with CAS containing 0.1X EQ™ Four Element Calibration Beads.

## 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).

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