

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 (400286).

Workflow Overview

Step	Time (approximate)
Day 1: Cell Staining	
1 Prepare reagents. Heparin blocking solution	20 min
2 Prepare sample. Heparin blocking	Variable
3 Stain, lyse, and fix red blood cells (RBCs). Surface marker staining, Cell-ID™ live/dead intercalator-103Rh staining, RBC lysis, and fresh fix	1 hr 20 min
4 Stain cells with Cell-ID™ Intercalator-Ir.*	10 min (hands-on) Incubate overnight.
Day 2: Data Acquisition	
1 Set up the instrument.†	
<ul style="list-style-type: none"> Helios™—Install WB Injector, warm up, tune, perform bead sensitivity test, and condition the instrument with Maxpar Cell Acquisition Solution. CyTOF® XT—Check nebulizer spray, load beads, warm up and tune. 	<ul style="list-style-type: none"> • 1 hr 35 min • 55 min
2 Wash and count cells.	25 min (variable)
3 Acquire data.† Helios or CyTOF XT™ system, Maxpar Direct Immune Profiling Assay template, and CyTOF Software:	(per sample):
<ul style="list-style-type: none"> Helios—v6.7.1016 (or later) CyTOF XT—v8.0 (or later) 	<ul style="list-style-type: none"> • 20–25 min‡ • 35–45 min‡
4 Perform post-run instrument maintenance.†	
<ul style="list-style-type: none"> Helios—Shut down system, remove injector, and clean parts. CyTOF XT—Shut down system and clean parts. 	<ul style="list-style-type: none"> • 1 hr • automated
5 Normalize data. CyTOF Software:	(variable):
<ul style="list-style-type: none"> Helios—v6.7.1016 (or later) CyTOF XT—v8.0 (or later) 	<ul style="list-style-type: none"> • 3–5 min/sample • automated
6 Analyze normalized data.	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.
- 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 for the number of samples to test. Protect 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)].

- 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.
 - Gently vortex to mix each tube and incubate at room temperature for 20 min.
 - Aliquot 270 µL of heparin-blocked whole blood into a 5 mL tube containing the dry antibody pellet. The final volume of the resuspended dry antibody pellet and blood is approximately 300 µL.
- 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.
- Gently vortex to mix each tube.
 - Incubate tube at room temperature for 30 min.

Red Blood Cell Lysis

- Immediately after staining is complete, add 250 µL of Cal-Lyse lysing solution to each tube.
- Gently vortex to mix each tube and incubate the tubes at room temperature in the dark for 10 min.
- Add 3 mL of Maxpar Water (201069) to each tube.
- Gently vortex to mix each tube and incubate at room temperature in the dark for 10 min.

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 at room temperature in the dark for an additional 5 min.

- 5 Centrifuge the tubes at 300 x *g* for 5 min and carefully aspirate the supernatant.
- 6 Wash cells by adding 3 mL of Maxpar Cell Staining Buffer [CSB (201068)] 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.
- 10 Gently vortex to resuspend cells in residual volume.

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 µm 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 and gently vortex to mix well.
- 4 Incubate at room temperature for 10 min.
- 5 Centrifuge cells at 800 x *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 µM 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 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 to mix. 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 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 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 (Helios only) or CAS Plus (CyTOF XT only) to each tube and gently vortex.
- 9 (CyTOF XT only) Filter cells through 35 µm cell strainer cap tubes into a new 5 mL polypropylene tube.
- 10 Reserve a small volume (approximately 10 µL) from each tube to count cells.
- 11 Centrifuge tubes at 800 x *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 1×10^6 cells/mL.
- **3** Immediately before data acquisition, completely resuspend cells to the maximum recommended cell concentration of 1×10^6 cells/mL.
- **4** Filter the cells through a 35 µm cell strainer into a new 5 mL polypropylene tube.
- **5** Acquire at least 400,000 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 **400** in the Events (x 1000) field to acquire 400,000 events.
 - c Enter the resuspension volume of each sample in the **Volume (µL)** column.
 - d Select **Medium** 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** Transfer the automatically normalized FCS files with the suffix **_Processed** after the Sample Name from their saved folder to a location for further analysis.
- **6** 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 and search for the SDS using either the product name or the part or catalog number.

For technical support visit fluidigm.com/support. For general support visit fluidigm.com/support.

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