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 (PN 400286).

Workflow Overview

Day 1: Cell Staining

	Step	Time
1	**Prepare sample.** Aliquot, count, and determine PBMC viability.	Variable
2	**FcR-block, stain cells, and fix cells.** Surface marker and live/dead intercalator-103Rh staining	80 min
3	**Stain cells with Cell-ID™ Intercalator-Ir.***	10 min (hands-on) Incubate overnight.

Day 2: Data Acquisition

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

* Potential stopping point

† Instrument operator: See Appendix A in the Maxpar Direct Immune Profiling Assay User Guide (PN 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.

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
- 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.

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 pipetting.
- ☐ **4** Wash cells by adding 10 mL of Maxpar Cell Staining Buffer (CSB).
- ☐ **5** Centrifuge each sample at 300 *x g* for 5 min, carefully aspirate supernatant, and resuspend in residual volume by gently pipetting.

FcR-Block Cells

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

Antibody Staining

- ☐ **1** Add 215 µL of CSB to each tube of PBMC, for a final volume of 270 µL.
- ☐ **2** Transfer the 270 µL (3 x 10^6 cells) of the FcR-blocked PBMC directly into one 5 mL tube containing the dry antibody pellet. Final volume of the Ab pellet and blood is 300 µL.

  NOTE Open assay foil packet no more than 1 hr before use. Open cap only when ready to add sample.
- ☐ **3** Gently vortex to mix each tube and incubate for 30 min at room temperature.
- ☐ **4** Wash cells by adding 3 mL of CSB to each tube, gently vortex, and centrifuge at 300 *x 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 two 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 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 Maxpar 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 (containing 3 x 10^6 cells in suspension) and gently vortex to mix.
- ☐ **4** Incubate for 10 min at room temperature.
- ☐ **5** Centrifuge cells 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 Cell-ID Intercalator-Ir

DANGER Maxpar Fix and Perm Buffer contains formaldehyde. Read the safety data sheet.

- ☐ **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 assay 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 the Helios system.

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 10^6 cells/mL with CAS containing 0.1X EQ™ Four Element Calibration Beads .

- ☐ **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 10^6 cells/mL.
- ☐ **4** Filter the cells through a 35 µm cell strainer.
- ☐ **5** Acquire at least 300,000 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.

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.

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