Maxpar Human Immune Monitoring Panel Kit Cell Staining

IMPORTANT Before using this quick reference, read and understand the detailed instructions and safety guidelines in the Maxpar® Human Immune Monitoring Panel Kit Cell Staining and Data Acquisition Protocol (PN PRD027).

Workflow Overview

	Step	Time
1	**Prepare reagents.** Antibody cocktail and cell culture media	20 min
2	**Prepare cells.** Aliquot, count, and determine PBMC viability.	Variable
3	**Stain, FcR-block, and fix cells.** Cisplatin and surface marker stains	80 min
4	**Stain cells with Intercalator-Ir.***	Incubate overnight
5	**Set up instrument.**† Install Helios™ WB Injector, warm up, tune.	1 hr 10 min
6	**Wash and count cells.**	25 min (variable)
7	**Acquire data.**† Helios or upgraded CyTOF® 2 system	17–22 min per sample
8	**Analyze normalized data.**	Variable

* Potential stopping point

† Instrument operator: See Appendix B in the Maxpar Human Immune Monitoring Panel Kit Protocol for information. Even if you do not plan to operate the instrument, we recommend that you read and understand the procedures in Appendix B before using the kit and before transferring this information to those responsible for instrument operation.

Prepare the Reagents

IMPORTANT To ensure reliable results:

- Retrieve, mix and centrifuge reagents as directed.
- Use frozen aliquots of Cell-ID™ Intercalator-Ir and Cell-ID Cisplatin only once and immediately after thawing.
- Open the single-use Pierce™ 16% Formaldehyde (FA) ampule and prepare the FA solution immediately before use in the fixation process.

Prepare the Cell Culture Media

Prewarm serum-free and serum-containing complete media at 37 °C. Use the same media that are normally used for cell culture.

Prepare the Antibody Cocktail

☐ **1** Label the Maxpar metal-conjugated antibody vials with "1" for the 1 µL per test group, and "2" for the 2 µL per test group according to Appendix A in the Maxpar Human Immune Monitoring Panel Kit Protocol to ensure proper tracking of antibodies added into the cocktail mix.

☐ **2** Briefly centrifuge each vial before opening. Keep vials on ice.

☐ **3** Prepare the antibody cocktail in a 1.5 mL tube on ice by first adding Maxpar Cell Staining Buffer and then adding each of the antibodies, as instructed in Appendix A of the Maxpar Human Immune Monitoring Panel Kit Protocol.

☐ **4** Briefly vortex to mix the complete antibody cocktail. Avoid creating air bubbles.

NOTE The antibody cocktail can be stored at 4 °C for up to 4 hours before use in cell staining, as instructed in Surface-Stain Cells. If your PBMC preparation exceeds 4 hours or if cell viability is a concern, you can prepare the antibody cocktail after you count viable cells (see Prepare Cells).

Prepare Cells

☐ **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 (RBCs) to ensure maximum PBMC recovery. Dispense the PBMC into individual 5 mL tubes for each sample.

☐ **2** Count cells and determine cell viability of each sample. For best results, we recommend using samples with ≥80% cell viability and minimal to no RBC contamination.

Viability-Stain Cells with Cell-ID Cisplatin

DANGER Cell-ID Cisplatin contains cisplatin. Read the safety data sheet (see Safety).

☐ **1** Centrifuge each sample at 300 x *g* for 5 minutes, carefully aspirate supernatant, and mix well by gently pipetting.

☐ **2** (Optional) If cells were prepared in serum-containing medium, wash cells to remove residual protein by adding 1 mL of prewarmed serum-free medium. Centrifuge at 300 x *g* for 5 minutes, carefully aspirate supernatant, and gently pipet to mix.

☐ **3** Resuspend cells to 2 x 10^7 cells/mL in prewarmed serum-free medium.

☐ **4** Prepare a working solution of 10 µM cisplatin by diluting the Cell-ID Cisplatin in pre-warmed serum-free medium (500X dilution from 5 mM stock). For example, add 2 µL of 5 mM stock to 1 mL of pre-warmed serum-free medium.

☐ **5** Add an equal volume of 10 µM cisplatin working solution to the cell suspension (final concentration of cisplatin is 5 µM).

☐ **6** Mix well and incubate at room temperature for 5 minutes.

- ☐ **7** Quench the cisplatin stain by washing with serum-containing medium, using 5x the volume of the cistplatin-stained cells. Centrifuge at 300 x *g* for 5 minutes, aspirate supernatant, and gently pipet to mix.
- ☐ **8** Wash cells by adding 4 mL of Maxpar Cell Staining Buffer. Centrifuge at 300 x *g* for 5 minutes, aspirate supernatant, and gently pipette to mix.
- ☐ **9** Resuspend cells in Maxpar Cell Staining Buffer to a final concentration of 6 x 10^7 cells/mL and aliquot 50 µL (3 x 10^6 cells) into a 1.5 mL tube for the sample to be stained.

FcR-Block Cells

- ☐ **1** Add 5 µL of Human TruStain FcX™ (FcX) to each tube. Gently pipet to mix.
- ☐ **2** Incubate the tubes at room temperature for 10 minutes.
- ☐ **3** Continue with cell staining without washing cells.

Surface-Stain Cells

- ☐ **1** Add 45 µL of the antibody cocktail to each tube so the total staining volume is 100 µL (50 µL of cell suspension + 5 µL FcX + 45 µL antibody cocktail; see Appendix A in the Maxpar Human Immune Monitoring Panel Kit Protocol for antibody mixing volumes).
- ☐ **2** Gently pipet to mix each tube and incubate at room temperature for 15 minutes.
- ☐ **3** Gently vortex to mix each tube and incubate the tubes at room temperature for an additional 15 minutes, for a total antibody cocktail incubation time of 30 minutes.
- ☐ **4** Wash cells by adding 1 mL of Maxpar Cell Staining Buffer to each tube, and centrifuge at 300 x *g* for 5 minutes.
- ☐ **5** Carefully aspirate and discard supernatant. Gently pipet to resuspend cells in residual volume.
- ☐ **6** Repeat Steps 4 and 5 for a total of two washes.

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 Maxpar PBS. Include 10% volume overage for multiple samples.
- ☐ **2** 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.
- ☐ **3** Incubate for 10 minutes at room temperature.
- ☐ **4** Centrifuge at **800 x *g*** for 5 minutes.

  NOTE The increased centrifugation speed after cell fixation results in greater cell recovery.
- ☐ **5** Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.

Stain Cells with Cell-ID 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 (containing 3 x 10^6 cells in suspension) and gently vortex.
- ☐ **3** Incubate at 4 °C overnight.

  STOPPING POINT Samples can be stored in intercalation solution for up to 48 hours before data acquisition.

Set Up the Instrument

Make sure the Helios or upgraded CyTOF 2 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. See Appendix B in the Maxpar Human Immune Monitoring Panel Kit Protocol for information on instrument use and troubleshooting for instrument operators.

IMPORTANT Even if you do not plan to operate the instrument, we recommend that you read and understand the procedures in Appendix B 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 minutes.
- ☐ **2** Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- ☐ **3** Wash cells by adding 1 mL of Maxpar Cell Staining Buffer to each tube and gently vortex. Centrifuge tubes at 800 x *g* for 5 minutes.
- ☐ **4** Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.
- ☐ **5** Wash cells by adding 1 mL of Maxpar PBS 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 minutes. While tubes are in the centrifuge, go to Step 6.

☐ **6** Count cells in the reserved volume from each tube. Make sure to note the cell concentration for each tube.

NOTE Cell loss during wash steps leads to a lower cell concentration than the initial 3 x 10^6 cells/mL aliquoted after cisplatin-staining.

☐ **7** When centrifugation is complete, carefully aspirate and discard supernatant.

☐ **8** Leave cells pelleted at 4 °C until you are ready to run them on Helios or upgraded CyTOF 2 system.

NOTE Cells should be run on same day they are washed from intercalation solution. Immediately before data acquisition, the instrument operator will adjust samples to the maximum recommended cell concentration of 1.0 x 10^6 cells/mL with Maxpar Cell Acquisition Solution containing 0.1X EQ™ Four Element Calibration Beads (see Appendix B in the Maxpar Human Immune Monitoring Panel Kit Protocol).

Prepare to Acquire Data

Samples are resuspended in Maxpar Cell Acquisition Solution containing 0.1X EQ beads immediately before data acquisition on the Helios or upgraded CyTOF 2 system. Appendix B in the Maxpar Human Immune Monitoring Panel Kit Protocol outlines the instrument setup and data acquisition workflow for cells stained with the Maxpar Human Immune Monitoring Panel Kit.

IMPORTANT Even if you do not plan to operate the instrument, we recommend that you read and understand the procedures in Appendix B before using the kit and before transferring this information to those responsible for instrument operation.

Provide the following materials to the instrument operator (see also Appendix B in the protocol).

☑	Material
☐	Washed and pelleted samples
☐	Maxpar Cell Acquisition Solution*
☐	EQ Four Element Calibration Beads (Cat. No. 201078)
☐	Polypropylene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm
☐	Helios WB Injector (Cat. No. 107950)
☐	Maxpar Human Immune Monitoring Panel Kit acquisition template†

* Supplied with Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324). See Fluidigm Kit Contents in the protocol.

† Go to fluidigm.com/productsupport/cytof-helios-support-hub to download the acquisition template, or contact your local Fluidigm field application specialist.

Analyze Normalized Data

After sample acquisition and data normalization is complete (see Appendix B in the Maxpar Human Immune Monitoring Panel Kit Protocol), transfer the normalized .fcs files to the cytometry data analysis software of your choice, such as the latest version of GemStone™ software by Verity Software House or Premium Cytobank, for further evaluation. For more information, contact your local Fluidigm field application specialist.

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.

North America +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@fluidigm.com **Latin America** +1 650 266 6100 | techsupportlatam@fluidigm.com
Europe/Middle East/Africa/Russia +44 1223 859941 | techsupporteurope@fluidigm.com **China (excluding Hong Kong)** +86 21 3255 8368 | techsupportchina@fluidigm.com
Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com **All other Asian countries/India/Australia** +1 650 266 6100 | techsupportasia@fluidigm.com