PN 400286 B1

Maxpar Direct Immune Profiling Assay Cell Staining and Data Acquisition

User Guide

For use on Helios with WB Injector

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About This Document

IMPORTANT This user guide describes how to use the Maxpar® Direct™ Immune Profiling Assay™ on the Helios™ system. For detailed instructions on instrument and software operation, see the Helios User Guide (PN 400250).

IMPORTANT Before using this kit, read and understand the safety guidelines in this document. For complete safety information, see Appendix D.

Safety Alert Conventions

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.

Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
!	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
DANGER	Signal word that indicates more severe hazards.
WARNING	Signal word that indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
!	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the system user guide for the applicable pictograms and hazards pertaining to system usage.
DANGER	Signal word that indicates an imminent hazard that will result in severe injury or death if not avoided.
WARNING	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.
CAUTION	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
IMPORTANT	Signal word that indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Introduction

This document outlines the Maxpar Direct Immune Profiling Assay cell surface staining and data acquisition workflow for mass cytometry, using a validated panel of dry metal-conjugated antibodies for deep immune profiling of human whole blood or peripheral blood mononuclear cells (PBMC).

To ensure compatibility of this staining panel with your cell preparation workflow, we recommend performing a pilot experiment using noncritical samples. Refer to Day 1: Cell Staining for specific instructions on the design of this preliminary experiment. The product was tested using peripheral whole blood collected in sodium heparinized tubes.

Workflow Overview

Times shown here are estimates. Actual times may vary.

	Workflow Step	Hands-On Time	Run Time
Day 1: Cell Staining			
For Whole Blood Staining (up to 270 µL per tube)			
1	**Prepare reagents.** Heparin blocking solution	20 min	—
2	**Prepare sample.** Heparin blocking	Variable	—
3	**Stain cells, lyse red blood cells (RBC), and fix cells.** Surface marker staining, live/dead intercalator-103Rh staining, and RBC lysis	80 min	—
4	**Stain cells with Cell-ID™ Intercalator-Ir.***	10 min	Incubate overnight
For PBMC Staining ($3x10^6$ PBMC per tube)			
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	Incubate overnight
Day 2: Data Acquisition			
1	**Set up Helios.†** Install WB Injector, warm up, tune, perform bead sensitivity test, and condition with Maxpar Cell Acquisition Solution (CAS).	15 min	1 hr 30 min
2	**Wash and count cells.**	25 min (variable)	—
3	**Acquire data.†** Helios system **Maxpar Direct Immune Profiling Assay** template CyTOF® Software v6.7.1016 (or higher)	2 min per sample	10–15 min per sample
4	**Perform post-run instrument maintenance.†** Shut down system, remove injector, and clean parts.	30 min	1 hr 25 min
5	**Normalize data.†** CyTOF Software v6.7.1016 (or higher)	1 min	20 min (variable)
6	**Analyze normalized data.**	**Variable**	**—**

* Potential stopping point

† Instrument operator: See Appendix A, which outlines the Helios system setup and data acquisition workflow for cells stained with the Maxpar Direct Immune Profiling Assay. Even if you do not plan to operate the instrument, we recommend that you read and understand the procedures in Appendix A: Instrument Setup and Data Acquisition before using the kit and before transferring this information to those responsible for instrument operation.

Materials

Store reagents as soon as they are received according to the manufacturer's storage recommendations.

Fluidigm Kit Contents

IMPORTANT Upon receiving the Cell-ID Intercalator-Ir, divide into single-use aliquots and freeze them at –20 °C.

The following reagents are included in Maxpar Direct Immune Profiling Assay (Cat. No. 201325), which provides the necessary reagents to stain 25 test samples.

Product Name	Catalog Number	Storage
Cell Staining Reagents		
Cell-ID Intercalator-Ir—125 µM, 25 µL	S00093	–20 °C in single-use aliquots
Maxpar Cell Staining Buffer—500 mL	201068	2–8 °C. Do not freeze.
Maxpar Fix and Perm Buffer—25 mL	S00092	
Maxpar Direct Immune Profiling Assay Foil Packet	S00124	
Maxpar PBS—100 mL	S00125	
Maxpar Cell Acquisition Solution—200 mL	201240	

Fluidigm Materials Required But Not Supplied

The following Fluidigm products are required to perform red blood cell (RBC) lysis or data acquisition of test samples on a Helios system (see Appendix A for more information on acquisition).

Product Name	Catalog Number	Storage
Maxpar Water	201069	2–8 °C. Do not freeze.
EQ™ Four Element Calibration Beads—100 mL	201078	
Tuning Solution—250 mL	201072	Room temperature
Helios WB Injector	107950	

Required Reagents from Other Suppliers

The following reagents are required to perform cell staining using this protocol.

Product	Catalog Number	Source
Human TruStain FcX™ (Fc-Receptor Blocking Solution)	422301 (50 tests)/ 422302 (200 tests)	BioLegend®
Pierce™ 16% Formaldehyde (w/v), Methanol-free	28906 (10 x 1 mL)*/ 28908 (10 x 10 mL)	Thermo Fisher Scientific

Product	Catalog Number	Source
Cal-Lyse™ Lysing Solution (with formaldehyde and EGTA)	GAS-010 (25 mL)/ GAS-010S100 (100 mL)	Thermo Fisher Scientific
Sodium Heparin Salt	H3149-10KU	Sigma Aldrich™

* 1 mL of 16% FA is sufficient for 5–7 test samples stained on the same day.

Required Consumables from Other Suppliers

Product
1 mL Norm-Ject® latex-free syringes and compatible 0.1 µm syringe filters (Cat. No. 53548-001)
BD Vacutainer® glass blood collection tubes with sodium heparin (Cat. No. 366480)
Corning® polypropylene round-bottom tubes, 5 mL capacity, 12 x 75 mm (Cat. No. 352063)
Corning polystyrene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm (Cat. No. 352235)
1.5 mL microfuge tubes
Pipet tips with aerosol barrier

Required Equipment

Product
Two centrifuges, one for 5 mL tubes and one for 1.5 mL microfuge tubes
Vacuum aspirator
Vortexer

Required Software

CyTOF SW v6.7.1016 or higher with the **Maxpar Direct Immune Profiling Assay.tem** template file, or a template derived from it, are required to acquire and normalize data on samples stained using this protocol for Maxpar Pathsetter™ analysis. Contact your local Fluidigm Field Applications Specialist for information on CyTOF Software and acquisition template for the Maxpar Direct Immune Profiling Assay. See Appendix A for more information.

Suggested Analysis Software

We suggest the use of Maxpar Pathsetter software to perform immune population analysis on the normalized FCS files.

For more information, contact your local Fluidigm Field Applications Specialist or refer to the Maxpar Pathsetter User Guide in the Maxpar Pathsetter software Help tab.

Day 1: Cell Staining

Before You Begin

IMPORTANT Read and understand the safety information in Appendix D.

IMPORTANT All staining on whole blood or PBMC should be performed in a biological safety cabinet while wearing appropriate personal protective equipment (PPE).

- Follow your institution's safety protocols when handling biological specimens.
- Take all necessary safety precautions while handling whole blood.
- Ensure that tubes are tightly capped between centrifugation and washes.

Required cell number/volume: Each Maxpar Direct Immune Profiling Assay tube stains whole blood or PBMC with 30 surface markers (see Appendix B for the complete list of antibodies) and rhodium-viability staining. Each tube can stain:

- 270 µL of peripheral whole blood
- 3 x 10^6 PBMC

IMPORTANT For optimal results, whole blood should be stained within 24 hr after collection.

Reagent handling: Open assay foil packet no more than 1 hr before use. The assay tubes should be stored at 2–8 °C when not in use. Label each tube and place it on a rack at room temperature. Frozen aliquots of Cell-ID Intercalator-Ir should be used only once, immediately after thawing. Avoid multiple freeze/thaw cycles.

Centrifuge speeds: For cell centrifuge steps, centrifuge for 5 min at 300 x *g* before cell fixation and for 5 min at 800 x *g* after cell fixation. The increased centrifuge speed after cell fixation results in greater cell recovery.

Formaldehyde solution: It is critical to prepare a fresh formaldehyde (FA) solution to effectively fix cells stained with the Maxpar Direct Immune Profiling Assay. Be sure to open the single-use 16% formaldehyde ampule and prepare the FA solution immediately before use in the fixation process (see Fix Cells).

Pilot experiments: Before you perform this protocol on valuable samples, we recommend performing a pilot experiment using this protocol on noncritical samples. Run an initial pilot experiment on noncritical samples to test the success of antibody staining and Cell-ID Intercalator-Ir (DNA) staining and to check for contaminants in your cell preparation. Contaminants include common metals found in the environment (for example, Ba and Pb), and those from experimental treatment of tissue (for example, Pt). Run cells stained with Cell-ID Intercalator Ir (only) using the panel kit acquisition template and check for high signal from Sn, Xe, Cs, Ba, Gd, Pb, Pt, I, and Os (shown as BCKG in the template).

For PBMC:

FcR-blocking with Human TruStain FcX: The FcR-blocking step is recommended in the protocol to prevent nonspecific background signal by preventing binding of Maxpar metal-conjugated antibodies to Fc receptors. Fc receptors specific for IgG, including FcγR1 (CD64), FcγRII (CD32), and FcγRIII (CD16), are present on many cell types, with particularly high expression on monocytes, granulocytes, and B cells.

NOTE For more information, contact your local Fluidigm Field Applications Specialist.

For Whole Blood:

Best practices for shipping whole blood are a consistent temperature of 2–8 °C for the duration of the shipment. Peripheral whole blood should be collected in sodium heparinized tubes.

Reagents and Solutions to Prepare in Advance

(For whole blood) Heparin blocking solution: Prepare the 10 KU/mL heparin solution by adding 1 mL of Maxpar PBS solution to 10 KU of sodium heparin salt. We recommend using 10 µL per 1 mL of whole blood. This solution can be stored at 2–8 °C while you prepare samples for blocking. See manufacturer's recommendation for long-term storage.

Retrieve the Cell Staining Reagents

Step	Reagent	Preparation
(For whole blood) Heparin	10 KU/mL sodium heparin blocking solution	No preparation required.
(For whole blood) RBC lysis	Cal-Lyse lysing solution	Aliquot enough for the number of samples to test. Protect solution from light.
	Maxpar Water	Bring to room temperature before use.
(For PBMC) FcR block	Human TruStain FcX	No preparation required.
Antibody staining	Maxpar Direct Immune Profiling Assay Foil Packet	No preparation required.
Antibody staining	Maxpar Cell Staining Buffer	Bring to room temperature before use.
Cell fixation	Pierce 16% Formaldehyde	Remove enough single-use ampules from the packaging for the number of samples to test, protecting ampules from light.
	Maxpar PBS	Bring to room temperature before use.
Intercalator-Ir stain	Cell-ID Intercalator-Ir	Remove a single-use aliquot from freezer and thaw it to room temperature immediately before use.
	Maxpar Fix and Perm Buffer	No preparation required.

For Whole Blood Staining

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

Antibody Staining

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 for 20 min at room temperature.
3. 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.

   NOTE The recommended starting volume of peripheral whole blood required per sample for efficient staining is 270 µL. If using less than 270 µL of blood, top up with Maxpar Cell Staining Buffer to a total volume to 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 tubes 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 in the dark.

   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.

Figure 1. Whole blood solution before (left) and after (right) 10 min incubation with water following Cal-Lyse treatment.

NOTE Fluidigm recommends aspirating supernatant instead of decanting.

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 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 Steps 6–7 twice for a total of 3 washes.
9. Visually inspect the cell pellet and the cell supernatant after each wash. (See Figure 2 for an example of an appropriate supernatant and pellet color.)

Figure 2. Poorly hemolyzed sample (left). Cell pellet should be white and supernatant should be clear (right) before proceeding to Fix Cells procedure.

NOTE If the cell pellet is still mostly red after 3 washes with Maxpar Cell Staining Buffer, wash the pellet once with Maxpar Water and once more with Cell Staining Buffer.

10. Gently vortex to resuspend cells in residual volume.
11. Proceed to Fix Cells.

For PBMC Staining

1. Prepare PBMC from frozen PBMC aliquots using your preferred method to minimize environmental and experimental contaminants, making sure to lyse and remove 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.
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

NOTE See Before You Begin for FcR-blocking consideration points.

1. Resuspend cells in Maxpar Cell Staining Buffer to a final concentration of 6 x 10^7 cells/mL.
2. Aliquot 50 µL (3 x 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 Maxpar Cell Staining Buffer 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 a 5 mL tube containing the dry antibody pellet. The final volume of the resuspended dry antibody pellet and PBMC is approximately 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 Maxpar Cell Staining Buffer to each tube and gently vortex. 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 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 µm syringe filter to filter the stock formaldehyde, and then dilute 1 part of the 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 for PBMC) and gently vortex to mix.

4 Incubate tubes 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. For complete safety information, see the Appendix D: Safety.

1 Prepare 1 mL of intercalation solution for each sample by adding Cell-ID Intercalator-Ir into 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 the samples 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. See Appendix A: Instrument Setup and Data Acquisition for information on instrument use and troubleshooting for instrument operators.

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** template file.

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.

Retrieve the Reagents

Step	Reagent	Preparation
Wash and count cells	Cells in intercalation solution (from Day 1)	Remove from 2–8 °C.
	Maxpar Cell Staining Buffer	
	Maxpar Cell Acquisition Solution	
Acquire data	Maxpar Cell Acquisition Solution	
	EQ Four Element Calibration Beads	

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 Maxpar Cell Staining Buffer 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 Steps 3–4 once for a total of 2 washes with Maxpar Cell Staining Buffer.
6. Wash cells by adding 2 mL of Maxpar Cell Acquisition Solution 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 Maxpar Cell Acquisition Solution 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 9.
9. 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 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.

   NOTE Run cells on the same day they are washed from intercalation solution. 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 Maxpar Cell Acquisition Solution containing 0.1X EQ beads (see Appendix A).

Prepare to Acquire Data

Samples are resuspended in Maxpar Cell Acquisition Solution containing 0.1X EQ beads immediately prior to data acquisition on Helios. Appendix A outlines the instrument setup and data acquisition workflow for cells stained with the Maxpar Direct Immune Profiling Assay Kit.

1. Verify CyTOF Software version for acquisition and normalization is v6.7.1016 (or higher) for Maxpar Direct Immune Profiling Assay.
2. Provide the following materials to the instrument operator (see also Required Materials in Appendix A).

☑	Material	Notes
☐	Washed and pelleted samples	Remove from 2–8 °C (see Wash and Count Cells).
☐	Maxpar Cell Acquisition Solution*	Provide 1 mL buffer for each sample to be run, plus additional volume to resuspend each sample to 1.0 x 10^6 cells/mL in buffer containing 0.1X EQ beads.
☐	EQ Four Element Calibration Beads	Provide sufficient volume to resuspend each sample to 1.0 x 10^6 cells/mL in Maxpar Cell Acquisition Solution containing 0.1X EQ beads
☐	Polypropylene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm	Provide sufficient number to run each sample.
☐	Helios WB Injector (if operator is using HT Injector)	Cat. No.107950
☐	Maxpar Direct Immune Profiling Assay acquisition template†	Provide the operator with a copy of the electronic file (Maxpar Direct Immune Profiling Assay acquisition template.tem).

* Supplied with Maxpar Direct Immune Profiling Assay (Cat. No. 201325). See Fluidigm Kit Contents.
† Contact your local Fluidigm Field Applications Specialist.

Analyze Normalized Data

After sample acquisition and data normalization are complete (see Helios User Guide, PN 400250), transfer the normalized FCS files to the latest version of Maxpar Pathsetter software for further evaluation. For more information, see the Maxpar Pathsetter User Guide in the Help tab of the Maxpar Pathsetter software or contact your local Fluidigm Field Applications Specialist.

Appendix A: Instrument Setup and Data Acquisition

This appendix outlines the Helios system setup and data acquisition workflow for cells stained with the Maxpar Direct Immune Profiling Assay.

Before using this workflow, read and understand the detailed instructions and safety guidelines in the Helios User Guide (PN 400250).

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.

IMPORTANT Before starting Helios, ensure that you are using CyTOF Software v6.7.1016 (or higher) for Maxpar Direct Immune Profiling Assay.

Required Materials

Product	Catalog Number	Source	Storage
CyTOF Software v6.7 for Maxpar Direct Immune Profiling Assay	108544	Fluidigm	NA
Maxpar Cell Acquisition Solution—200 mL*	201240	Fluidigm	2–8 °C
Helios WB Injector	107950	Fluidigm	Room temperature
Tuning Solution—250 mL	201072	Fluidigm	Room temperature
EQ Four Element Calibration Beads—100 mL	201078	Fluidigm	2–8 °C
Polypropylene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm	352235	Corning	NA
Maxpar Direct Immune Profiling Assay acquisition template*	–	See Important Notes Before Starting	NA

* Supplied with Maxpar Direct Immune Profiling Assay (Cat. No. 201325). See Fluidigm Kit Contents.

Workflow Overview

Times shown are estimates. Actual times may vary.

	Workflow Step	Hands-On Time	Run Time
1	**Install Helios WB Injector.**	5 min	—
2	**Start plasma and warm up Helios.**	—	45 min
3	**Tune Helios.**	—	20 min
4	**Perform the Bead Sensitivity Test.**	5 min	5 min
5	**Condition Helios with Maxpar Cell Acquisition Solution.**	1 min	15 min
6	**Acquire data.**	2 min per sample	15–20 min per sample
7	**Perform post-run instrument maintenance.** Shut down system, remove injector, clean parts.	30 min	1 hr 25 min
8	**Normalize data.** CyTOF Software v6.7 for Maxpar Direct Immune Profiling Assay	1 min	3–5 min/file

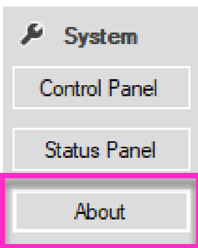
Before You Begin

Important Notes Before Starting

Required software: CyTOF Software v6.7 for Maxpar Direct Immune Profiling Assay (v6.7.1016 or higher) and the Maxpar Direct Immune Profiling Assay Kit acquisition template (**Maxpar Direct Immune Profiling Assay.tem**) are required in order to run and normalize samples stained with the Maxpar Direct Immune Profiling Assay Kit.

To verify the version of software installed on your instrument workstation

1. Open CyTOF Software.
2. In the Toolbar, under System, click **About**.



IMPORTANT If an earlier version of CyTOF Software is installed, refer to the CyTOF Software v6.7.1016 Release Notes (PN 400295) for installation instructions. For more information, contact your Fluidigm Field Applications Specialist.

Condition of instrument: Be sure to clean and maintain all instrument parts, particularly the Nebulizer, the injector, and vacuum interface cones, as instructed in the Helios User Guide (PN 400250) and check for contamination before starting the instrument and using the Helios WB Injector.

NOTE For specific recommendations on cleaning the vacuum interface cones, contact your local Fluidigm Field Applications Specialist.

Retrieve the Reagents

Step	Product	Preparation
Tune instrument.	Tuning Solution	Keep at room temperature.
Condition instrument, acquire data.	Maxpar Cell Acquisition Solution	Remove from 2–8 °C.
Acquire data.	EQ Four Element Calibration Beads	Remove from 2–8 °C.

Install the Helios WB Injector and Tune Instrument

Refer to the Helios User Guide (PN 400250) for details on installing the Helios WB Injector and preparing the instrument for use before acquiring samples stained with the Maxpar Direct Immune Profiling Assay.

IMPORTANT The makeup gas must be adjusted to + 0.2 L/min higher than the current value for the HT injector prior to warming up and tuning the instrument.

Acquire Data

Before You Begin

Samples stained with the Maxpar Direct Immune Profiling Assay antibody panel must be run using **Maxpar Direct Immune Profiling Assay.tem** template file. Contact your local Fluidigm Field Applications Specialist for more information.

NOTE Before you start acquiring data using this template:

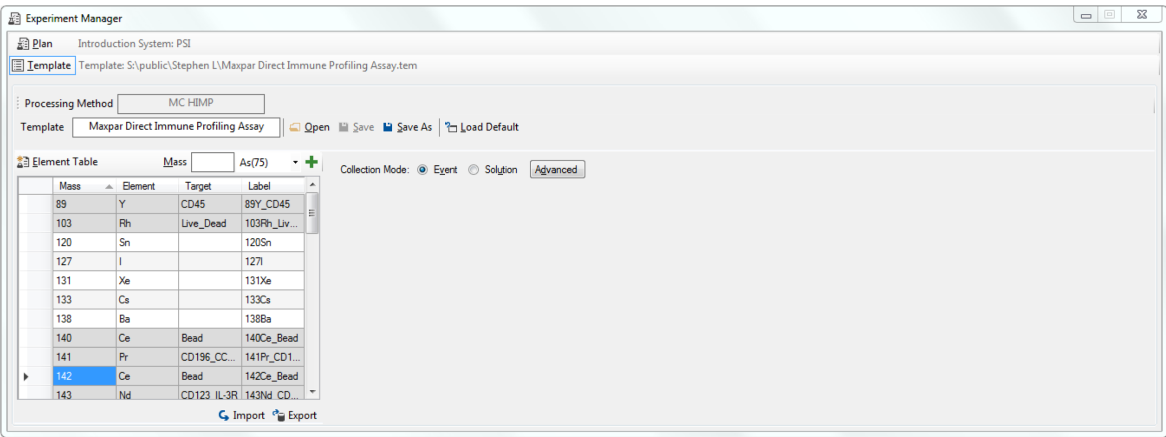
- See the Data Acquisition section in the Helios User Guide (PN 400250) for information on how to import a template and run samples using CyTOF Software.
- The acquisition template has been configured to acquire all channels corresponding to the Maxpar Direct Immune Profiling Assay antibody panel. All cell markers have been appropriately labeled.
- Samples to analyze using Maxpar Pathsetter software must be acquired using the acquisition template without altering any existing settings (specifically, do not edit any Target labels). Additional channels can be added to the template (see the Maxpar Pathsetter User Guide).

Test Bead Sensitivity and Condition Sample Introduction

1. Follow the instructions in the Bead Sensitivity Test and Analyze Bead Data sections in the Helios User Guide (PN 400250) to test EQ bead sensitivity and to verify system cleanliness and tuning.
2. Load a 5 mL tube containing at least 1 mL of Maxpar Cell Acquisition Solution on the sample loader and start sample introduction. Run for at least 15 min prior to acquisition of samples.

Load Acquisition Template

1. Open the Experiment Manager.
   In the Toolbar, click the **Acquire** tab and then click **Experiment Manager**.
2. Open the Maxpar Direct Immune Profiling Assay.tem template file acquisition template.



3. *(Optional)* Choose additional markers.

   NOTE The original Maxpar Direct Immune Profiling Assay template cannot be edited but additional elements can be added. The modified template can be saved as a new template.

   a. Click **Element Table** and then click each applicable element. Click **Apply**.
   b. Enter a name for the new target in the Target column. Click **Save As**.
   c. Choose a unique name for the acquisition template and click **Save**.
4. Close Experiment Manager.
5. Set an event limit for the acquisition. This determines when to stop data acquisition.
   a. On the Acquire tab, under Acquisition, click **Stop At**.
   b. Click Event and in the Limit textbox enter **400000** for whole blood samples or **300000** for PBMC samples.
   c. Click **OK**.
6. To specify where to save your date, click the folder icon. Navigate to an existing folder or create a new one.
7. Enter a name for the output FCS data file.

Prepare and Acquire Cells for Analysis

NOTE Run cells on the same day they are washed from intercalation solution (see Wash and Count Cells).

1. Shake the 1X EQ Four Element Calibration Beads vigorously to resuspend.
2. Prepare enough volume of 0.1X EQ Four Element Calibration Beads (by diluting 1 part beads to 9 parts Maxpar Cell Acquisition Solution) to completely resuspend cells to the maximum recommended cell concentration of 1.0 x 10^6 cells/mL.

3 Immediately before data acquisition, completely resuspend cells to the maximum recommended cell concentration of 1 x 10^6 cells/mL.

4 Filter cells through 35 µm cell strainer cap tubes.

5 In the Acquisition window, set the Stop at Event limit for each sample to **300,000** events for PBMC or **400,000** events for whole blood. The expected acquisition rate at the maximum cell concentration of 1.0 x 10^6 cells/mL is 250–500 events/ second.

6 Place the sample into the Sample Loader.

7 Click

to start acquisition.

NOTE We recommend cleaning the instrument by running Maxpar Cell Acquisition Solution for at least 5 min between samples to minimize sample carryover.

Normalize Sample Data

1 When you have acquired data from all test samples, use the CyTOF Software v6.7 for Maxpar Direct Immune Profiling Assay (v6.7.1016 or higher) with default FCS Processing settings to normalize the final FCS files, and then, if required, concatenate files according to the instructions in the Normalization of Mass Cytometry Data Using EQ Four Element Calibration Beads (UG13-02_150501). For more information, contact your local Fluidigm Field Applications Specialist.

2 (Optional) Transfer the normalized FCS files from their saved location to a location for further analysis.

NOTE For further information see the Maxpar Pathsetter User Guide in the Maxpar Pathsetter software Help tab.

Perform Post-Run Instrument Maintenance

After all samples stained with the Maxpar Direct Immune Profiling Assay are acquired for the day, perform the following post-run instrument maintenance tasks:

1 Follow the instructions in the End-of-Day Cleaning and Shutdown: Turning Off Plasma sections of the Helios User Guide (PN 400250), making sure to clean the injector daily with deionized water (DIW) according to the instructions in the Maintenance chapter of the Helios User Guide.

2 Clean the sample introduction system with washing solution and DIW.

   a Load a 5 mL tube containing at least 1 mL of washing solution on the Sample Loader and start sample introduction. Run for at least 5 min.

   b Load a 5 mL tube containing at least 1 mL of DIW on the sample loader and start sample introduction. Run for at least 10 min.

3 When the instrument is cooled down (approximately 15–30 min), remove and rinse the Helios WB Injector daily with DIW.

   NOTE Rinsing the WB injector daily is in addition to the weekly cleaning with 10% Contrad 100 in DIW as instructed in the Maintenance chapter of the Helios User Guide.

IMPORTANT The heater may still be hot. Carefully remove the heater using the heater tray and place on the upper support pins to perform instrument maintenance.

Appendix B: Related Documentation

Go to fluidigm.com/documents to download these related documents.

Title	Part Number
Helios User Guide	400250
CyTOF Software 6.7.1016 Release Notes	400295
Maxpar Direct Immune Profiling Assay Product Information Sheet	PRD036
Maxpar Direct Immune Profiling Assay Cell Staining in Whole Blood Quick Reference	400287
Maxpar Direct Immune Profiling Assay Cell Staining in PBMC Quick Reference	400288
Maxpar Pathsetter: Analyze Data in Method Run Mode Quick Reference	400297
Data Normalization for Maxpar Pathsetter	400290
Maxpar Pathsetter User Guide	Available in the Maxpar Pathsetter Software (PN 401018)

Go to fluidigm.com/documents and log in to download these additional related documents. Contact your local Fluidigm Field Applications Specialist for more information.

Appendix C: Maxpar Direct Immune Profiling Assay Panel

Antibody (clone)	Mass
CD45 (HI30)	89Y
CD196/CCR6 (G034E3)	141Pr
CD123 (6H6)	143Nd
CD19 (HIB19)	144Nd
CD4 (RPA-T4)	145Nd
CD8a (RPA-T8)	146Nd
CD11c (Bu15)	147Sm
CD16 (3G8)	148Nd
CD45RO (UCHL1)	149Sm
CD45RA (HI100)	150Nd
CD161 (HP-3G10)	151Eu
CD194/CCR4 (L291H4)	152Sm
CD25 (BC96)	153Eu
CD27 (O323)	154Sm
CD57 (HCD57)	155Gd
CD183/CXCR3 (G025H7)	156Gd
CD185/CXCR5 (J252D4)	158Gd
CD28 (CD28.2)	160Gd
CD38 (HB-7)	161Dy
CD56/NCAM (NCAM16.2)	163Dy
TCRgd (B1)	164Dy
CD294 (BM16)	166Er
CD197/CCR7 (G043H7)	167Er
CD14 (63D3)	168Er
CD3 (UCHT1)	170Er
CD20 (2H7)	171Yb
CD66b (G10F5)	172Yb
HLA-DR (LN3)	173Yb
IgD (IA6-2)	174Yb
CD127 (A019D5)	176Yb
Live/dead intercalator-103Rh	103 Rh

Appendix D: Safety

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use the appropriate personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and nitrile gloves, according to your laboratory safety practices.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Instrument Safety

For complete instrument safety information, including a full list of the symbols on the instrument, refer to the Helios User Guide (PN 400250).

WARNING BIOHAZARD. If you are putting biohazardous material on the instrument or system, use appropriate personal protective equipment and adhere to Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines online at cdc.gov/biosafety/publications/index.htm.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that system operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier. When handling any chemical, the following safe-handling guidelines should be strictly observed:

- Do not inhale fumes from chemicals. Use adequate ventilation and return caps to bottles immediately after use.
- Use, store, and dispose of chemicals according to manufacturer recommendations and to regulations applicable to the locality, state, province, and/or country.
- When preparing chemical solutions, always work in a fume hood that is suitable for those chemicals.

- Conduct sample preparation away from the system to minimize corrosion and contamination.
- Store solvents in an approved cabinet (with the appropriate ventilation) away from the system.

DANGER Maxpar Fix and Perm Buffer contains formaldehyde. Causes skin irritation. May cause allergic skin reaction. Causes serious eye irritation. May cause respiratory irritation. Suspected of causing genetic defects. Suspected of causing cancer. Read the safety data sheet (SDS) before using this reagent.

DANGER Tuning solution contains nitric acid. May intensify fire; oxidizer. Causes severe skin burns and eye damage. Read the safety data sheet (SDS) before using this reagent.

Disposal of Products

Used reagents should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

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For technical support visit
fluidigm.com/support.