Maxpar Human Immune Monitoring Panel Kit Cell Staining and Data Acquisition

For use with Helios or upgraded CyTOF 2 system and Helios WB Injector

Contents

About This Protocol

This protocol describes how to use the Maxpar® Human Immune Monitoring Panel Kit with the Helios™ or upgraded CyTOF® 2 system. For detailed instructions on instrument and software operation, see the Helios User Guide (PN 400250).

IMPORTANT Before using the Maxpar Human Immune Monitoring Panel Kit, read and understand the detailed instructions and safety guidelines in this document.

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 instrument user guide for the applicable pictograms and hazards pertaining to instrument 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 Corporation, 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 or catalog 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 cell surface staining and data acquisition workflow for mass cytometry using a validated panel of metal-conjugated antibodies for deep immune profiling of human peripheral blood mononuclear cells (PBMC).

To ensure compatibility of this staining panel with your cell preparation workflow, we recommend performing a small series of pilot experiments using noncritical samples. Refer to Day 1: Cell Staining for specific instructions on the design of these preliminary experiments.

Workflow Overview

Times shown here are estimates. Actual times may vary.

	Workflow Step	Hands-On Time	Run Time
Day 1: Cell Staining			
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 stain.***	10 min	Incubate overnight
Day 2: Data Acquisition			
5	**Set up instrument.**† Install Helios WB Injector, warm up, tune.	5 min	1 hr 5 min
6	**Wash and count cells.**	25 min/variable	—
7	**Acquire data.**† Helios or upgraded CyTOF 2 system	2 min per sample	15–20 min per sample
8	**Perform post-run instrument maintenance.**† Shut down system, remove injector, clean parts.	30 min	1 hr 25 min
9	**Normalize data.**† Latest build of CyTOF Software v6.7	1 min	20 min/variable
10	**Analyze normalized data.**	Variable	—

* Potential stopping point

† Instrument operator: See Appendix B, which outlines the Helios or upgraded CyTOF 2 system setup and data acquisition workflow for cells stained with the Maxpar Human Immune Monitoring Panel Kit. 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.

Materials

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

Fluidigm Kit Contents

DANGER Cell-ID™ Cisplatin contains cisplatin. For complete safety information, see Appendix D: Safety.

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

The following reagents are included in Maxpar Human Immune Monitoring Panel Kit, 29 Marker–25 Tests (Cat. No. 201324), which provides the necessary reagents to stain 25 test samples.

Product Name	Catalog Number	Storage
Cell Staining Reagents		
Cell-ID Cisplatin—100 µL	201064	–20 °C in single-use aliquots
Cell-ID Intercalator-Ir—125 µM, 25 µL	S00093	–20 °C in single-use aliquots
Maxpar Cell Staining Buffer—500 mL	201068	4 °C. Do not freeze.
Maxpar Fix and Perm Buffer—25 mL	S00092	4 °C. Do not freeze.
Maxpar Metal-Conjugated Antibodies	Various (provided in individual tubes; see Appendix A for panel)	4 °C. Do not freeze.
Maxpar PBS—100 mL	S00125	4 °C. Do not freeze.
Data Acquisition Reagents (see Appendix B for more information)		
Maxpar Cell Acquisition Solution—200 mL	201240	4 °C. Do not freeze.

Fluidigm Materials Required But Not Supplied

The following Fluidigm products are required to perform data acquisition of test samples on a Helios or upgraded CyTOF 2 system (see Appendix B for more information).

Product Name	Catalog Number	Storage
EQ™ Four Element Calibration Beads—100 mL	201078	4 °C
Tuning Solution—250 mL	201072	Room temperature
Helios WB Injector	107950	Room temperature

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
Serum-free and serum-containing complete cell culture media	–	User-supplied

* One 1 mL vial is sufficient for 5–7 test samples stained on the same day.

Required Consumables

Product
1 mL Norm-Ject® rubber-free syringes and compatible 0.1 µm syringe filters
Polypropylene round-bottom tubes, 5 mL capacity, 12 x 75 mm
Polypropylene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm
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

The latest build of CyTOF Software v6.7 and the Maxpar Human Immune Monitoring Panel Kit acquisition template (Hu_ImmuneMonitoring_Panel_201324_Acq.tem) are required to acquire data on samples stained using this protocol. See Prepare to Acquire Data for more information.

Suggested Software

We suggest the use of the latest version of GemStone™ software by Verity Software House or Premium Cytobank to perform cell population frequency analysis on the normalized .fcs data file generated from CyTOF Software. For more information, contact your local Fluidigm field application specialist.

Day 1: Cell Staining

Before You Begin

IMPORTANT Read and understand the safety information in Appendix D.

Reagent handling: Retrieve, mix, and centrifuge reagents as directed. Frozen aliquots of Cell-ID Intercalator-Ir and Cell-ID Cisplatin should be used only once and immediately after thawing. Avoid multiple freeze/thaw cycles.

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

FcR-blocking with Human TruStain FcX: The FcR-blocking step is recommended in the protocol to prevent binding of Maxpar metal-conjugated antibodies to Fc receptors. Binding results in high nonspecific background signal. 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.

Formaldehyde solution: It is critical to prepare fresh formaldehyde (FA) solution to effectively fix cells stained with the Maxpar Human Immune Monitoring Panel Kit. 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 use this protocol on valuable samples, we recommend performing two pilot experiments using this protocol on noncritical samples:

- **Experiment 1 (without antibody staining):** Run an initial pilot experiment on a subset of noncritical samples to test the success of Cell-ID Cisplatin (viability) 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 unstained cells using the panel kit acquisition template and check for high signal from Sn, Xe, Cs, Ba, Pb, Pt, I, and Os (shown as BCKG in the template).
- **Experiment 2 (with antibody staining):** Run a final pilot experiment on a subset of noncritical samples to familiarize yourself with the complete protocol workflow, including antibody staining and data analysis.

NOTE For more information, contact your local Fluidigm field application specialist.

Reagents and Solutions to Prepare in Advance

Media for cell viability staining: Before the FcR-blocking step of the protocol, cells are stained with Cell-ID Cisplatin to identify viable cells. To facilitate cell viability staining with Cell-ID Cisplatin, pre-warm serum-free and serum-containing complete media at 37 °C before beginning protocol. Use the same media that are normally used for cell culture.

Antibody cocktail: The 29 antibodies provided in the Maxpar Human Immune Monitoring Panel Kit are split into two groups: Group 1 uses 1 µL antibody per test, and Group 2 uses 2 µL per test. Label the antibody vials and prepare the antibody cocktail for surface markers as instructed in Appendix A: Antibody Cocktail Table.

NOTE The antibody cocktail can be stored at 4 °C for up to 4 hours before use in cell staining. 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).

Retrieve the Cell Staining Reagents

Step	Reagent	Preparation
Viability stain	Cell-ID Cisplatin	Remove a single-use aliquot from –20 °C and then thaw it to room temperature immediately before use.
Viability and surface stains	Maxpar Cell Staining Buffer	Remove from 4 °C
FcR block	Human TruStain FcX	Remove from 4 °C
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	Remove from 4 °C
Intercalator-Ir stain	Cell-ID Intercalator-Ir	Remove a single-use aliquot from –20 °C and then thaw it to room temperature immediately before use.
	Maxpar Fix and Perm Buffer	Remove from 4 °C

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

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.

Viability-Stain Cells with Cell-ID Cisplatin

NOTE See Reagents and Solutions to Prepare in Advance for cell viability staining consideration points.

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 a serum-containing medium, wash cells to remove residual protein by adding 1 mL of pre-warmed serum-free medium. Centrifuge at 300 x *g* for 5 minutes, carefully aspirate the supernatant, and gently pipet to mix.

3 Resuspend cells to 2 x 10^7 cells/mL in pre-warmed 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 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

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

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 the 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: Antibody Cocktail Table for antibody mixing volumes).

2 Gently pipet to mix each tube and incubate the tubes 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 cells at 300 x *g* for 5 minutes.

5 Carefully aspirate and discard supernatant. Gently vortex to resuspend cells in residual volume.

6 Repeat Steps 4 and 5 for a total of two washes.

Fix Cells

NOTE See Reagents and Solutions to Prepare in Advance for cell fixation consideration points.

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 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 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 tubes for 10 minutes at room temperature.

4 Centrifuge cells at **800 x *g*** for 5 minutes.

   NOTE The increased centrifuge 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. For complete safety information, see Appendix D: 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.

Day 2: 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: Instrument Setup and Data Acquisition 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.

Retrieve the Reagents

Step	Reagent	Preparation
Wash and count cells	Cells in intercalation solution (from Day 1)	Remove from 4 °C.
	Maxpar Cell Staining Buffer	
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 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 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 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 centrifuging is complete, carefully aspirate and discard supernatant.
8. Leave cells pelleted at 4 °C until ready to run on Helios or upgraded CyTOF 2 system.

   NOTE Cells should be run on the 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 beads (see Appendix B).

Prepare to Acquire Data

Samples are resuspended in Maxpar Cell Acquisition Solution containing 0.1X EQ beads immediately prior to data acquisition on the Helios or upgraded CyTOF 2 system. Appendix B 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 Required Materials in Appendix B).

☑	Material	Notes
☐	Washed and pelleted samples	Remove from 4 °C (see Wash and Count Cells).
☐	Maxpar Cell Acquisition Solution*	Need 1 mL buffer for each sample that will 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	Need 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	Need sufficient number to run each sample
☐	Helios WB Injector	Make sure to use Cat. No. 107950.
☐	Maxpar Human Immune Monitoring Panel Kit acquisition template†	Provide the operator with a copy of the electronic file (Hu_ImmuneMonitoring_Panel_201324_Acq.tem).

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

† 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), 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.

Appendix A: Antibody Cocktail Table

The following tables can be used to prepare the Maxpar metal-conjugated antibody cocktail for surface marker staining in Maxpar Cell Staining Buffer.

Retrieve the Reagents

Reagent	Preparation
Maxpar Metal-Conjugated Antibodies	Remove each vial from 4 °C, keep on ice.
Maxpar Cell Staining Buffer	Remove from 4 °C.

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 Table 1 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 Table 2.
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).

Table 1. Metal-conjugated antibodies provided in the Human Immune Monitoring Panel Kit. To use this table: Label each antibody vial with the appropriate group number as shown, and then check off each antibody as you add the appropriate volume to the antibody cocktail as instructed in Table 2.

Group Label	Catalog No.	Antibody	Mass	☑
Group "1" (1 µL antibody/sample)	S3089003C	Anti-Human CD45 (HI30)	89Y	☐
	S3142001C	Anti-Human CD19 (HIB19)	142Nd	☐
	S3143012C	Anti-Human CD127/IL-7Ra (A019D5)	143Nd	☐
	S3144014C	Anti-Human CD38 (HIT2)	144Nd	☐
	S3146005C	Anti-Human IgD (IA6-2)	146Nd	☐
	S3147008C	Anti-Human CD11c (Bu15)	147Sm	☐
	S3148004C	Anti-Human CD16 (3G8)	148Nd	☐
	S3149029C	Anti-Human CD194/CCR4 (L291H4)	149Sm	☐
	S3151001C	Anti-Human CD123/IL-3R (6H6)	151Eu	☐
	S3152008C	Anti-Human TCRgd (11F2)	152Sm	☐
	S3153020C	Anti-Human CD185/CXCR5 (RF8B2)	153Eu	☐
	S3154003C	Anti-Human CD3 (UCHT1)	154Sm	☐
	S3155011C	Anti-Human CD45RA (HI100)	155Gd	☐
	S3158010C	Anti-Human CD27 (L128)	158Gd	☐
	S3160003C	Anti-Human CD28 (CD28.2)	160Gd	☐
	S3162023C	Anti-Human CD66b (80H3)	162Dy	☐
	S3163004C	Anti-Human CD183/CXCR3 (G025H7)	163Dy	☐
	S3164009C	Anti-Human CD161 (HP-3G10)	164Dy	☐
	S3165011C	Anti-Human CD45RO (UCHL1)	165Ho	☐
	S3166007C	Anti-Human CD24 (ML5)	166Er	☐
	S3167009C	Anti-Human CD197/CCR7 (G043H7)	167Er	☐
	S3168002C	Anti-Human CD8 (SK1)	168Er	☐
	S3169003C	Anti-Human CD25 (2A3)	169Tm	☐
	S3171012C	Anti-Human CD20 (2H7)	171Yb	☐
	S3173005C	Anti-Human HLA-DR (L243)	173Yb	☐
	S3174004C	Anti-Human CD4 (SK3)	174Yb	☐
	S3176008C	Anti-Human CD56 (NCAM16.2)	176Yb	☐
Group "2" (2 µL antibody/sample)	S3141003C	Anti-Human CD196/CCR6 (G034E3)	141Pr	☐
	S3175015C	Anti-Human CD14 (M5E2)	175Lu	☐

Table 2. To use this antibody cocktail table: 1) Locate the row matching the Number of Samples to be stained (a) and then add the required Volume of Cell Staining Buffer (b) to your master mix tube. 2) Again locate the row matching the Number of Samples to be stained (a) and then add the indicated Volume of Antibody (c) in each group to the master mix tube, using Table 1 as a guide to check off the antibodies used to stain the samples as you add them from each group. All volumes include 10% overage as reflected in the total expected volume in the master mix tube (d).

(a) Number of Samples	(b) Volume of Cell Staining Buffer (µL)	(c) Volume of Antibody (µL)		(d) Total Volume in Tube (µL)
		Group 1	Group 2	
1	15.4	1.1	2.2	49.5
2	30.8	2.2	4.4	99.0
3	46.2	3.3	6.6	148.5
4	61.6	4.4	8.8	198.0
5	77.0	5.5	11.0	247.5
6	92.4	6.6	13.2	297.0
7	107.8	7.7	15.4	346.5
8	123.2	8.8	17.6	396.0
9	138.6	9.9	19.8	445.5
10	154.0	11.0	22.0	495.0
11	169.4	12.1	24.2	544.5
12	184.8	13.2	26.4	594.0
13	200.2	14.3	28.6	643.5
14	215.6	15.4	30.8	693.0
15	231.0	16.5	33.0	742.5
16	246.4	17.6	35.2	792.0
17	261.8	18.7	37.4	841.5
18	277.2	19.8	39.6	891.0
19	292.6	20.9	41.8	940.5
20	308.0	22.0	44.0	990.0
21	323.4	23.1	46.2	1,039.5
22	338.8	24.2	48.4	1,089.0
23	354.2	25.3	50.6	1,138.5
24	369.6	26.4	52.8	1,188.0
25	385.0	27.5	55.0	1,237.5

Appendix B: Instrument Setup and Data Acquisition

This appendix outlines the Helios or upgraded CyTOF 2 system setup and data acquisition workflow for cells stained with the Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324).

IMPORTANT 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 following procedures before using the kit and before transferring this information to those responsible for instrument operation.

Required Materials

Product	Catalog Number	Source	Storage
Maxpar Cell Acquisition Solution—200 mL*†	201240	Fluidigm	4 °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	4 °C
Polypropylene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm*	–	Major laboratory supplier (MLS)	–
Maxpar Human Immune Monitoring Panel Kit acquisition template (Hu_ImmuneMonitoring_Panel_201324_Acq.tem)*	–	See Important Notes Before Starting	–

* Instrument operator: These materials may be supplied by cell staining personnel.

† Supplied with Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324). See Fluidigm Kit Contents.

Workflow Overview

Times shown here 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 instrument.**	—	45 min
3	**Tune the instrument.**	—	20 min
4	**Acquire data.**	2 min per sample	15–20 min per sample
5	**Perform post-run instrument maintenance.** Shut down system, remove injector, clean parts.	30 min	1 hr 25 min
6	**Normalize data.** Latest build of CyTOF Software v6.7	1 min	20 min/variable

Before You Begin

Important Notes Before Starting

Required software: The latest build of CyTOF Software v6.7 and the Maxpar Human Immune Monitoring Panel Kit acquisition template (Hu_ImmuneMonitoring_Panel_201324_Acq.tem) are required to run samples stained with the Maxpar Human Immune Monitoring Panel Kit.

NOTE The CyTOF Software build is shown in the **CyTOF Login** dialog box or under **About** in the left navigation bar of the main software window. To update to the latest software release, refer to the Install CyTOF Software 6.7 for Stand-Alone Processing Workstations section in the CyTOF Software 6.7 Release Notes (PN 400314). If the acquisition template is not supplied by cell staining personnel, go to fluidigm.com/productsupport/cytof-helios-support-hub to download the template. For more information, contact your local Fluidigm field application specialist.

Condition of instrument: Be sure to clean and maintain all instrument parts, particularly the nebulizer and vacuum interface cones, as instructed in the Maintenance chapter of the Helios User Guide (PN 400250) 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 application specialist.

Retrieve the Reagents

Step	Product	Preparation
Tune instrument	Tuning Solution	Keep at room temperature.
Tune instrument, acquire data	Maxpar Cell Acquisition Solution	
Acquire data	EQ Four Element Calibration Beads	Remove from 4 °C.

Install the Helios WB Injector

Installation of the Helios WB Injector (Cat. No. 107950, see Figure 1 below) is the same as for the Helios HT Injector provided with the Helios or upgraded CyTOF 2 system.

Figure 1. Helios WB Injector (Cat. No. 107950)—full length view (left) and close-up of "WB" etched on surface (right).

See the Reassemble the Torch Assembly section of the Helios User Guide (PN 400250) for installation instructions, making sure to:

1. Install the Helios WB Injector by pushing and turning until it is fully inserted.
2. Confirm that the injector is 1.5–2 mm from the end of the inner portion of the torch.

Start Plasma and Warm Up Instrument

Follow the procedures in the Preparation and Startup section of the Helios User Guide (PN 400250) to prepare the instrument for use before running samples stained with the Maxpar Human Immune Monitoring Panel Kit.

IMPORTANT Make sure the argon pressure in the Sample Loader is within 14 psi for sample delivery using the Helios WB Injector.

1. Load a 5 mL tube containing at least 1 mL of deionized water (DIW) onto the Sample Loader.
2. In the Control Bar of the CyTOF Software, click **ON** in Sample Introduction.
3. From the Menu Panel:
   a. Click the **Status Panel**, and then toggle Sample Introduction to expand the view.
   b. Verify the Pressure is within 14 psi.

   NOTE See the Helios User Guide (PN 400250) for information on how to troubleshoot the Helios or upgraded CyTOF 2 system.

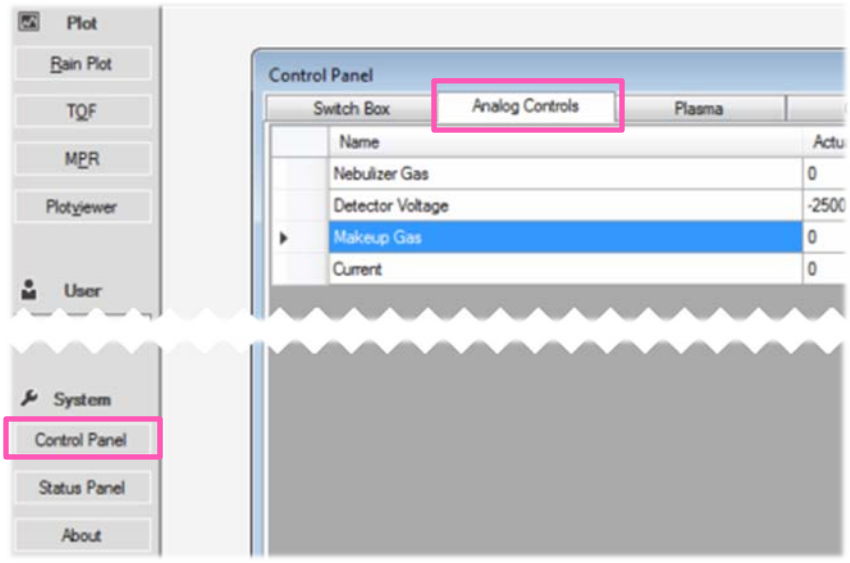
Tune the Instrument

Tune (calibrate) the Helios or upgraded CyTOF 2 system using a custom protocol before running any samples stained with the Maxpar Human Immune Monitoring Panel Kit to ensure optimal data quality.

NOTE See the Tuning section of the Helios User Guide (PN 400250) for additional information about the tuning procedure.

Change the Makeup Gas Flow Value

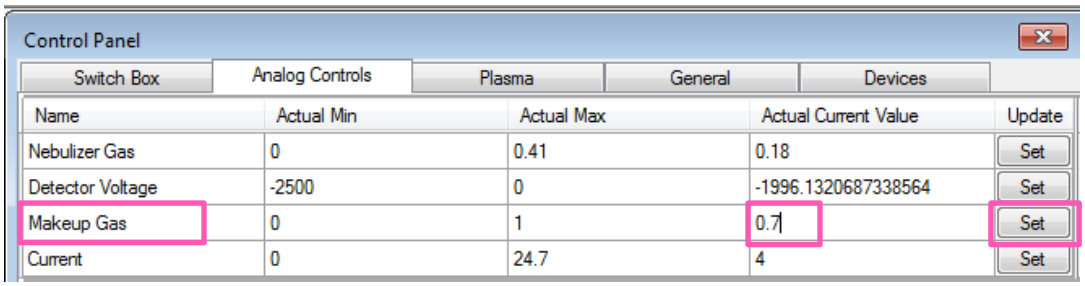
1 Click **Control Panel** in the menu panel of the CyTOF Software, and then click **Analog Controls**.



2 Record the Actual Current Value for Makeup Gas as shown in the Analog Controls tab. You will reset to this value if you choose to reinstall the Helios HT Injector (see Perform Post-Run Instrument Maintenance).

3 Set the Actual Current Value for Makeup Gas to the actual current value for the Helios HT Injector plus 0.2 L/min, and then click **Set**.

NOTE For example, if the actual current value for the Helios HT injector is 0.5 L/min, the value for the Helios WB Injector should be set to 0.7 L/min (0.5 L/min + 0.2 L/min).

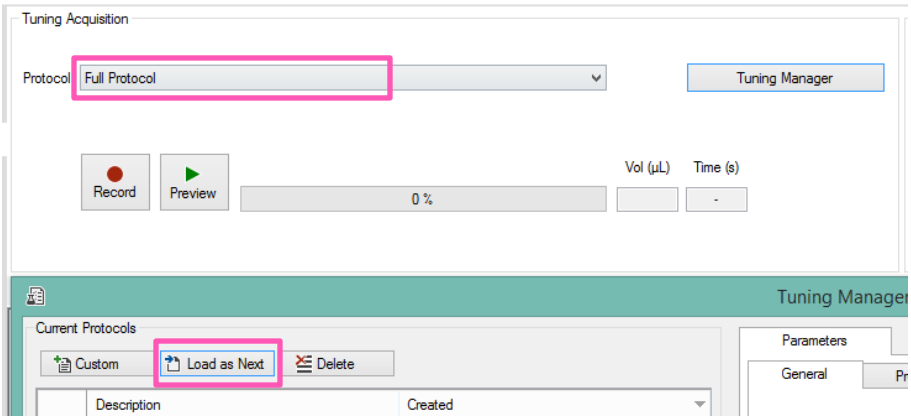


Run the Tuning Procedure

DANGER Tuning solution contains nitric acid. For complete safety information, see Appendix D: Safety.

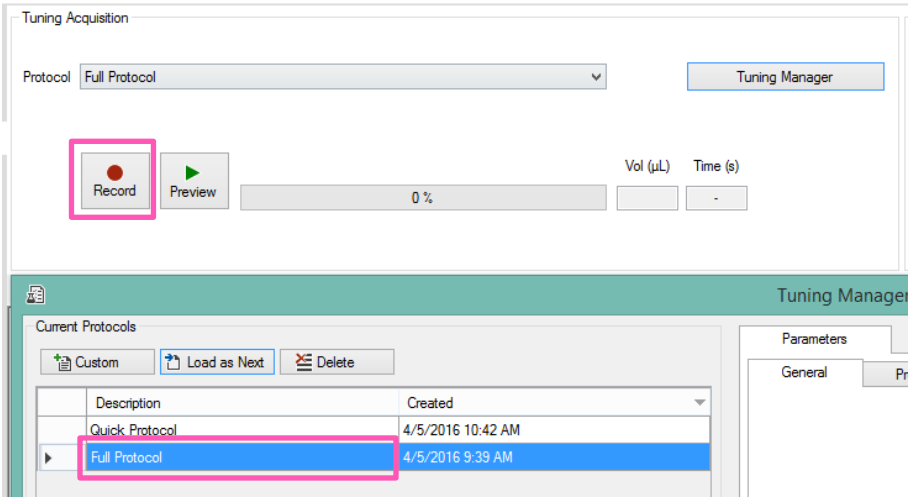
1 Click **Tune** in the menu panel of the software.

2 Select **Full Protocol** from the Protocol dropdown menu, and then click **Load as Next**.



3 Load 1 mL of tuning solution onto the PSI sample loader.

4 Confirm that **Full protocol** is selected under Current Protocols, and then click **Record** to start the protocol.



The software displays the progress of the tuning procedure.

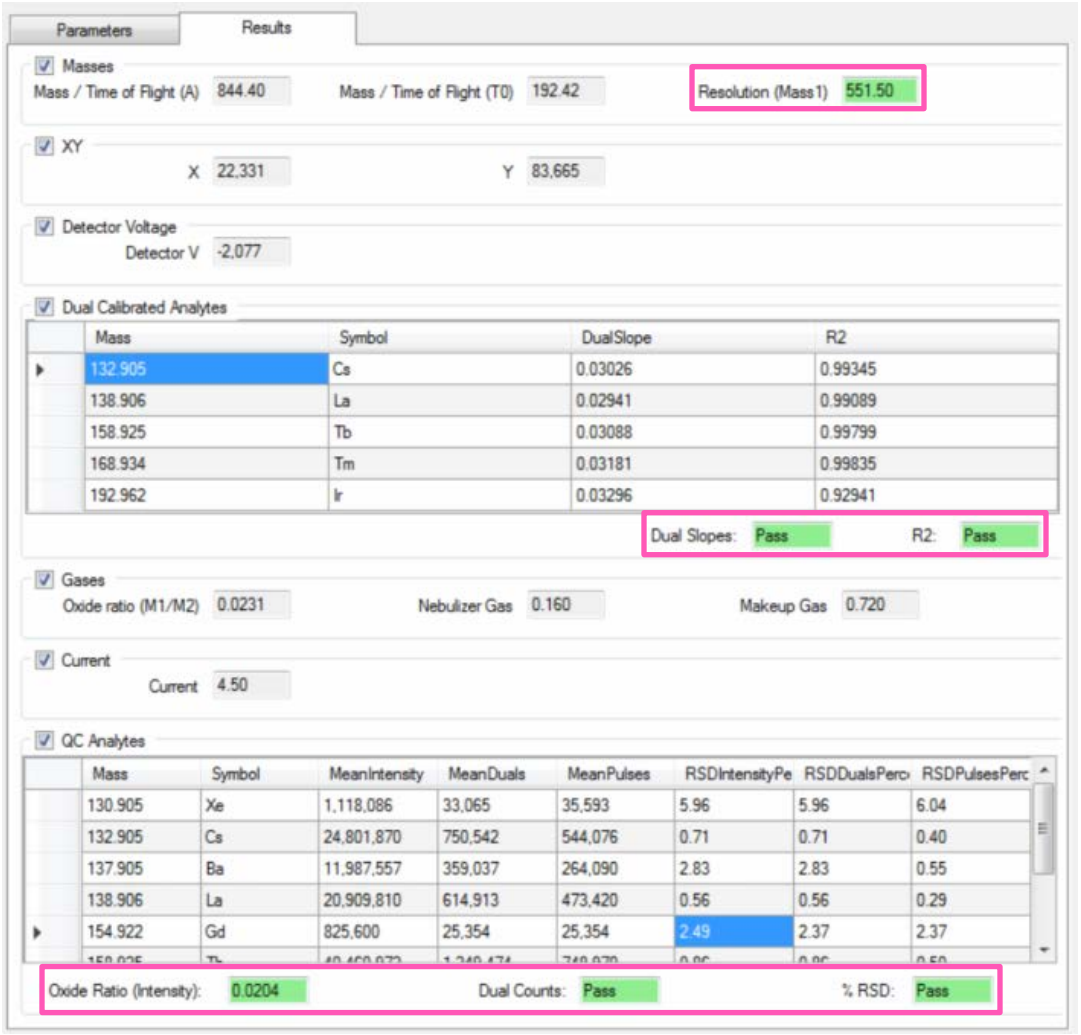
5 Click **OK** to dismiss the Tuning is complete dialog box.

6 Click **Results** below the Tuning Sequence table to view the Results tab.

NOTE You can click **Results** repeatedly during the tuning procedure to refresh the tuning results report, if needed.

7 View the tuning results report generated at the end of the tuning procedure and verify that the tuning results show a Pass (green) status.

NOTE If the tuning results do not pass, see the Helios User Guide (PN 400250) for troubleshooting information or contact Fluidigm Technical Support for assistance.



Acquire Data

Before You Begin

Samples stained with the Maxpar Human Immune Monitoring Panel Kit antibody panel must be run using the acquisition template (Hu_ImmuneMonitoring_Panel_201324_Acq.tem). If the acquisition template is not supplied by cell staining personnel, go to fluidigm.com/productsupport/cytof-helios-support-hub to download the template, or contact your local Fluidigm field application specialist.

Note the following before you start acquiring data using this template:

- See the Sample Acquisition section in the Helios User Guide (PN 400250) for information on how to import a template and run samples using CyTOF Software.

  IMPORTANT Do not select or edit any fields in the template (open the Experiment Manager, select the template to import, and then close the Experiment Manager).

- The acquisition template has been configured to acquire all channels corresponding to the Maxpar Human Immune Monitoring Panel Kit antibody panel. All cell markers have been appropriately labeled.
- Samples to analyze using GemStone software must be acquired using the acquisition template without altering any settings (specifically, do not edit any Target labels).

Test Bead Sensitivity and Start Sample Introduction

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

Acquire and Normalize Samples

NOTE Cells should be run 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 a sufficient 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 PBMC concentration of 1.0×10^6 cells/mL.
3. Immediately before data acquisition, resuspend cells with 0.1X EQ Four Element Calibration beads in Maxpar Cell Acquisition Solution and filter cells through 35 µm cell strainer cap tubes.

4 Acquire samples on a Helios or upgraded CyTOF 2 system using the acquisition template (Hu_ImmuneMonitoring_Panel_201324_Acq.tem).

IMPORTANT Do not select or edit any fields in the template.

NOTE See the Troubleshooting section in the Helios User Guide (PN 400250) if you encounter any issues during the sample acquisition process.

5 In the Acquisition window, set the **Stop at Event** limit for each sample to 300,000 events. The expected acquisition rate at the maximum cell concentration of 1.0 x 10^6 cells/mL is 250–350 events/second.

NOTE We recommend cleaning the instrument with Maxpar Cell Acquisition Solution for at least 5 minutes between samples to minimize carryover.

Normalize Data

1 When you have acquired data from all test samples, use the CyTOF Software Method with default FCS Processing settings to normalize the final .fcs files in the CyTOF Software, 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 application specialist.

2 (Optional) Transfer the normalized .fcs files from their saved location to an analysis location for further analysis (see Analyze Normalized Data).

Perform Post-Run Instrument Maintenance

After all samples stained with the Maxpar Human Immune Monitoring Panel Kit 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 nebulizer *daily* with 10% Contrad® 100 in DIW according to the instructions in the Maintenance chapter of the Helios User Guide.

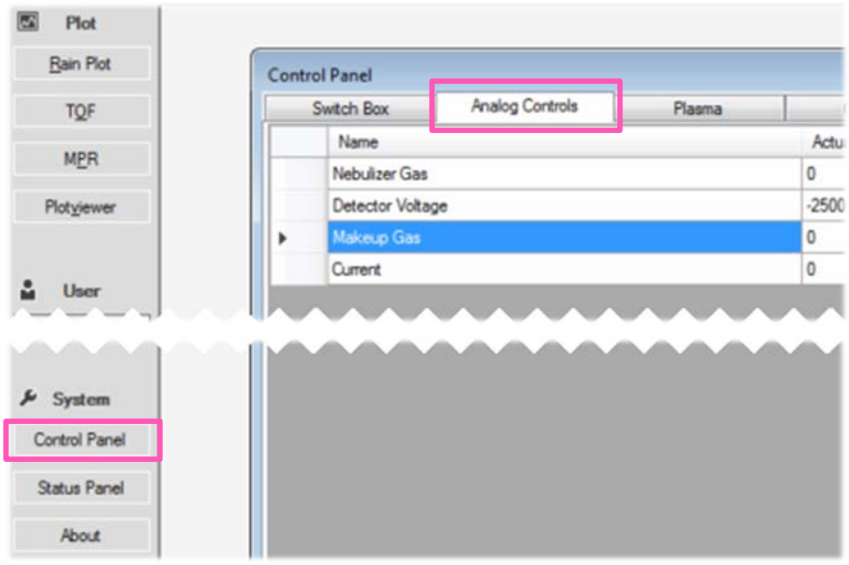
2 When the instrument is cooled down (approximately 15–30 minutes), remove and clean the Helios WB Injector *daily* with DIW, in addition to the weekly cleaning with 10% Contrad 100 in DIW as instructed in the Maintenance chapter of the Helios User Guide.

3 Install the appropriate injector based on the next samples to be acquired:

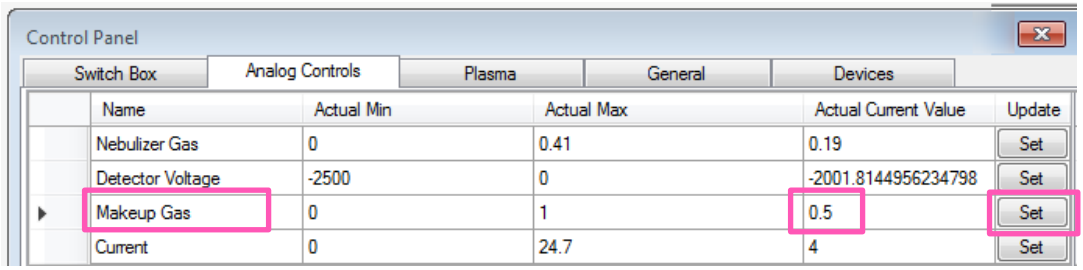
- **Option 1: Helios WB Injector**—Install the Helios WB Injector (Cat. No. 107950) if you will continue to acquire samples stained with the Maxpar Human Immune Monitoring Panel Kit.
- **Option 2: Helios HT Injector**—Install the Helios HT Injector (Cat. No. 107018) provided with the Helios or upgraded CyTOF 2 system if you will acquire samples stained with other reagents. Go to Step 4.

4 (Option 2: Helios HT Injector only) Change the Makeup Gas flow value:

a Click **Control Panel** in the menu panel of the CyTOF Software, and then click **Analog Controls**.



b Set the **Actual Current Value** for Makeup Gas to the actual current value for the Helios HT Injector that you recorded before installing the Helios WB Injector (see Step 2 in Change the Makeup Gas Flow Value), and then click **Set**.



Appendix C: Related Documentation

Go to fluidigm.com to download these related documents.

Title	Part Number
Helios User Guide	400250
CyTOF Software 6.7 Release Notes	400314
Maxpar Human Immune Monitoring Panel Kit Product Information Sheet	PRD033
Maxpar Human Immune Monitoring Panel Kit Cell Staining Quick Reference	PRD034

Go to Premium.Cytobank.org and log in to download these additional related documents. Contact your local Fluidigm field application specialist for more information.

Title	Part Number
Normalization of Mass Cytometry Data using EQ Four Element Calibration Beads User Guide	UG13-02_150903
Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit Technical Note	400270

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 personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and 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 Do not modify this instrument. Unauthorized modifications may create a safety hazard.

WARNING BIOHAZARD. If you are putting biohazardous material on the instrument, 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 instrument 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.

DANGER Cell-ID Cisplatin contains cisplatin. Combustible liquid. Causes skin irritation. May cause genetic defects. May cause cancer. Read the safety data sheet (SDS) before using this reagent.

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.

For technical support visit fluidigm.com/support.

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