Maxpar Phosphoprotein Staining with Fresh Fix

For use with CyTOF 2, Helios, and CyTOF XT

Contents

About This Document

This protocol describes how to use Maxpar® metal-conjugated antibodies to stain activation-induced phosphorylated antigens and to fresh fix cells for detection on CyTOF® 2, Helios™, and CyTOF XT™. For detailed instructions on system and software operation, see the user guide for your instrument (see Appendix B).

IMPORTANT Before using the reagents in this protocol, read and understand the detailed instructions and safety guidelines in this document. For complete safety information, see Appendix C.

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, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Introduction

This staining protocol should be followed for the detection of activation-induced phosphorylated antigens using Fluidigm suspension mass cytometers. For staining antigens located on the cell surface, follow the Maxpar Cell Surface Staining with Fresh Fix Protocol (400276). For staining antigens located within the nucleus, including transcription factors, follow the Maxpar Nuclear Staining with Fresh Fix Protocol (400277). For staining secreted proteins, including cytokines, or antigens located in the cytoplasm, follow the Maxpar Cytoplasmic/Secreted Antigen Staining with Fresh Fix Protocol (400279).

Materials

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

Required Reagents from Fluidigm

Product Name	Catalog Number	Storage
Cell Staining Reagents		
Cell-ID™ Intercalator-Ir—125 µM, 25 µL	201192A	–20 °C in single-use aliquots
Maxpar® Cell Staining Buffer—500 mL	201068	4 °C. Do not freeze.
Maxpar Fix I Buffer (5X)	201065	
Maxpar Fix and Perm Buffer	201067	
Maxpar metal-conjugated antibodies	Various	
Maxpar PBS—500 mL	201058	
Cell Acquisition Reagents for CyTOF 2 and Helios with HT Injector		
Maxpar Water	201069	4 °C. Do not freeze.
EQ™ Four Element Calibration Beads—100 mL	201078	
Tuning Solution—250 mL	201072	Room temperature
Cell Acquisition Reagents for Helios with WB Injector		
EQ Four Element Calibration Beads—100 mL	201078	4 °C. Do not freeze.
Maxpar Cell Acquisition Solution—200 mL or 6-pack (6 x 200 mL)	201240 or 201241	
Tuning Solution—250 mL	201072	Room temperature
Cell Acquisition Reagents for CyTOF XT		
EQ Six Element Calibration Beads—100 mL	201245	2–8 °C
Maxpar Cell Acquisition Solution Plus for CyTOF XT—1 L	201244	

Suggested Reagents from Fluidigm

Product Name	Catalog Number	Storage
Cell Viability Reagents		
Cell-ID Cisplatin—100 µL	201064	–20 °C in single-use aliquots
Cell-ID Cisplatin-194Pt—100 µL	201194	
Cell-ID Cisplatin-195Pt—100 µL	201195	
Cell-ID Cisplatin-196Pt—100 µL	201196	
Cell-ID Cisplatin-198Pt—100 µL	201198	

Required Reagents from Other Suppliers

Product Name	Source	Part Number
Methanol	Fisher Scientific™	BP1105-4
Pierce™ 16% Formaldehyde (w/v), Methanol-free	Thermo Fisher Scientific™	28906 (10 x 1 mL) or 28908 (10 x 10 mL)
Human TruStain FcX™ (Fc Receptor Blocking Solution)	BioLegend®	422301 (50 tests) or 422302 (200 tests)
Serum-Free and Complete Media	Major laboratory supplier (MLS)	—

Required Consumables

Product Name	Source	Part Number
1.5 mL microfuge tubes	MLS	—
Pipette tips with aerosol barrier	MLS	—
Polypropylene round-bottom tubes, 5 mL capacity, 12 x 75 mm	Falcon™	352063
Polypropylene round-bottom tubes with 35 µm cell-strainer cap, 5 mL capacity, 12 x 75 mm	Falcon	352235
1 mL Norm-Ject® latex-free syringes and compatible 0.1 µm syringe filters	VWR (or MLS)	53548-001
Carousel tubes for CyTOF XT only:		
Polypropylene skirted conical tube with screw cap, 15 mL capacity, 20 x 76 mm	Sarstedt, Inc.	60.732.001

Required Equipment

Product Name	Source	Part Number
Centrifuge capable of holding 5 mL polypropylene tubes	MLS	—
Vacuum aspirator	MLS	—
Vortexer	MLS	—

Before You Begin

IMPORTANT Read and understand the safety information in Appendix C.

For the overall success of the protocol, we recommend the following best practices.

Fix- and perm-sensitive surface epitopes: The protocol recommends staining of surface markers prior to cell fixation to achieve optimal signal-to-noise ratio in surface marker detection. Surface staining may also be performed either following fixation, or concurrently with intracellular staining, following both fixation and permeabilization. However, staining surface antigens post-fixation and/or permeabilization may result in decreased signal-to-noise ratio and should be evaluated for individual antigens/clones.

Antibody compatibility: Antibodies labeled with 106Cd and 110Cd metal isotopes are not compatible for use with the Cell-ID 20-Plex Pd Barcoding Kit (201060) due to direct mass overlap with the 106Pd and 110Pd metal isotopes in the kit.

Staining pre-fixed cells: For cells fixed prior to this protocol, shorter fixation times and reduced concentration of fixative may result in improved detection of surface markers. This should be evaluated for individual antigens and antibody clones. Centrifugation should be performed at 800 x *g* for 5 min after cell fixation.

Buffers: All Fluidigm products containing saponin are tested for compatibility with our staining workflows. The use of alternative saponin-based reagents may result in high background and/or nonspecific staining on Fluidigm CyTOF suspension mass cytometry systems. We recommend that you perform a pilot test with any saponin-based reagents from other suppliers to determine their compatibility with this workflow.

Reagent handling: Retrieve, mix, and centrifuge reagents as directed. See the individual reagent Product Information Sheet or Technical Data Sheet for usage instructions.

Cell viability staining: Cells can be stained with Cell-ID Cisplatin to identify viable cells. Search for the applicable Product Information Sheet or Technical Data Sheet available at fluidigm.com for usage instructions (see Appendix B: Related Documents).

NOTE the following:

- Samples resuspended in serum-containing media, for example complete RPMI with FBS, should be washed in serum-free media or PBS before beginning viability staining with Cell-ID Cisplatin.
- Cell-ID Cisplatin reagent channels may not be compatible with platinum (Pt)-labeled antibodies due to direct mass overlap. Pt-labeled antibodies are compatible with Cell-ID Intercalator-103Rh and non-overlapping, monoisotopic Cell-ID Cisplatin reagents.

Reagent titration: We recommend titrating antibodies and other reagents for optimal performance and data quality in individual experiments. See the individual reagent Product Information Sheet or Technical Data Sheet for usage instructions. Cell surface antibodies and phosphoprotein antibodies are diluted in Maxpar Cell Staining Buffer.

Fc receptor blocking with Human TruStain FcX: An optional Fc receptor (FcR) blocking step is recommended in the protocol to prevent nonspecific background signal by blocking binding of Maxpar metal-conjugated antibodies to FcRs. FcRs 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 Maxpar antibodies. Be sure to open the single-use

formaldehyde ampule and prepare the FA solution immediately before use in the fixation process [see Fix Cells (Fresh Fix)].

Centrifuge speeds: For cell centrifugation steps, centrifugation should be performed at 300 x *g* for 5 min before cell fixation, and at 800 x *g* for 5 min after cell fixation. The increased centrifugation speed after cell fixation results in greater cell recovery. When centrifuging sample volumes greater than 5 mL in a single tube, increase centrifugation time to 10 min for greater cell recovery.

Reagents and Solutions to Prepare in Advance

Antibodies: Centrifuge the stock antibody at 12,000 x *g* for 5 min to sediment antibody aggregates.

Antibody cocktail: Prepare separate antibody cocktails for surface markers and phosphoproteins in Maxpar Cell Staining Buffer for use within the same day. The antibody cocktail should be temporarily stored at 2–8 °C before use. We recommend preparing 2X antibody cocktails with 10% in excess volume for multiple samples, such that the prepared volume for each test is 55 µL (see Appendix A: Antibody Cocktail Preparation Guide). When 50 µL of the 2X antibody cocktail is added to 50 µL of cells, the total staining volume is 100 µL.

Intercalation solution: Prepare 1 mL of cell intercalation solution for each 1–3 million cell 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. Include 10% volume overage for multiple samples processed together. Prepare fresh for same day use.

NOTE For example, to prepare intercalation solution for 10 samples, add 11 µL of 125 µM Intercalator-Ir to 11 mL of Maxpar Fix and Perm Buffer.

1.6% FA solution: Prepare a fresh 1.6% formaldehyde (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 1 sample, add 100 µL of filtered 16% stock formaldehyde to 900 µL of Maxpar PBS.

(Optional) Serum-Free and Complete Media: Optional cisplatin viability stain can be performed prior to cell stimulation for sensitive phosphorylation experiments. If performing, warm **serum-free** and complete media at 37 ˚C prior to beginning the protocol. Use the same media (without serum) that is normally used for cell culture. See the individual Cell-ID Cisplatin Product Information Sheet or Technical Data Sheet for additional usage instructions.

Prepare Cells

IMPORTANT Perform cell viability staining on cells prior to fixation.

1. Prepare cells of interest from cell culture or primary tissue and activate desired signaling pathways by adding stimulus to cells for appropriate length of time.
2. At the end of stimulation, stop the signaling reaction by adding 5X Fix I Buffer to a final concentration of 1X.
3. Mix gently and thoroughly, and incubate at room temperature for 10 min.
4. Transfer cells to an appropriate tube and wash with Maxpar Cell Staining Buffer, using 5–10X the volume of the cell suspension. Centrifuge at 800 *x g* for 5 min and remove supernatant by aspiration.

   NOTE The increased centrifuge speed after cell fixation results in greater cell recovery.
5. Resuspend cells in Maxpar Cell Staining Buffer and aliquot 1–3 million cells, in a volume of 50 µL, into individual 5 mL polypropylene tubes for each sample to be stained.

   NOTE Adjust the volume in which cells are resuspended to account for volume of FcR blocking solution, if used.

Stain Cells with Surface Antibodies

1. (Optional) FcR blocking: Add 5 µL of Human TruStain FcX to each tube of 1–3 million cells in 45 µL and incubate at room temperature for 10 min. Without washing off FcR blocking solution, continue with the protocol.
2. Add 50 µL of the 2X antibody cocktail to each tube so the total staining volume is 100 µL (50 µL of cell suspension + 50 µL 2X antibody cocktail). (See Appendix A: Antibody Cocktail Preparation Guide for mixing volumes.)
3. Gently vortex to mix each tube and incubate the tubes at room temperature for 15 min.
4. Gently vortex samples and incubate at room temperature for an additional 15 min.
5. Following the incubation, wash by adding 2 mL Maxpar Cell Staining Buffer to each tube, centrifuge at 800 x *g* for 5 min, and remove supernatant by aspiration.
6. Repeat Step 5 for a total of 2 washes with Maxpar Cell Staining Buffer, and resuspend cells thoroughly in residual volume by gently vortexing.

Stain Cells with Phosphoprotein Antibodies

1. Place cells on ice for 10 min to chill sample.
2. Add 1 mL of 4 ˚C methanol to each sample, mix gently, and incubate on ice for 15 min.
3. Wash cells with 2 mL of Maxpar Cell Staining Buffer, centrifuge at 800 x *g* for 5 min, and remove supernatant by aspiration.

4 Repeat Step 3 for a total of 2 washes with Maxpar Cell Staining Buffer.

5 Resuspend pellet in residual volume with gentle vortexing.

6 Add 50 µL of the 2X phosphoprotein antibody cocktail to each tube so the total staining volume is 100 µL (50 µL of cell suspension + 50 µL 2X antibody cocktail).

7 Gently vortex samples and incubate the tubes at room temperature for 30 min.

8 Following the incubation, wash by adding 2 mL Maxpar Cell Staining Buffer to each tube, centrifuge at 800 x *g* for 5 min and remove supernatant by aspiration.

9 Repeat Step 8 for a total of 2 washes with Maxpar Cell Staining Buffer, and resuspend cells thoroughly in residual volume by gently vortexing after final wash/aspiration.

Fix Cells (Fresh Fix)

IMPORTANT It is essential to thoroughly disrupt the pellet by vortexing before adding the fresh fixative at this step.

1 Add 1 mL of the 1.6% FA solution to each tube and gently vortex to mix well.

2 (Optional) Reserve a small volume (approximately 10 µL) from each tube to count cells, to ensure optimal Cell-ID Intercalator-Ir staining.

3 Incubate cells at room temperature for 10 min. If you reserved volume, go to Step 4.

4 (Optional) During incubation, count cells in the reserved volume from each tube. Make sure to note the cell concentration for each tube.

5 After incubation is complete, centrifuge cells at 800 x *g* for 5 min.

6 Carefully aspirate and remove supernatant. Gently vortex to resuspend cells in residual volume.

Stain Cells with Cell-ID Intercalator-Ir

1 Add 1 mL of intercalation solution to each 1–3 million cell sample and gently vortex. Ensure that cells are well resuspended.

2 Incubate the samples at room temperature for 1 hr or leave at 4 °C overnight.

STOPPING POINT Samples can be stored in intercalation solution at 2–8 °C for up to 48 hr before data acquisition.

Prepare Cells for Acquisition

IMPORTANT Run cells on the same day they are washed from intercalation solution.

Stained samples can be acquired on CyTOF 2, Helios (HT or WB Injector), or CyTOF XT systems. Follow the applicable section below to prepare cells for acquisition. For more

information, refer to your instrument user guide or select the Help tab in CyTOF Software v7.0 (108520) or later (see Appendix B: Related Documents).

For Helios (HT or WB Injector) and CyTOF 2

IMPORTANT For Helios users, check with your Helios operator to confirm whether the HT or WB Injector is in use.

1. After incubation in the intercalation solution, thoroughly resuspend the cells by gently vortexing. Count a 10 µL aliquot of the cells, then centrifuge tubes containing cells in intercalation solution at 800 x *g* for 5 min. Carefully aspirate supernatant and resuspend cells in residual volume by gently vortexing.

   NOTE Wash steps after staining may lead to cell loss and therefore a lower cell count than the initial cell count before staining.

2. Wash cells by adding 2 mL of Maxpar Cell Staining Buffer per sample and gently vortex to mix. Centrifuge at 800 x *g* for 5 min, carefully aspirate supernatant, and resuspend cells in residual volume by gently vortexing.

3. Repeat wash by adding 2 mL of Maxpar Cell Staining Buffer per sample and gently vortex to mix.

4. Centrifuge at 800 x *g* for 5 min and carefully aspirate supernatant.

   STOPPING POINT Pelleted samples can be stored in Maxpar Cell Staining Buffer at 2–8 °C on the day of acquisition until ready to acquire samples.

5. Gently vortex to resuspend cells in residual volume. Wash cells by adding 2 mL of the appropriate acquisition reagent per sample, as shown below. Gently vortex to mix, centrifuge at 800 x *g* for 5 min and carefully aspirate supernatant.

Instrument	Acquisition Reagent
Helios (WB Injector)	Maxpar Cell Acquisition Solution
CyTOF 2 or Helios (HT Injector)	Maxpar Water

6. Repeat Step 5 for a total of 2 washes.

   NOTE The second wash is essential to remove residual Maxpar Cell Staining Buffer from samples before acquisition.

7. Prepare a sufficient volume of 0.1X EQ Four Element Calibration Beads to resuspend all samples to the recommended cell concentration for acquisition by diluting 1 part beads to 9 parts acquisition reagent, as shown below:

Instrument	Acquisition Reagent	Cell Concentration
Helios (WB Injector)	Maxpar Cell Acquisition Solution	0.5–1 x 10^6 cells/mL
Helios (HT Injector)	Maxpar Water	0.5–1 x 10^6 cells/mL
CyTOF 2	Maxpar Water	0.25–0.5 x 10^6 cells/mL

8. Gently vortex to resuspend cells in residual volume.

9. Immediately before sample acquisition, resuspend cells in the diluted 0.1X EQ Four Element Calibration Beads and filter sample through appropriately-sized cell strainers (for example, 35–45 µm mesh).

10 Acquire data on Helios (HT or WB Injector) or CyTOF 2.

For CyTOF XT

1 After incubation in the intercalation solution, thoroughly resuspend the cells in the intercalation solution by gently vortexing. Count a 10 µL aliquot of the cells, then centrifuge tubes at 800 x *g* for 5 min.

NOTE Wash steps after staining may lead to cell loss and therefore a lower cell count than the initial cell count before staining.

2 Carefully aspirate supernatant and resuspend cells in residual volume by gently vortexing.

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 supernatant and resuspend cells in residual volume by gently vortexing.

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 Plus for CyTOF XT to each tube and gently vortex. Centrifuge tubes at 800 x *g* for 5 min.

7 Carefully aspirate supernatant and resuspend cells in residual volume by gently vortexing.

8 Repeat wash by adding another 2 mL of Maxpar Cell Acquisition Solution Plus for CyTOF XT to each tube and gently vortex.

NOTE The additional wash with Maxpar Cell Acquisition Solution Plus for CyTOF XT is essential to remove residual Maxpar Cell Staining Buffer from samples.

9 Prepare samples for acquisition according to the sample format:

Sample Format	Sample Preparation Procedure
Pelleted	**1** Filter cells through 35 µm cell strainer cap tubes into new 5 mL polypropylene tubes. **IMPORTANT** Cells must be filtered before CyTOF XT acquisition. **2** Centrifuge tubes at 800 x *g* for 5 min. **3** Carefully aspirate (leaving approximately 100 µL residual volume in the tube) and discard supernatant. **4** Leave cells pelleted at 2–8 °C in the chilled Autosampler carousel until ready to run. Load EQ Six Element Calibration Beads in carousel Location 14 before startup. **NOTE** The instrument operator should keep the samples pelleted when loading them into the Autosampler carousel.

Sample Format	Sample Preparation Procedure
Suspension	**1** Centrifuge tubes at 800 x *g* for 5 min. **2** Carefully aspirate and discard supernatant. **3** Prepare a sufficient volume of 0.1X EQ Six Element Calibration Beads to completely resuspend all samples to a recommended cell concentration of 0.5–1 x 10^6 cells/mL (including approximately 100 µL extra volume per sample), by diluting 1 part beads to 9 parts Maxpar Cell Acquisition Solution Plus for CyTOF XT. **4** Immediately prior to data acquisition, resuspend cells in the diluted 0.1X EQ Six Element Calibration Beads. **5** Filter cells through 35 µm cell strainer cap tubes into new 5 mL polypropylene tubes. For samples larger than 4 mL, use a new 15 mL skirted polypropylene carousel tube. **IMPORTANT** Cells must be filtered before CyTOF XT acquisition.

10 Acquire data on CyTOF XT.

Appendix A: Antibody Cocktail Preparation Guide

The following guide can be used to prepare the separate Maxpar metal-conjugated antibody cocktails in Maxpar Cell Staining Buffer. We recommend preparing 2X antibody cocktails with 10% in excess volume for multiple samples. Prepare the separate antibody cocktails in individual 1.5 mL tubes by first adding Maxpar Cell Staining Buffer and then adding each of the antibodies. Combine 50 µL of the complete 2X antibody cocktail with each 50 µL sample to be stained.

(a) Number of Samples	(d) Volume of Antibody (µL)	(c) Volume of Cell Staining Buffer (µL)	(b) Number of Antibodies																																		
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	1.1		53.9	52.8	51.7	50.6	49.5	48.4	47.3	46.2	45.1	44	42.9	41.8	40.7	39.6	38.5	37.4	36.3	35.2	34.1	33	31.9	30.8	29.7	28.6	27.5	26.4	25.3	24.2	23.1	22	20.9	19.8	18.7	17.6
2	2.2		108	106	103	101	99	96.8	94.6	92.4	90.2	88	85.8	83.6	81.4	79.2	77	74.8	72.6	70.4	68.2	66	63.8	61.6	59.4	57.2	55	52.8	50.6	48.4	46.2	44	41.8	39.6	37.4	35.2
3	3.3		162	158	155	152	149	145	142	139	135	132	129	125	122	119	116	112	109	106	102	99	95.7	92.4	89.1	85.8	82.5	79.2	75.9	72.6	69.3	66	62.7	59.4	56.1	52.8
4	4.4		216	211	207	202	198	194	189	185	180	176	172	167	163	158	154	150	145	141	136	132	128	123	119	114	110	106	101	96.8	92.4	88	83.6	79.2	74.8	70.4
5	5.5		270	264	259	253	248	242	237	231	226	220	215	209	204	198	193	187	182	176	171	165	160	154	149	143	138	132	127	121	116	110	105	99	93.5	88
6	6.6		323	317	310	304	297	290	284	277	271	264	257	251	244	238	231	224	218	211	205	198	191	185	178	172	165	158	152	145	139	132	125	119	112	106
7	7.7		377	370	362	354	347	339	331	323	316	308	300	293	285	277	270	262	254	246	239	231	223	216	208	200	193	185	177	169	162	154	146	139	131	123
8	8.8		431	422	414	405	396	387	378	370	361	352	343	334	326	317	308	299	290	282	273	264	255	246	238	229	220	211	202	194	185	176	167	158	150	141
9	9.9		485	475	465	455	446	436	426	416	406	396	386	376	366	356	347	337	327	317	307	297	287	277	267	257	248	238	228	218	208	198	188	178	168	158
10	11		539	528	517	506	495	484	473	462	451	440	429	418	407	396	385	374	363	352	341	330	319	308	297	286	275	264	253	242	231	220	209	198	187	176
11	12.1		593	581	569	557	545	532	520	508	496	484	472	460	448	436	424	411	399	387	375	363	351	339	327	315	303	290	278	266	254	242	230	218	206	194
12	13.2		647	634	620	607	594	581	568	554	541	528	515	502	488	475	462	449	436	422	409	396	383	370	356	343	330	317	304	290	277	264	251	238	224	211
13	14.3		701	686	672	658	644	629	615	601	586	572	558	543	529	515	501	486	472	458	443	429	415	400	386	372	358	343	329	315	300	286	272	257	243	229
14	15.4		755	739	724	708	693	678	662	647	631	616	601	585	570	554	539	524	508	493	477	462	447	431	416	400	385	370	354	339	323	308	293	277	262	246
15	16.5		809	792	776	759	743	726	710	693	677	660	644	627	611	594	578	561	545	528	512	495	479	462	446	429	413	396	380	363	347	330	314	297	281	264
16	17.6		862	845	827	810	792	774	757	739	722	704	686	669	651	634	616	598	581	563	546	528	510	493	475	458	440	422	405	387	370	352	334	317	299	282
17	18.7		916	898	879	860	842	823	804	785	767	748	729	711	692	673	655	636	617	598	580	561	542	524	505	486	468	449	430	411	393	374	355	337	318	299
18	19.8		970	950	931	911	891	871	851	832	812	792	772	752	733	713	693	673	653	634	614	594	574	554	535	515	495	475	455	436	416	396	376	356	337	317
19	20.9		1024	1003	982	961	941	920	899	878	857	836	815	794	773	752	732	711	690	669	648	627	606	585	564	543	523	502	481	460	439	418	397	376	355	334
20	22		1078	1056	1034	1012	990	968	946	924	902	880	858	836	814	792	770	748	726	704	682	660	638	616	594	572	550	528	506	484	462	440	418	396	374	352
21	23.1		1132	1109	1086	1063	1040	1016	993	970	947	924	901	878	855	832	809	785	762	739	716	693	670	647	624	601	578	554	531	508	485	462	439	416	393	370
22	24.2		1186	1162	1137	1113	1089	1065	1041	1016	992	968	944	920	895	871	847	823	799	774	750	726	702	678	653	629	605	581	557	532	508	484	460	436	411	387
23	25.3		1240	1214	1189	1164	1139	1113	1088	1063	1037	1012	987	961	936	911	886	860	835	810	784	759	734	708	683	658	633	607	582	557	531	506	481	455	430	405
24	26.4		1294	1267	1241	1214	1188	1162	1135	1109	1082	1056	1030	1003	977	950	924	898	871	845	818	792	766	739	713	686	660	634	607	581	554	528	502	475	449	422
25	27.5		1348	1320	1293	1265	1238	1210	1183	1155	1128	1100	1073	1045	1018	990	963	935	908	880	853	825	798	770	743	715	688	660	633	605	578	550	523	495	468	440

To use the table: Locate the row matching the number of samples to be processed (a) and the column for the number of antibodies used to stain the sample (b). Use the table to determine the total volume of Cell Staining Buffer needed (c). Add this volume of Cell Staining Buffer to your master mix tube. Again locate the row matching the number of samples to be processed (a) and in the adjacent column determine the volume per antibody (d). Add the indicated volume of each antibody solution to the master mix tube.

Appendix B: Related Documents

Go to fluidigm.com to download these related documents.

Title	Document Number
Cell-ID 20-Plex Pd Barcoding Kit User Guide	PRD023
Cell-ID Cisplatin Product Information Sheet	PRD018
Cell-ID Cisplatin-194Pt Technical Data Sheet	TDS-00013
Cell-ID Cisplatin-195Pt Technical Data Sheet	TDS-00014
Cell-ID Cisplatin-196Pt Technical Data Sheet	TDS-00015
Cell-ID Cisplatin-198Pt Technical Data Sheet	TDS-00016
Cell-ID Intercalator-Ir Technical Data Sheet	TDS-00703
CyTOF Software v8.0 Help for CyTOF XT (installed with CyTOF Software v8.0)	FLDM-00045
CyTOF XT User Guide	FLDM-00254
Helios User Guide	400250
Maxpar Cell Surface Staining with Fresh Fix Protocol	400276
Maxpar Cytoplasmic/Secreted Antigen Staining with Fresh Fix Protocol	400279
Maxpar Nuclear Antigen Staining with Fresh Fix Protocol	400277

Appendix C: 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 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.

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.

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 techsupport.fluidigm.com.

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