FLUIDIGM®

PRD002 Rev 14

Maxpar Antibody Labeling

User Guide

For Use with Maxpar MCP9 Antibody Labeling Kits and Maxpar X8 Antibody Labeling Kits

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About This Guide

This guide describes how to use the Maxpar® MCP9 Antibody Labeling Kits and Maxpar X8 Antibody Labeling Kits to label antibodies with compatible metal isotopes in order to produce metal-conjugated antibodies intended for use only with Fluidigm mass cytometry systems, with MCP9 cadmium-conjugated antibodies intended for use in suspension mass cytometry **only**. For a summary of the available metal isotopes for each kit, see Select a Maxpar Labeling Kit and Protocol. For detailed instructions on instrument and software operation, refer to the user guide for your system (see Related Documents).

IMPORTANT Before using the kits, 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 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 safety data sheets (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 document may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

Chapter 1: Overview

The Maxpar® Antibody Labeling Kits provide necessary reagents to conjugate antibodies using a specific Maxpar polymer and compatible Maxpar metal isotopes.

Select a Maxpar Labeling Kit and Protocol

Table 1 summarizes the available metal isotopes and labeling method, with the compatible Maxpar labeling kits and recommended protocol to use for each kit. Based on this criteria, select the appropriate Maxpar labeling kit and protocol to use for your experiment.

Table 1. Maxpar Antibody Labeling Kits and Protocols

Metal	Isotopes (Da)	Labeling Method	Maxpar Labeling Kit	Maxpar Labeling Protocol
Cd	106–116	Maxpar MCP9 Polymer	Maxpar MCP9 Antibody Labeling Kits	See Chapter 2
Lanthanide series: Pr Nd Sm Eu Gd Tb Dy Ho Er Tm Yb Lu	 141 142–150 147–154 151–153 155–160 159 161–164 165 166–170 169 171–176 175	Maxpar X8 Polymer	Maxpar X8 Antibody Labeling Kits	See Chapter 3

Chapter 2: Maxpar MCP9 Antibody Labeling Kits

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IMPORTANT Metal-conjugated antibodies produced using the Maxpar® MCP9 Antibody Labeling protocol are intended for use in Fluidigm's suspension mass cytometry **only**.

NOTE This protocol has been optimized for a multitude of immunoglobulin G (IgG) isotypes. This protocol has variable success with IgM antibodies. Each reaction is optimized for labeling 100 µg of antibody with a cadmium (Cd) metal. For information on selecting antibodies for Cd metal labeling, see Before You Begin in this chapter.

Workflow Overview

Figure 1 summarizes the Maxpar MCP9 Antibody Labeling procedure. Figure 2 shows an overview of the workflow and estimated times used in this protocol. For additional labeling procedures in this user guide, see Select a Maxpar Labeling Kit and Protocol.

Loading of the Maxpar metal-chelating polymer (MCP9) with Cd metal solution (Figure 1A) and partial reduction of the antibody (Figure 1B) should be performed simultaneously. **It is imperative, however, not to exceed the recommended reduction time**, and not to allow the partially reduced antibody to remain free of the loaded polymer.

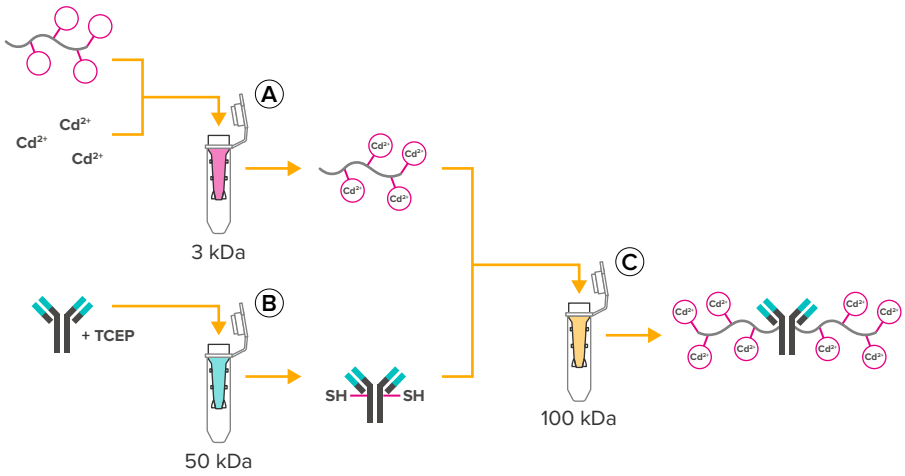

Figure 1. This procedure involves first loading the MCP9 polymer with Cd metal solution (A) and partially reducing the antibody (B), then conjugating the antibody with the Cd-loaded polymer (C).

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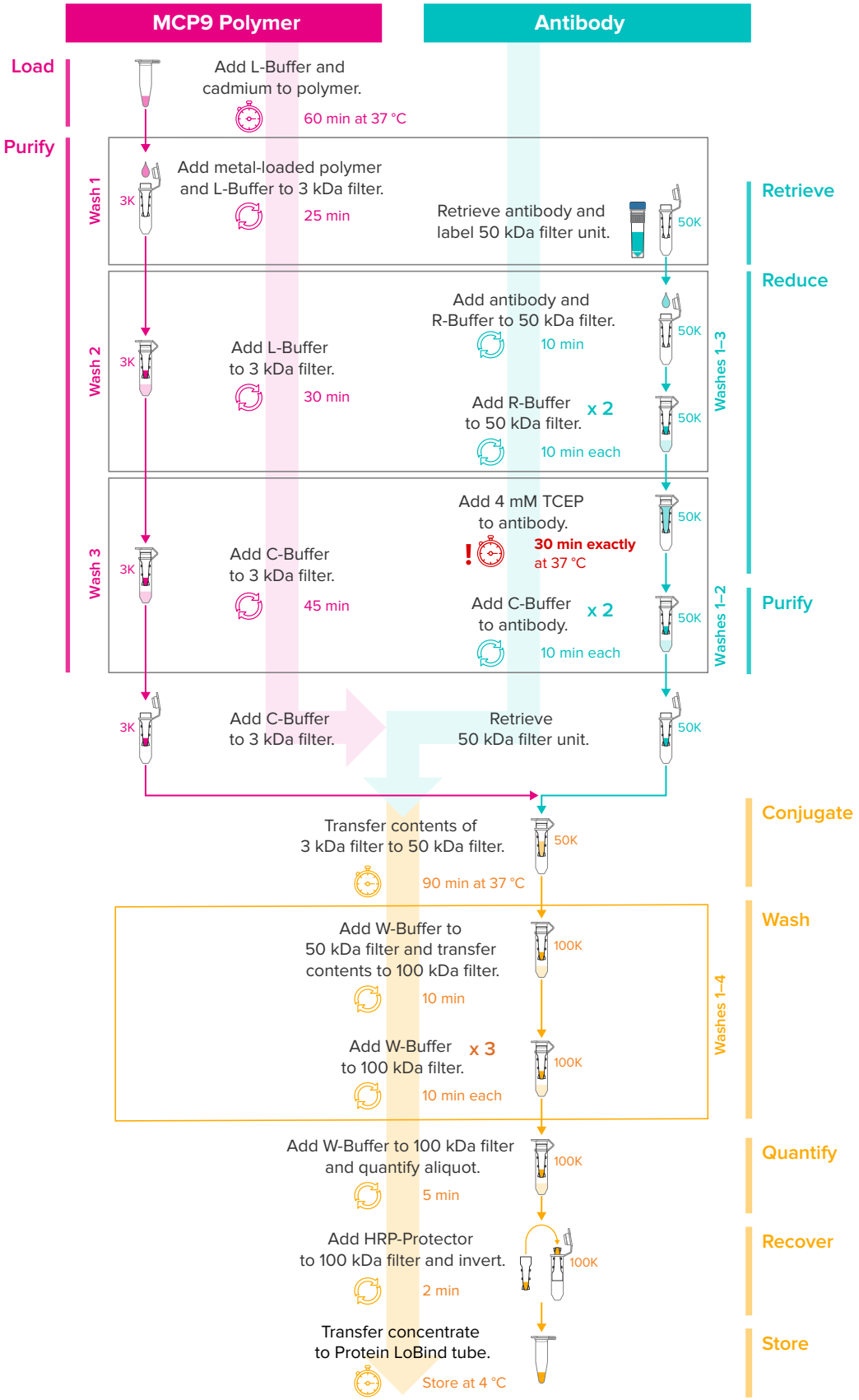

Figure 2. Overview of the Maxpar MCP9 Antibody Labeling procedural workflow

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Materials

Required Reagents

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

Fluidigm Kit Contents

The Maxpar MCP9 Antibody Labeling Kit is available in a four-reaction (4 Rxn) configuration with the following Cd metal isotopes.

Product Name	Cd Metal Isotope	Cat. No. (4 Rxn)
Maxpar MCP9 Antibody Labeling Kit	106Cd	201106A
	110Cd	201110A
	111Cd	201111A
	112Cd	201112A
	113Cd	201113A
	114Cd	201114A
	116Cd	201116A

IMPORTANT The Maxpar MCP9 Antibody Labeling Kit contains reagents with different storage conditions. Store reagents according to Fluidigm recommendations.

The following reagents are included in the Maxpar MCP9 Antibody Labeling Kit (4 Rxn), which provides the necessary reagents to label four antibodies in 100 µg amounts with a specific Cd metal isotope.

Product Name	Cd Metal Isotope	Part No. (4 Rxn)	Storage
Maxpar Cadmium Nitrate—50 mM, 52 µL (4 Rxn kit contains one of the following Cd metal isotopes)	106Cd	S00126	4 °C. Do not freeze.
	110Cd	S00128	
	111Cd	S00130	
	112Cd	S00132	
	113Cd	S00134	
	114Cd	S00136	
	116Cd	S00138	
Maxpar R-Buffer—6 mL (2 bottles)	—	S00001	4 °C. Do not freeze.
Maxpar C-Buffer—5.5 mL (1 bottle)	—	S00003	
Maxpar W-Buffer—8 mL (1 bottle)	—	S00005	
Maxpar L-Buffer—1.4 mL (2 tubes)	—	S00007	
Maxpar MCP9 Polymer—0.4 mg (4 tubes)	—	S00122	**–20 °C** sealed with desiccant

IMPORTANT Maxpar MCP9 Polymer is moisture-sensitive. Upon receipt, immediately store the single-use polymer tubes at –20 °C with provided desiccant and sealed container.

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Required Reagents from Other Suppliers

Product Name	Source	Part Number
Purified IgG antibodies: glycerol-free and carrier-free (no BSA, hydrolyzed protein, or gelatin for stabilization)	Major laboratory supplier (MLS)	—
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) solution, pH 7.0 (10 × 1 mL, 0.5 M)	MilliporeSigma*	646547
HRP-Protector™ peroxidase stabilizer **IMPORTANT** **Do not** supplement with sodium azide after purchase.	Boca Scientific†	222 050 (50 mL) or 222 125 (125 mL)

* Recommended if MilliporeSigma is not available in your location: Merck
† Recommended if Boca Scientific is not available in your location: CANDOR® Bioscience

Required Consumables

Product Name	Source	Part Number
Amicon® Ultra-0.5 Centrifugal Filter Unit, 0.5 mL V-bottom, 8-pack*	MilliporeSigma†	UFC500308 (3 kDa) UFC505008 (50 kDa) UFC510008 (100 kDa)
Eppendorf® Protein LoBind Tubes, 1.5 mL, 100 tubes	Eppendorf	022431081

* Additional pack sizes are available from MilliporeSigma
† Recommended if MilliporeSigma is not available in your location: Merck

Required Equipment

Product Name	Source	Part Number
2 microcentrifuges capable of 12,000 × *g* with fixed angle rotor compatible with 1.5 mL tubes	MLS	—
Mini-centrifuge compatible with 1.5 mL tubes	MLS	—
Water bath capable of 37 ±1.5 °C and compatible with 0.2 mL and 1.5 mL tubes **IMPORTANT** Optionally for polymer loading **only**, you can use a well-calibrated dry heat block if it meets the above criteria. **Do not** use a metal bead bath with this protocol.	MLS	—
Method to assess protein quantity*	MLS	—
Pipettes (P10–P1000) and appropriate aerosol barrier (filter) tips	MLS	—

* Recommended: NanoDrop™ spectrophotometer that measures purified protein by A280 method and IgG sample type option (Thermo Fisher Scientific)

Before You Begin

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Antibody Requirements

- Maxpar MCP9 Antibody Labeling Kits use seven Cd metal isotopes in the 106–116 Da range to label antibodies, and Maxpar X8 Antibody Labeling Kits use lanthanide (Ln) metal isotopes (see Select a Maxpar Labeling Kit and Protocol). Due to the ion optics of the mass cytometer, these lower-mass Cd metal isotopes are detected at a lower relative sensitivity than metal isotopes in the 153–176 Da range. As a result, ideal antibody candidates for Cd labeling should consist of antibody clones with high antigen expression and high antibody sensitivity. For example, human peripheral blood mononuclear cell (PBMC) surface markers such as CD45 (HI30), CD8 (RPA-T8), CD3 (UCHT1), and CD20 (2H7) are suitable targets.

  NOTE The Cd metal isotopes of 106 Da and 110 Da in the Maxpar MCP9 Antibody Labeling Kits are **not** compatible for use with the Cell-ID™ 20-Plex Pd Barcoding Kit (PN 201060) due to direct mass overlap of metal isotopes in the kits.
- To effectively assign targets and Cd metals, contact your local Fluidigm field application specialist.
- Antibodies used with this kit must be purified, glycerol-free, and carrier-free (no BSA, hydrolyzed protein, or gelatin for stabilization).

Expected Results

- The expected yield from Maxpar antibody labeling will vary from clone to clone. For the same antibody clone, the expected yield from Maxpar Cd antibody labeling is typically lower than the expected yield from Maxpar Ln antibody labeling. The average expected recovery with this kit is approximately 50%, as compared to the average expected recovery of 60% with the Maxpar Antibody Labeling Kit for lanthanide metals.
- The use of saponin-based reagents with Cd-labeled antibodies generated by this kit may result in high background and/or nonspecific staining on Helios™ or CyTOF® 2-to-Helios systems. All Fluidigm products containing saponin are tested for compatibility with Cd-labeled antibodies generated by this kit. We recommend that you perform a pilot test with any saponin-based reagents from other suppliers to determine their compatibility with Cd-labeled antibodies generated by this kit.
- For optimal results, titrate the conjugated antibody with appropriate positive and negative controls on the mass cytometry system you will use, and stain the same concentration of cells in the experimental samples that you will use in the final panel (see How to Use Cd-Labeled Antibodies for more information).

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Best Practices

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

- **Do not** perform the Maxpar MCP9 Antibody Labeling protocol for Cd metals at the same time as the Maxpar X8 Antibody Labeling protocol for Ln metals. If you attempt to perform both protocols simultaneously, the differences in materials and procedures between the kits may result in user error or procedural delays that may yield variable or poor results. For ease of use, figures illustrating the polymer ( ), antibody ( ), and conjugated antibody ( ) steps are color-coded in this document (see Figure 1).
- To avoid procedural delays, initially perform only 2 antibody conjugations at a time, and then scale up to no more than 8 conjugations once you are familiar with the protocol.
- Make sure to use the correct polymer for your experiment. The Maxpar MCP9 polymer is for use with cadmium nitrate (Cd metal solution). See Fluidigm Kit Contents in this chapter for available Cd metals.
- MCP9 polymer is moisture-sensitive. Upon receipt, store the single-use polymer tubes at –20 °C with provided desiccant and sealed container.
- The single-use tubes of MCP9 polymer and TCEP solution should be used only once and immediately after thawing to room temperature (RT). Avoid multiple freeze-thaw cycles.
- Retrieve, mix, and centrifuge reagents as directed.
- Use filter tips in all pipetting steps to prevent cross-contamination between metal stocks and reagents.
- The 37 °C incubation reactions in this protocol are temperature-sensitive. **We recommend using a water bath for polymer loading, partial reduction of the antibody, and antibody conjugation.** Before placing tubes in a water bath, make sure all tubes are tightly sealed (for example, with a waterproof sealing film). Optionally for polymer loading **only**, you can use a well-calibrated dry heat block that is compatible with 0.2 mL tubes, but it must have an operational temperature range of 37 ±1.5 °C. If your dry heat block cannot function within this range, use a water bath instead.
- For best results with the Amicon Ultra-0.5 mL Centrifugal Filter Unit (filter device and 1.5 mL collection tube):
  - For every wash step, use a P100 pipette to pipet wash buffers down the inside wall of the filter device and ensure that the pipette tip **does not touch** the delicate filter membrane. For antibody washes, try to minimize the contact of pipette tips with antibody solution in order to increase yield.
  - Make sure to place the flat white filter section so that it faces the cap strap, and place the cap strap so that it faces the center of the centrifuge rotor (see Figure 3).

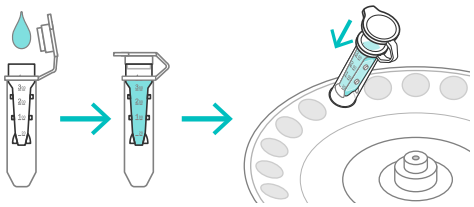

Figure 3. Orientation of the Amicon Ultra-0.5 mL Centrifugal Filter Unit.

  - To avoid damage to the filter unit during centrifugation, check the vertical clearance of an assembled unit before centrifugation.

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Materials to Prepare in Advance

IMPORTANT It is critical to prepare the following materials before starting the Maxpar protocol.

- Centrifuge the stock antibody at 12,000 × *g* for 5 min to sediment antibody aggregates, and then verify the stock antibody concentration by NanoDrop spectrophotometer (or your preferred method) after blanking against the **antibody suspension buffer**. The composition of the buffer can be found on the technical data sheet supplied by the antibody vendor.

  NOTE If the stock antibody volume is >400 µL, pre-concentrate the antibody approximately 10 min before you start the first antibody wash (see Step 14).

- Ensure that the water bath is equilibrated to 37 °C by verifying the instrument reading with a compatible thermometer. Optionally for polymer loading **only**, you can use a dry heat block (see Best Practices for temperature specifications).

- Verify that the centrifuge rotor and diameter settings in the microcentrifuges are correctly set up.

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MCP9 Protocol Steps

IMPORTANT Make sure to read the information in the Before You Begin section in this chapter and familiarize yourself with the entire protocol before proceeding, as there are several incubations and conjugation steps that must be performed in parallel. Times shown are estimates for one conjugation.

MCP9 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
START: **Preload the polymer with cadmium.**	**0:00**	**–**	
1 Retrieve from –20 °C **only** the number of single-use MCP9 polymer tubes that are required for the experiment, thaw to RT before opening to avoid moisture condensation, and then use immediately.			
2 Once thawed to RT, centrifuge the MCP9 polymer tube and the tube containing 50 mM cadmium nitrate (Cd metal solution) for 10 sec in a mini-centrifuge to collect contents at the bottom of each tube. **IMPORTANT** Make sure to use the MCP9 polymer with Cd metal solution. Label the MCP9 polymer tube with the specific Cd metal isotope.	 (10 sec)		
3 Add 87 µL of L-Buffer to the MCP9 polymer tube to resuspend the polymer.			
4 Mix thoroughly by pipetting until the polymer is completely dissolved (approximately 1 min).			
5 Add 13 µL of 50 mM Cd metal solution to the MCP9 polymer tube.			
6 Mix thoroughly by pipetting.			

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MCP9 Polymer Steps	Elapsed Time (hr:min)	Antibody Steps
7 Incubate at 37 °C for 60 min in a water bath or dry heat block. During the polymer incubation, label a new 3 kDa filter unit with the specific Cd metal isotope. **NOTE** The 37 °C incubation reactions in this protocol are temperature-sensitive. We recommend using a water bath (see Best Practices in this chapter).	 (60 min)	–
Perform polymer wash 1. **8** After the 60 min polymer incubation is complete, add 100 µL of L-Buffer to the newly labeled 3 kDa filter unit from Step 7. **9** (Polymer wash 1) Retrieve the metal-loaded polymer mixture from Step 7 and then transfer all contents (approximately 100 µL) to the 3 kDa filter containing L-Buffer. **10** Add 100 µL of L-Buffer to the polymer tube, mix thoroughly by pipetting to wash the sides of the tube, and then transfer all contents (approximately 100 µL) of the wash mixture to the 3 kDa filter. **NOTE** The filter should now contain approximately 300 µL of L-Buffer–metal-loaded polymer solution. **11** Use a P100 pipette to mix thoroughly, being careful not to touch the delicate filter.	**1:05**	
12 **a** Centrifuge at 12,000 × *g* for 25 min at RT. During centrifugation, proceed to Step 12b.	 (25 min)	**1:10**  (1 min) **Retrieve antibody.** **12** **b** During polymer wash 1, retrieve the stock antibody and label a new 50 kDa filter unit with the specific antibody clone. **NOTE** If the stock antibody volume is >400 µL, pre-concentrate the antibody approximately 10 min before you start antibody wash 1 (see Step 14).

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MCP9 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
13 After polymer wash 1 in Step 12a is complete: **a** Aspirate to discard column flow-through from centrifugation of the 3 kDa filter unit in Step 12a. **b** Proceed to Step 14 and start antibody wash 1.	–	–	
		1:35	**Perform antibody wash 1.** **14** (Antibody wash 1) Add 100 µg of stock antibody (up to 400 µL) to the labeled 50 kDa filter from Step 12b. Adjust the volume in the filter to 400 µL with R-Buffer. **NOTE** If the stock antibody volume is >400 µL, centrifuge the 50 kDa filter at 12,000 × *g* for 10 min at RT to pre-concentrate the antibody, and then adjust the volume in the filter to 400 µL with R-Buffer. **15** Centrifuge at 12,000 × *g* for 10 min at RT. During centrifugation, proceed to Step 16 and start polymer wash 2.
		(10 min)	
Perform polymer wash 2. **16** (Polymer wash 2) Add 300 µL of L-Buffer to the 3 kDa filter, and centrifuge at 12,000 × *g* for 30 min at RT. During polymer centrifugation, continue with the remaining antibody washes 2 and 3 (see Step 17).	1:40		
	(30 min)	1:45	**Perform antibody washes 2 and 3.** **IMPORTANT** To ensure that functionally reproducible quantities of Cd-loaded polymer are conjugated to the antibody using this MCP9 protocol, you must perform washes of the purified antibody. **17** Discard column flow-through from centrifugation of the 50 kDa filter unit in Step 15. **NOTE** To discard flow-through, we recommend aspiration or careful decanting to ensure that no flow-through is left in the cap of the tube.

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MCP9 Polymer Steps	Elapsed Time (hr:min)	Antibody Steps
	–	**18** (Antibody wash 2) Add 400 µL of R-Buffer to the filter and centrifuge at 12,000 × *g* for 10 min at RT.
	(10 min)	**19** Discard column flow-through from centrifugation.
		20 (Antibody wash 3) Repeat Steps 18 and 19. During centrifugation, prepare a fresh 4 mM TCEP solution by diluting 8 µL of 0.5 M TCEP stock with 992 µL of R-Buffer.
	(10 min)	**NOTE** For each 100 µg of antibody being labeled, 100 µL of freshly prepared 4 mM TCEP solution is required.
	2:05	**Partially reduce the antibody.**
		21 (Antibody reduction) Add 100 µL of the 4 mM TCEP solution to the antibody in the 50 kDa filter and mix quickly and thoroughly by pipetting.
		22 Immediately incubate at 37 °C in a water bath for **30 min**.
	(30 min)	**IMPORTANT** **Proceed quickly and carefully, and do not exceed 30 min.**
Perform polymer wash 3.	2:10	During the antibody reduction, proceed to Step 23 and start polymer wash 3.
23 (Polymer wash 3) After starting the 30 min antibody reduction in Step 21, perform polymer wash 3:		**NOTE** The 37 °C incubation reactions in this protocol are temperature-sensitive. We recommend using a water bath for partial reduction of the antibody (see Best Practices in this chapter).
a Discard column flow-through from centrifugation of the 3kDa filter unit in Step 16.		
b Add 400 µL of C-Buffer to the 3 kDa filter and centrifuge at 12,000 × *g* for 45 min at RT. During polymer centrifugation, proceed to Step 24 and start to purify the partially reduced antibody.	(45 min)	

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MCP9 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
	–	2:35	**Purify the partially reduced antibody.** **24** (Reduced antibody wash 1) After the 30 min antibody reduction is complete, retrieve the 50 kDa filter containing the partially reduced antibody from the 37 °C water bath (see Step 21). **25** Add 300 µL of C-Buffer to the 50 kDa filter and gently mix by pipetting to carefully wash the antibody.  (10 min) **26** Centrifuge at 12,000 × *g* for 10 min at RT. **NOTE** We recommend using a second microcentrifuge for this step to avoid a timing conflict with polymer wash 3 (see Step 23). **27** Discard column flow-through from centrifugation.  (10 min) **28** (Reduced antibody wash 2) Wash again by adding 400 µL of C-Buffer to the 50 kDa filter and centrifuge at 12,000 × *g* for 10 min at RT. **NOTE** Reduced antibody wash 2 will finish **slightly after** polymer wash 3 (see Step 23).
Retrieve the purified cadmium-loaded polymer. **29** Retrieve 3 kDa filter unit containing the purified Cd-loaded polymer from the centrifuge (see Step 23).	2:55 	2:55 	**Retrieve the purified partially reduced antibody.** **30** Retrieve 50 kDa filter unit containing the purified partially reduced antibody from the centrifuge (see Step 28) and discard column flow-through.

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Elapsed Time (hr:min)	Combined Steps
3:00	**Conjugate the antibody with cadmium-loaded polymer.** **IMPORTANT** Before starting conjugation, verify you have retrieved the correct metal and antibody combination. **31** Using a pipette, resuspend the Cd-loaded polymer from Step 29 (residual volume approximately 20 µL) in 60 µL of C-Buffer (total volume approximately 80 µL). **32** Transfer the resuspended contents to the corresponding partially reduced antibody in the 50 kDa filter from Step 30 (final conjugation volume approximately 100 µL). **33** Mix gently by pipetting.
(90 min)	**34** Incubate at 37 °C for 90 min in a water bath. During the incubation, label a new 100 kDa filter unit with the metal and antibody information. **NOTE** The 37 °C incubation reactions in this protocol are temperature-sensitive. We recommend using a water bath for antibody conjugation (see Best Practices in this chapter).
4:35	**Wash the metal-conjugated antibody.** **35** (Conjugated antibody wash 1) After the incubation is complete, retrieve the 50 kDa filter unit from the water bath, and then add 200 µL of W-Buffer to the 50 kDa filter containing 100 µL antibody conjugation mixture (total volume approximately 300 µL). **36** Mix gently by pipetting, and then transfer contents to the newly labeled 100 kDa filter unit from Step 34. **37** Add another 100 µL of W-Buffer to the 50 kDa filter, mix gently by pipetting to rinse the filter, and then transfer all contents to the 100 kDa filter.
(10 min)	**38** Centrifuge at 5,000 × *g* for 10 min. **NOTE** If an antibody conjugation mixture fails to flow through the filter, verify the correct orientation of the filter in the centrifuge (see Figure 3) and try again, or centrifuge the mixture at 8,000 × *g* for all wash steps.
	39 Discard column flow-through from centrifugation.
(10 min)	**40** (Conjugated antibody washes 2–4) Repeat wash 3 more times with W-Buffer (for a total of 4 washes). **a** Add 400 µL of W-Buffer, centrifuge at 5,000 × *g* for 10 min, and then discard flow-through.
(10 min)	**b** Add 400 µL of W-Buffer, centrifuge at 5,000 × *g* for 10 min, and then discard flow-through.
(10 min)	**c** Add 400 µL of W-Buffer, centrifuge at 5,000 × *g* for 10 min, and then discard flow-through.

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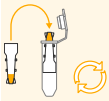
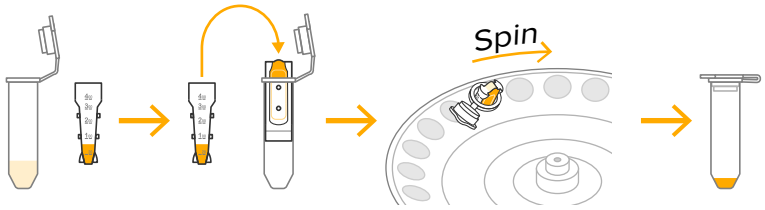
Elapsed Time (hr:min)	Combined Steps
5:20	**Determine yield.** **41** After the final wash with W-buffer, add approximately 75 µL of W-Buffer to the 100 kDa filter to dilute the conjugate (approximate volume of 25 µL) to a total volume of 100 µL. Pipet to mix and carefully rinse the walls of the filter, ensuring that the pipette tip **does not touch** the delicate filter membrane (see Best Practices in this chapter). **42** Quantify the conjugated antibody by using the NanoDrop spectrophotometer (or your preferred method) to measure the absorbance of a 2 µL aliquot at 280 nm against a W-Buffer blank. **NOTE** If using the NanoDrop, make sure to select the Protein A280 module and the IgG sample type option.  **43** Centrifuge the 100 kDa filter at 12,000 × *g* for 5 min to remove the W-Buffer. (5 min)
5:40	**Recover and store the metal-conjugated antibody.** **44** Calculate the volume of HRP-Protector (antibody stabilization buffer **without** sodium azide) required to obtain a final concentration of 0.5 mg/mL of conjugated antibody, or that yields a solution that is at least 50% HRP-Protector by volume. **45** Add the calculated volume of HRP-Protector minus the residual volume (approximately 25 µL) to the 100 kDa filter to obtain a final concentration of 0.5 mg/mL of conjugated antibody. Pipet to mix and carefully rinse the walls of the filter, ensuring that the pipette tip **does not touch** the delicate filter membrane.  **46** Label a new collection tube, invert the 100 kDa filter over to the clean collection tube, and then centrifuge the inverted filter/collection tube assembly at 1,000 × *g* for 2 min (see Figure 4). (2 min)  Figure 4. Invert the filter into a clean collection tube and centrifuge the assembly.  **47** Transfer the conjugated antibody into a new labeled Protein LoBind tube, seal tightly, and store at 4 °C until ready to titrate. **48** Titrate the antibody on the suspension mass cytometry system you will use. **IMPORTANT** Metal-conjugated antibodies produced using the Maxpar MCP9 Antibody Labeling protocol are intended for use in Fluidigm's suspension mass cytometry **only**. For titration guidelines, see How to Use Cd-Labeled Antibodies.  **49** After the conjugated antibody has been titrated, if necessary dilute it to the optimum working concentration in HRP-Protector in a Protein LoBind tube, and then seal tightly and store it at 4 °C.

How to Use Cd-Labeled Antibodies

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For optimal results with Cd-conjugated antibodies, we recommend the following guidelines:

- Add applicable Cd metal isotopes to your CyTOF acquisition template (.tem) prior to acquisition of samples. Refer to the user guide for your system for information on how to add elements to the acquisition template and run samples using CyTOF Software.
- Ensure that the samples used with 116Cd-labeled antibodies do not contain high levels of tin (Sn). Perform a test by acquiring unstained cells to confirm the absence of this environmental contaminant before performing any experiments, including titrations.
- Validate and titrate the conjugated antibody with appropriate positive and negative controls. Stain the same concentration of cells in the experimental samples that you will use in the final panel. We recommend titrating the antibody with relevant positive and negative controls for the experimental system in which the antibody will be used. Set up the antibody titration as follows: 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL, and 0.125 µg/mL.
- When using the Cell-ID 20-Plex Pd Barcoding Kit (PN 201060) for multiplex sample staining with Cd-conjugated antibodies, perform a pilot barcoding experiment to determine the amount of signal spillover into Cd channels due to abundance sensitivity and metal impurity. If there is a negative impact on Cd channels, titrate the barcoding reagent to adjust for this potential signal spillover. See the Cell-ID 20-Plex Pd Barcoding Kit User Guide (PRD023) for information on barcoding samples.

  NOTE The Cd metal isotopes 106Cd and 110Cd in the Maxpar MCP9 Antibody Labeling Kits are not compatible for use with the Cell-ID 20-Plex Pd Barcoding Kit, due to direct mass overlap of metal isotopes in the kits.
- When using the Cell-ID 127 IdU labeling reagent (PN 201127), oxides from 111Cd staining may spillover into the 127I (iodine) channel. Perform a pilot experiment with titrated 111Cd-labeled antibodies to determine the impact of oxide spillover into the 127I channel and compatibility with 111Cd-labeled antibodies. If spillover is observed, titrate the 111Cd antibody to adjust for this potential signal spillover.
- For existing high-parameter antibody panels, ideal antibody candidates for Cd labeling should consist of antibody clones with high antigen expression and high antibody sensitivity. This is due to the ion optics of the mass cytometer, where these lower-mass Cd metal isotopes are detected at a lower relative sensitivity than metal isotopes in the 153–176 Da range. To expand an existing panel with antibodies targeting low expression antigens or lower sensitivity, consider opening up channels in the higher relative sensitivity range by moving existing ideal antibody clones labeled with metals in the 153–176 Da range into the lower-mass Cd metal isotopes.

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

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

166–170 Er

169 Tm

171–176 Yb

175 Lu

Chapter 3: Maxpar X8 Antibody Labeling Kits

IMPORTANT Metal-conjugated antibodies produced using the Maxpar® X8 Antibody Labeling protocol are intended for use in Fluidigm's suspension mass cytometry and Imaging Mass Cytometry™ systems.

NOTE This protocol has been optimized for a multitude of immunoglobulin G (IgG) isotypes, and it works well for affinity-purified polyclonal preparations. This protocol has variable success with IgM antibodies. Each reaction is optimized for labeling 100 µg of antibody with a lanthanide (Ln) metal. For information on selecting antibodies for Ln metal labeling, see Before You Begin in this chapter.

Workflow Overview

Figure 5 summarizes the Maxpar X8 Antibody Labeling procedure. Figure 6 shows an overview of the workflow and estimated times used in this protocol. For additional labeling procedures in this user guide, see Select a Maxpar Labeling Kit and Protocol.

Loading of the Maxpar X8 polymer with Ln metal solution (Figure 5A) and partial reduction of the antibody (Figure 5B) should be performed simultaneously. **It is imperative, however, not to exceed the recommended reduction time**, and not to allow the partially reduced antibody to remain free of the loaded polymer.

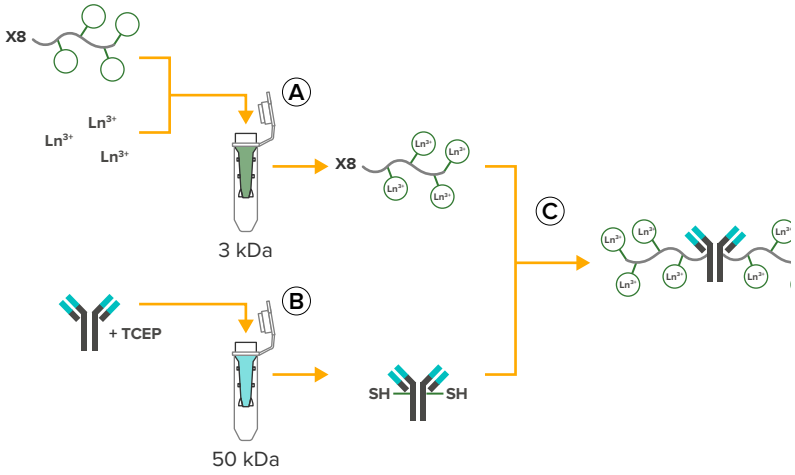


Figure 5. This procedure involves first loading the X8 polymer with Ln metal solution (A) and partially reducing the antibody (B), then conjugating the antibody with the Ln-loaded polymer (C).

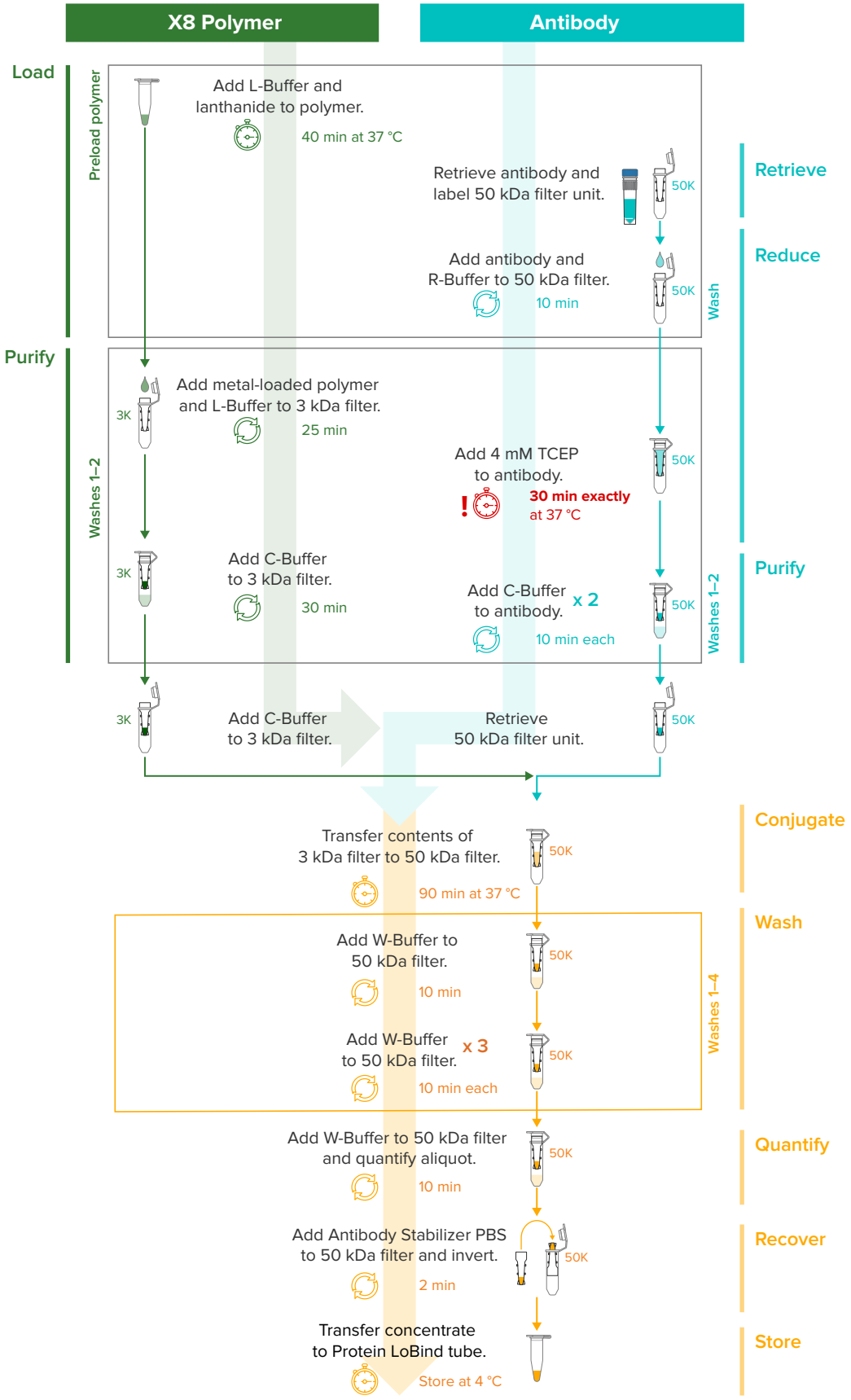


Figure 6. Overview of the Maxpar X8 Antibody Labeling procedural workflow

Use with Isotopes:

141 Pr
142–150 Nd
147–154 Sm
151–153 Eu
155–160 Gd
159 Tb
161–164 Dy
165 Ho
166–170 Er
169 Tm
171–176 Yb
175 Lu

Use with Isotopes:
141 Pr
142–150 Nd
147–154 Sm
151–153 Eu
155–160 Gd
159 Tb
161–164 Dy
165 Ho
166–170 Er
169 Tm
171–176 Yb
175 Lu

Materials

Required Reagents

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

Fluidigm Kit Contents

The Maxpar X8 Antibody Labeling Kit is available in both 4-reaction (4 Rxn) and bundled 40 Rxn configurations with the following Ln metal isotopes. For a list of the bundled 40 Rxn labeling kits and components, see Appendix A.

Table 2. Maxpar X8 Antibody Labeling Kits (4 Rxn)

Ln Metal Isotope	Cat. No. (4 Rxn)	Ln Metal Isotope	Cat. No. (4 Rxn)	Ln Metal Isotope	Cat. No. (4 Rxn)	Ln Metal Isotope	Cat. No. (4 Rxn)
141Pr	201141A	151Eu 153Eu	201151A 201153A	161Dy 162Dy 163Dy 164Dy	201161A 201162A 201163A 201164A	169Tm	201169A
142Nd 143Nd 144Nd 145Nd 146Nd 148Nd 150Nd	201142A 201143A 201144A 201145A 201146A 201148A 201150A	155Gd 156Gd 158Gd 160Gd	201155A 201156A 201158A 201160A	165Ho	201165A	171Yb 172Yb 173Yb 174Yb 176Yb	201171A 201172A 201173A 201174A 201176A
147Sm 149Sm 152Sm 154Sm	201147A 201149A 201152A 201154A	159Tb	201159A	166Er 167Er 168Er 170Er	201166A 201167A 201168A 201170A	175Lu	201175A

IMPORTANT The Maxpar X8 Antibody Labeling Kit contains reagents with different storage conditions. Store reagents according to Fluidigm recommendations.

The following reagents are included in the Maxpar X8 Antibody Labeling Kit (4 Rxn), which provides the necessary reagents to label four antibodies in 100 µg amounts with a specific Ln metal isotope. For a list of the Part Number (Part No.) for the Ln solution in each kit, see Appendix A.

Product Name	Part No. (4 Rxn)	Storage
Maxpar Lanthanide Solution—50 mM, 20 µL	One per kit (see page 35)	4 °C. Do not freeze.
Maxpar R-Buffer—6 mL (1 bottle)	S00001	4 °C. Do not freeze.
Maxpar C-Buffer—5.5 mL (1 bottle)	S00003	
Maxpar W-Buffer—8 mL (1 bottle)	S00005	
Maxpar L-Buffer—1.4 mL (1 tube)	S00007	
Maxpar X8 Polymer—0.1 mg (4 tubes)	S00009	**–20 °C** sealed with desiccant

IMPORTANT Maxpar X8 Polymer is moisture-sensitive. Upon receipt, immediately store the single-use polymer tubes at –20 °C with provided desiccant and sealed container.

Required Reagents from Other Suppliers

Product Name	Source	Part Number
Purified IgG or polyclonal antibodies: glycerol-free and carrier-free (no BSA, hydrolyzed protein, or gelatin for stabilization)	Major laboratory supplier (MLS)	—
Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) solution, pH 7.0 (10 × 1 mL, 0.5 M)	MilliporeSigma*	646547
Antibody Stabilizer PBS **IMPORTANT** Supplement to 0.05% sodium azide after purchase.	Boca Scientific†	131 050 (50 mL) or 131 125 (125 mL)
Sodium azide, BioUltra, ≥99.5% (T)	MilliporeSigma*	71289

* Recommended if MilliporeSigma is not available in your location: Merck
† Recommended if Boca Scientific is not available in your location: CANDOR® Bioscience

Required Consumables

Product Name	Source	Part Number
Amicon Ultra-0.5 Centrifugal Filter Unit, 0.5 mL V-bottom, 8-pack*	MilliporeSigma†	UFC500308 (3 kDa) UFC505008 (50 kDa)
Eppendorf Protein LoBind Tubes, 1.5 mL, 100 tubes	Eppendorf	022431081

* Additional pack sizes are available from MilliporeSigma
† Recommended if MilliporeSigma is not available in your location: Merck

Required Equipment

Product Name	Source	Part Number
2 microcentrifuges capable of 12,000 × *g* with fixed angle rotor compatible with 1.5 mL tubes	MLS	—
Mini-centrifuge compatible with 1.5 mL tubes	MLS	—
Water bath capable of 37 ±1.5 °C and compatible with 0.2 mL and 1.5 mL tubes **IMPORTANT** Optionally for polymer loading **only**, you can use a well-calibrated dry heat block if it meets the above criteria. **Do not** use a metal bead bath with this protocol.	MLS	—
Method to assess protein quantity*	MLS	—
Pipettes (P10–P1000) and appropriate aerosol barrier (filter) tips	MLS	—

* Recommended: NanoDrop spectrophotometer that measures purified protein by A280 method and IgG sample type option (Thermo Fisher Scientific)

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

166–170 Er

169 Tm

171–176 Yb

175 Lu

Before You Begin

Antibody Requirements and Expected Results

- To effectively assign targets and Ln metals, we recommend using Fluidigm's Maxpar Panel Designer, an interactive web-based application that simplifies and optimizes panel design. For more information, contact your local Fluidigm field application specialist.
- Antibodies used with this kit must be purified, glycerol-free, and carrier-free (no BSA, hydrolyzed protein, or gelatin for stabilization).
- For optimal results, titrate the conjugated antibody with appropriate positive and negative controls on the mass cytometry system that you will use, and stain the same concentration of cells in the experimental samples that you will use in the final panel (see How to Use Ln-Labeled Antibodies for more information).

Best Practices

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

- **Do not** perform the Maxpar X8 Antibody Labeling protocol for Ln metals at the same time as the Maxpar MCP9 Antibody Labeling protocol for Cd metals. If you attempt to perform both protocols simultaneously, the differences in materials and procedures between the kits may result in user error or procedural delays that may yield variable or poor results. For ease of use, figures illustrating the polymer ( ), antibody ( ), and conjugated antibody ( ) steps are color-coded in this document (see Figure 5).
- To avoid procedural delays, initially perform only 2 antibody conjugations at a time, and then scale up to no more than 8 conjugations once you are familiar with the protocol.
- Make sure to use the correct polymer for your experiment. The Maxpar X8 polymer is for use with Ln metal solution. See Fluidigm Kit Contents in this chapter for available Ln metals.
- X8 polymer is moisture-sensitive. Upon receipt, store the single-use polymer tubes at –20 °C with provided desiccant and sealed container.
- The single-use tubes of X8 polymer and TCEP solution should be used only once and immediately after thawing to room temperature (RT). Avoid multiple freeze-thaw cycles.
- Retrieve, mix, and centrifuge reagents as directed.
- Use filter tips in all pipetting steps to prevent cross-contamination between metal stocks and reagents.
- The 37 °C incubation reactions in this protocol are temperature-sensitive. **We recommend using a water bath for polymer loading, partial reduction of the antibody, and antibody conjugation.** Before placing tubes in a water bath, make sure all tubes are tightly sealed (for example, with a waterproof sealing film). Optionally for polymer loading **only**, you can use a well-calibrated dry heat block that is compatible with 0.2 mL tubes, but it must have an operational temperature range of 37 ±1.5 °C. If your dry heat block cannot function within this range, use a water bath instead.

- For best results with the Amicon Ultra-0.5 mL Centrifugal Filter Unit (filter device and 1.5 mL collection tube):
  - For every wash step, use a P100 pipette to pipet wash buffers down the inside wall of the filter device and ensure that the pipette tip **does not touch** the delicate filter membrane. For antibody washes, try to minimize the contact of pipette tips with antibody solution in order to increase yield.
  - Make sure to place the flat white filter section so that it faces the cap strap, and place the cap strap so that it faces the center of the centrifuge rotor (see Figure 7).

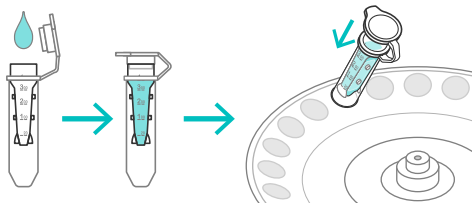


Figure 7. Orientation of the Amicon Ultra-0.5 mL Centrifugal Filter Unit

  - To avoid damage to the filter unit during centrifugation, check the vertical clearance of an assembled unit before centrifugation.

Materials to Prepare in Advance

IMPORTANT It is critical to prepare the following materials before starting the Maxpar protocol.

- Centrifuge the stock antibody at 12,000 × *g* for 5 min to sediment antibody aggregates, and then verify the stock antibody concentration by NanoDrop spectrophotometer (or your preferred method) after blanking against the antibody suspension buffer. The composition of the buffer can be found on the technical data sheet supplied by the antibody vendor.

  NOTE If the stock antibody volume is >400 µL, start to pre-concentrate the antibody approximately 10 min before you start antibody wash 1 (see Step 8).

- Ensure that the water bath is equilibrated to 37 °C by verifying the instrument reading with a compatible thermometer. Optionally for polymer loading **only**, you can use a dry heat block (see Best Practices in this chapter for temperature specifications).
- Verify the centrifuge rotor and diameter settings in the microcentrifuges are correctly set up.

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

166–170 Er

169 Tm

171–176 Yb

175 Lu

X8 Protocol Steps

IMPORTANT Make sure to read the information in the Before You Begin section in this chapter and familiarize yourself with the entire protocol before proceeding, as there are several incubations and conjugation steps that must be performed in parallel. Times shown are estimates for one conjugation.

X8 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
START: **Preload the polymer with lanthanide.**	**0:00**	**–**	
1 Retrieve from –20 °C **only** the number of single-use X8 polymer tubes that are required for the experiment, thaw to RT before opening to avoid moisture condensation, and then use immediately.			
2 Once thawed to RT, centrifuge the X8 polymer tube and the tube containing 50 mM Ln metal chloride solution for 10 sec in a mini-centrifuge to collect contents at the bottom of each tube. **IMPORTANT** Make sure to use the X8 polymer with Ln metal solution. Label the X8 polymer tube with the specific Ln metal isotope.	 (10 sec)		
3 Add 95 µL of L-Buffer to the X8 polymer tube to resuspend the polymer.			
4 Mix thoroughly by pipetting until the polymer is completely dissolved (approximately 1 min).			
5 Add 5 µL of 50 mM Ln metal solution to the X8 polymer tube.			
6 Mix thoroughly by pipetting.			

X8 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
7 Incubate at 37 °C for 40 min in a water bath or dry heat block. **a** During the polymer incubation, label a new 3 kDa filter unit. **b** Approximately **30 min** after starting the polymer incubation, proceed to Step 8 and start antibody wash 1. **NOTE** the following: • The 37 °C incubation reactions in this protocol are temperature-sensitive. We recommend using a water bath (see Best Practices in this chapter). • If the stock antibody volume is >400 µL, start to pre-concentrate the antibody approximately 10 min before you start antibody wash 1 (see Step 8).	 (40 min)	—	
		0:35	**Perform antibody wash 1.**
		 (1 min)	**8** Retrieve the antibody and label a new 50 kDa filter unit with the specific antibody clone. **9** (Antibody wash 1) Add 100 µg of stock antibody (up to 400 µL) to the labeled 50 kDa filter. Adjust the volume in the filter to 400 µL with R-Buffer. **NOTE** If the stock antibody volume is >400 µL, centrifuge the 50 kDa filter at 12,000 × *g* for 10 min at RT to pre-concentrate the antibody, and then adjust the volume in the filter to 400 µL with R-Buffer.
		 (10 min)	**10** Centrifuge at 12,000 × *g* for 10 min at RT. During centrifugation: **a** Prepare a fresh 4 mM TCEP solution by diluting 8 µL of 0.5 M TCEP stock with 992 µL of R-Buffer. **NOTE** For each 100 µg of antibody being labeled, 100 µL of freshly prepared 4 mM TCEP solution is required. **b** Proceed to Step 11 and start polymer wash 1.

Use with Isotopes:

141 Pr
142–150 Nd
147–154 Sm
151–153 Eu
155–160 Gd
159 Tb
161–164 Dy
165 Ho
166–170 Er
169 Tm
171–176 Yb
175 Lu

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

166–170 Er

169 Tm

171–176 Yb

175 Lu

X8 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
Perform polymer wash 1.	0:45		
11 After the 40 min polymer incubation is complete, add 200 µL of L-Buffer to the newly labeled 3 kDa filter unit from Step 7a. **12** (Polymer wash 1) Retrieve the metal-loaded polymer mixture from Step 7a and transfer all contents (approximately 100 µL) to the 3 kDa filter containing L-Buffer. **NOTE** The filter should now contain approximately 300 µL of L-Buffer–metal-loaded polymer solution. **13** Use a P100 pipette to mix thoroughly, being careful not to touch the delicate filter. **14** Centrifuge at 12,000 × *g* for 25 min at RT. During polymer centrifugation, proceed to Step 15 and start to partially reduce the antibody.	 (25 min)	0:50	**Partially reduce the antibody.** **15** Discard column flow-through from centrifugation of the 50 kDa filter unit (see Step 10). **NOTE** To discard flow-through, we recommend aspiration or careful decanting to ensure that no flow-through is left in the cap of the tube. **16** (Antibody reduction) Add 100 µL of the 4 mM TCEP solution to the antibody in the filter and mix quickly and thoroughly by pipetting.  (30 min) **17** Immediately incubate at 37 °C in a water bath for **30 min**. **IMPORTANT Proceed quickly and carefully, and do not exceed 30 min.** During antibody reduction, proceed to Step 18 and start polymer wash 2. **NOTE** The 37 °C incubation reactions in this protocol are temperature-sensitive. We recommend using a water bath for partial reduction of the antibody (see Best Practices in this chapter).

X8 Polymer Steps	Elapsed Time (hr:min)		Antibody Steps
Perform polymer wash 2. **18** (Polymer wash 2) After starting the 30 min antibody reduction in Step 17, perform polymer wash 2: **a** Aspirate to discard flow-through from centrifugation of the 3 kDa filter unit in Step 14. **b** Add 400 µL of C-Buffer to the 3 kDa filter and centrifuge at 12,000 × *g* for 30 min at RT. During polymer centrifugation, proceed to Step 19 and start to purify the partially reduced antibody. **NOTE** Polymer wash 2 will finish **slightly before** reduced antibody wash 2 (see Step 23).	1:15 (30 min)	–	
		1:20	**Purify the partially reduced antibody.** **19** (Reduced antibody wash 1) After the 30 min antibody reduction is complete, retrieve the 50 kDa filter containing the partially reduced antibody from the 37 °C water bath (see Step 17). **20** Add 300 µL of C-Buffer to the 50 kDa filter and gently mix by pipetting to carefully wash the antibody. **21** Centrifuge at 12,000 × *g* for 10 min at RT. (10 min) **22** Discard column flow-through from centrifugation. **23** (Reduced antibody wash 2) Wash again by adding 400 µL of C-Buffer to the 50 kDa filter and centrifuge at 12,000 × *g* for 10 min at RT. (10 min) **NOTE** Reduced antibody wash 2 will finish **slightly after** polymer wash 2 (see Step 18).
Retrieve the purified lanthanide-loaded polymer. **24** Retrieve 3 kDa filter unit containing the purified Ln-loaded polymer from the centrifuge (see Step 18).	1:45	1:45	**Retrieve the purified partially reduced antibody.** **25** Retrieve 50 kDa filter unit containing the purified partially reduced antibody from the centrifuge (see Step 21) and discard column flow-through.

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

166–170 Er

169 Tm

171–176 Yb

175 Lu

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

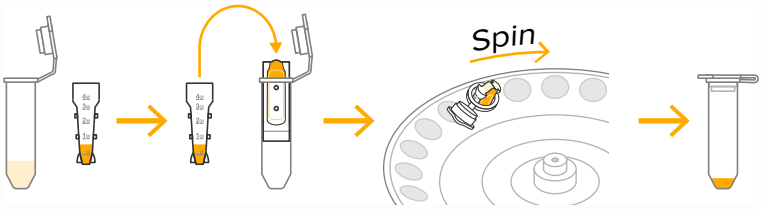
166–170 Er

169 Tm

171–176 Yb

175 Lu

Elapsed Time (hr:min)	Combined Steps
1:55	**Conjugate the antibody with lanthanide-loaded polymer.** **IMPORTANT** Before starting conjugation, verify you have retrieved the correct metal and antibody combination. **26** Using a pipette, resuspend the Ln-loaded polymer from Step 24 (residual volume approximately 20 µL) in 60 µL of C-Buffer (total volume approximately 80 µL). **27** Transfer the resuspended contents to the corresponding partially reduced antibody in the 50 kDa filter from Step 25 (final conjugation volume approximately 100 µL). **28** Mix gently by pipetting. **29** Incubate at 37 °C for 90 min in a water bath. **NOTE** The 37 °C incubation reactions in this protocol are temperature-sensitive. We recommend using a water bath for antibody conjugation (see Best Practices in this chapter).
 (90 min)	
3:30	**Wash the metal-conjugated antibody.** **30** (Conjugated antibody wash 1) After the incubation is complete, retrieve the 50 kDa filter unit from the water bath, and then add 200 µL of W-Buffer to the 50 kDa filter containing 100 µL antibody conjugation mixture (total volume approximately 300 µL). **31** Mix gently by pipetting, and then centrifuge at 12,000 × *g* for 10 min. **32** Discard column flow-through from centrifugation. **33** (Conjugated antibody washes 2–4) Repeat wash 3 more times with W-Buffer (for a total of 4 washes). **a** Add 400 µL of W-Buffer, centrifuge at 12,000 × *g* for 10 min, and then discard flow-through. **b** Add 400 µL of W-Buffer, centrifuge at 12,000 × *g* for 10 min, and then discard flow-through. **c** Add 400 µL of W-Buffer, mix by pipetting, and centrifuge at 12,000 × *g* for 10 min, and then discard flow-through.
 (10 min)	
 (10 min)	
 (10 min)	
 (10 min)	

Elapsed Time (hr:min)	Combined Steps
4:15	**Determine yield.**
	34 After the final wash with W-buffer, add approximately 80 µL of W-buffer to the 50 kDa filter to dilute the conjugate (approximate volume of 20 µL) to a total volume of 100 µL. Pipet to mix and carefully rinse the walls of the filter, ensuring that the pipette tip **does not touch** the delicate filter membrane (see Best Practices in this chapter).
	35 Quantify the conjugated antibody by using the NanoDrop spectrophotometer (or your preferred method) to measure the absorbance of a 2 µL aliquot at 280 nm against a W-Buffer blank (expected recovery is ≥60%).
	NOTE If using the NanoDrop, make sure to select the Protein A280 module and the IgG sample type option.
(10 min)	**36** Centrifuge the 50 kDa filter at 12,000 × *g* for 10 min to remove the W-Buffer.
4:30	**Recover and store the metal-conjugated antibody.**
	37 Calculate the volume of Antibody Stabilizer PBS (supplemented to 0.05% sodium azide after purchase) required to obtain a final concentration of 0.5 mg/mL of conjugated antibody, or that yields a solution that is at least 50% Antibody Stabilizer PBS by volume.
	38 Add the calculated volume of Antibody Stabilizer PBS (supplemented to 0.05% sodium azide after purchase) minus the residual volume (approximately 20 µL) to the 50 kDa filter to obtain a final concentration of 0.5 mg/mL of conjugated antibody. Pipet to mix and carefully rinse the walls of the filter, ensuring that the pipette tip **does not touch** the delicate filter membrane.
(2 min)	**39** Label a new collection tube, invert the 50 kDa filter over to the clean collection tube, and then centrifuge the inverted filter/collection tube assembly at 1,000 × *g* for 2 min (see Figure 8).
	
	Figure 8. Invert the filter into a clean collection tube and centrifuge the assembly.
	40 Transfer the conjugated antibody into a new labeled Protein LoBind tube, seal tightly, and store at 4 °C until ready to titrate.
	41 Titrate the antibody on the suspension or Imaging Mass Cytometry system you will use.
	IMPORTANT Metal-conjugated antibodies produced using the Maxpar X8 Antibody Labeling protocol are intended for use in Fluidigm's suspension mass cytometry or Imaging Mass Cytometry systems. For titration guidelines, see How to Use Ln-Labeled Antibodies.
	42 After the conjugated antibody has been titrated, if necessary dilute it to the optimum working concentration in Antibody Stabilizer PBS in a Protein LoBind tube, and then seal tightly and store it at 4 °C.

Use with Isotopes:
141 Pr
142–150 Nd
147–154 Sm
151–153 Eu
155–160 Gd
159 Tb
161–164 Dy
165 Ho
166–170 Er
169 Tm
171–176 Yb
175 Lu

Use with Isotopes:

141 Pr

142–150 Nd

147–154 Sm

151–153 Eu

155–160 Gd

159 Tb

161–164 Dy

165 Ho

166–170 Er

169 Tm

171–176 Yb

175 Lu

How to Use Ln-Labeled Antibodies

For optimal results with Ln-conjugated antibodies, we recommend the following guidelines:

- Ensure that the applicable Ln metal isotopes are included in your acquisition template prior to acquisition of samples. Refer to the user guide for your system for information on how to add elements to the acquisition template and run samples using CyTOF® Software (see Related Documents).
- For suspension mass cytometry experiments: Validate and titrate the conjugated antibody with appropriate positive and negative controls. Stain the same concentration of cells in the experimental samples that you will use in the final panel. We recommend titrating the antibody with relevant positive and negative controls for the experimental system in which the antibody will be used. Set up the antibody titration as follows: 8 µg/mL, 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/mL, 0.25 µg/mL, and 0.125 µg/mL.
- For Imaging Mass Cytometry experiments: Optimize each antibody concentration by performing a titration series on the relevant tissue type. We recommend setting up the serial dilution based on absolute concentration of conjugated antibodies. For example, set up the antibody titration as follows: 10 µg/mL, 3.3 µg/mL, 1.7 µg/mL. Refer to the Fluidigm technical data sheets for recommended starting concentrations.

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

Appendix A: Kit Contents

Maxpar X8 Antibody Labeling Kit (4 Rxn)

Maxpar Lanthanide Solution (4 Rxn)

Product Name	Ln Metal Isotope	Part No. (4 Rxn)	Ln Metal Isotope	Part No. (4 Rxn)	Ln Metal Isotope	Part No. (4 Rxn)	Ln Metal Isotope	Part No. (4 Rxn)
Maxpar® Lanthanide Solution—50 mM, 20 µL (4 Rxn kit contains one of the following Ln metal isotopes)	141Pr	S00014	151Eu 153Eu	S00024 S00026	161Dy 162Dy 163Dy 164Dy	S00102 S00032 S00104 S00033	169Tm	S00038
	142Nd 143Nd 144Nd 145Nd 146Nd 148Nd 150Nd	S00015 S00016 S00017 S00018 S00019 S00021 S00023	155Gd 156Gd 158Gd 160Gd	S00098 S00028 S00029 S00031	165Ho	S00034	171Yb 172Yb 173Yb 174Yb 176Yb	S00040 S00041 S00106 S00042 S00044
	147Sm 149Sm 152Sm 154Sm	S00020 S00022 S00025 S00027	159Tb	S00030	166Er 167Er 168Er 170Er	S00035 S00036 S00037 S00039	175Lu	S00043

Maxpar X8 Antibody Labeling Kit (40 Rxn)

Available Bundled Kits (40 Rxn)

Table 3. Maxpar X8 Antibody Labeling Kits (40 Rxn)

Ln Metal Isotope	Cat. No. (40 Rxn)	Ln Metal Isotope	Cat. No. (40 Rxn)	Ln Metal Isotope	Cat. No. (40 Rxn)	Ln Metal Isotope	Cat. No. (40 Rxn)
141Pr	201141B	151Eu 153Eu	201151B 201153B	161Dy 162Dy 163Dy 164Dy	201161B 201162B 201163B 201164B	169Tm	201169B
142Nd 143Nd 144Nd 145Nd 146Nd 148Nd 150Nd	201142B 201143B 201144B 201145B 201146B 201148B 201150B	155Gd 156Gd 158Gd 160Gd	201155B 201156B 201158B 201160B	165Ho	201165B	171Yb 172Yb 173Yb 174Yb 176Yb	201171B 201172B 201173B 201174B 201176B
147Sm 149Sm 152Sm 154Sm	201147B 201149B 201152B 201154B	159Tb	201159B	166Er 167Er 168Er 170Er	201166B 201167B 201168B 201170B	175Lu	201175B

Bundled Kit Components (40 Rxn)

IMPORTANT The Maxpar X8 Antibody Labeling Kit contains reagents with different storage conditions. Store reagents according to Fluidigm recommendations.

Product Name	Part No. (40 Rxn)	Storage
Maxpar® Lanthanide Solution—50 mM, 200 µL	One per kit (see page 37)	4 °C. Do not freeze.
Maxpar R-Buffer—60 mL (1 bottle)	S00002	4 °C. Do not freeze.
Maxpar C-Buffer—55 mL (1 bottle)	S00004	
Maxpar W-Buffer—80 mL (1 bottle)	S00006	
Maxpar L-Buffer—14 mL (1 bottle)	S00008	
Maxpar X8 Polymer—0.1 mg (10 pack of 4 tubes)	S00009	**–20 °C** sealed with desiccant

IMPORTANT Maxpar X8 Polymer is moisture-sensitive. Upon receipt, immediately store the single-use polymer tubes at –20 °C with provided desiccant and sealed container.

Maxpar Lanthanide Solution (40 Rxn)

Product Name	Ln Metal Isotope	Part No. (40 Rxn)	Ln Metal Isotope	Part No. (40 Rxn)	Ln Metal Isotope	Part No. (40 Rxn)	Ln Metal Isotope	Part No. (40 Rxn)
Maxpar® Lanthanide Solution—50 mM, 200 µL (40 Rxn kit contains one of the following Ln metal isotopes)	141Pr	S00049	151Eu 153Eu	S00059 S00061	161Dy 162Dy 163Dy 164Dy	S00103 S00067 S00105 S00068	169Tm	S00073
	142Nd 143Nd 144Nd 145Nd 146Nd 148Nd 150Nd	S00050 S00051 S00052 S00053 S00054 S00056 S00058	155Gd 156Gd 158Gd 160Gd	S00099 S00063 S00064 S00066	165Ho	S00069	171Yb 172Yb 173Yb 174Yb 176Yb	S00075 S00076 S00107 S00077 S00079
	147Sm 149Sm 152Sm 154Sm	S00055 S00057 S00060 S00062	159Tb	S00065	166Er 167Er 168Er 170Er	S00070 S00071 S00072 S00074	175Lu	S00078

Appendix B: Related Documents

Go to fluidigm.com to download these related documents.

Title	Document Number
Helios™ User Guide	400250
Hyperion™ Imaging System User Guide	400311
CyTOF® 2 Mass Cytometer User Manual	400200
Cell-ID™ 20-Plex Pd Barcoding Kit User Guide	PRD023
Maxpar® X8 Antibody Labeling Quick Reference	FLDM-00015
Maxpar MCP9 Antibody Labeling Quick Reference	FLDM-00016
Maxpar X8 Antibody Labeling Kits (4 Rxn) Product Information Sheet	FLDM-00017
Maxpar X8 Antibody Labeling Kits (40 Rxn) Product Information Sheet	FLDM-00029
Maxpar MCP9 Antibody Labeling Kits (4 Rxn) Product Information Sheet	FLDM-00018
Maxpar Panel Designer User Guide	100-9557

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 latex-free 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 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.

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