

Maxpar MCP9 Antibody Labeling

IMPORTANT Before using the Maxpar® MCP9 Antibody Labeling Kits (Cat. Nos. 201106A, 201110A–201114A, and 201116A), read and understand the detailed instructions and safety guidelines in the Maxpar Antibody Labeling User Guide (PRD002).

MCP9 Workflow Overview

Times shown are estimates for one conjugation. Actual times may vary.


Elapsed Time (hr:min)	Workflow Step
0:00	Preload the polymer with cadmium.
1:05	Perform at same time: <ul style="list-style-type: none"> Perform polymer wash 1. Retrieve antibody.
1:35	Perform at same time: <ul style="list-style-type: none"> Perform polymer washes 2–3. Perform antibody washes 1–3, partially reduce the antibody, and purify the partially reduced antibody.
3:00	Conjugate the antibody with cadmium-loaded polymer.
4:35	Wash the metal-conjugated antibody.
5:20	Determine yield.
5:40	Recover and store the metal-conjugated antibody.






Before You Begin




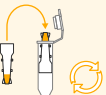

- Do not** perform the Maxpar MCP9 Antibody Labeling protocol for cadmium (Cd) metals at the same time as the Maxpar X8 Antibody Labeling protocol for lanthanide (Ln) metals.
- To avoid procedural delays, initially perform only 2 antibody conjugations at a time, and then scale up to no more than 8 conjugations 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 Maxpar Cadmium Nitrate (Cd metal solution).
- Retrieve, mix, and centrifuge reagents as directed.
- Use filter tips in all pipetting steps to prevent cross-contamination between metal stocks and reagents.
- 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 after blanking against the **antibody suspension buffer**.
- The 37 °C incubation reactions 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).
- For every wash step, use a P100 pipette to pipet wash buffers down the inside wall of the Amicon® Ultra-0.5 Centrifugal Filter Unit, 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.
- To discard column flow-through from centrifugation of Amicon filter units, aspirate or carefully decant to ensure that no flow-through is left in the cap of the Amicon collection tube.

MCP9 Protocol Steps

IMPORTANT Make sure to read the information in [Before You Begin](#) and familiarize yourself with the entire protocol before proceeding, as there are several incubations and conjugation steps that must be performed in parallel.

MCP9 Polymer Steps	Elapsed Time (hr:mm)	Antibody Steps
<p>START: Preload the polymer with cadmium.</p> <p>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.</p> <p>2 Centrifuge the MCP9 polymer tube and the tube containing 50 mM cadmium nitrate (Cd metal solution) for 10 sec in a mini-centrifuge.</p> <p>IMPORTANT Make sure to use the MCP9 polymer with Cd metal solution. Label the MCP9 polymer tube with the specific Cd metal isotope.</p>	<p>0:00</p> <p>–</p> <p> (10 sec)</p>	

MCP9 Polymer Steps	Elapsed Time (hr:mm)	Antibody Steps
<p>□ 3 Add 87 µL of L-Buffer to the MCP9 polymer tube.</p> <p>□ 4 Mix thoroughly by pipetting until the polymer is completely dissolved (approximately 1 min).</p> <p>□ 5 Add 13 µL of 50 mM Cd metal solution to the MCP9 polymer tube.</p> <p>□ 6 Mix thoroughly by pipetting.</p> <p>□ 7 Incubate at 37 °C for 60 min in a water bath. During the polymer incubation, label a new 3 kDa filter unit with the specific Cd metal isotope.</p>	<p>–</p> <p>–</p> <p> (60 min)</p>	
<p>Perform polymer wash 1.</p> <p>□ 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.</p> <p>□ 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.</p> <p>□ 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.</p> <p>□ 11 Use a P100 pipette to mix thoroughly, being careful not to touch the delicate filter.</p> <p>□ 12 a Centrifuge at 12,000 × g for 25 min at RT. During centrifugation, proceed to Step 12b.</p> <p>□ 13 After polymer wash 1 in Step 12a is complete:</p> <p>a Aspirate to discard column flow-through from centrifugation of the 3 kDa filter unit in Step 12a.</p> <p>b Proceed to Step 14 and start antibody wash 1.</p>	<p>1:05</p> <p> (25 min)</p>	<p>1:10 Retrieve antibody.</p> <p>□ 12 b During polymer wash 1, retrieve the stock antibody and label a new 50 kDa filter unit with the specific antibody clone.</p> <p> (1 min)</p>
<p>Perform polymer wash 2.</p> <p>□ 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).</p>	<p>1:40</p> <p> (30 min)</p>	<p>1:35 Perform antibody wash 1.</p> <p>□ 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.</p> <p>□ 15 Centrifuge at 12,000 × g for 10 min at RT. During centrifugation, proceed to Step 16 and start polymer wash 2.</p> <p> (10 min)</p>
		<p>1:45 Perform antibody washes 2 and 3.</p> <p>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.</p> <p>□ 17 Discard column flow-through from centrifugation of the 50 kDa filter unit in Step 15.</p>

Elapsed Time (hr:min)	Combined Steps
4:35	<p>Wash the metal-conjugated antibody.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 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. <input type="checkbox"/> 36 Mix gently by pipetting, and then transfer contents to the newly labeled 100 kDa filter unit from Step 34. <input type="checkbox"/> 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. <input type="checkbox"/> 38 Centrifuge at 5,000 \times g for 10 min. <input type="checkbox"/> 39 Discard column flow-through from centrifugation. <input type="checkbox"/> 40 (Conjugated antibody washes 2–4) Repeat wash 3 more times: add 400 μL of W-Buffer, centrifuge at 5,000 \times g for 10 min, and then discard flow-through. <ul style="list-style-type: none"> <input type="checkbox"/> Wash 2 <input type="checkbox"/> Wash 3 <input type="checkbox"/> Wash 4
 (10 min)  (10 min each \times 3)	
5:20	<p>Determine yield.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 41 After the final wash with W-buffer, add approximately 75 μL of W-Buffer to the 100 kDa filter to dilute the conjugate to a total volume of 100 μL. Pipette to mix and carefully rinse the walls of the filter, ensuring that the pipette tip does not touch the delicate filter membrane. <input type="checkbox"/> 42 Quantify the conjugated antibody by using the NanoDrop spectrophotometer to measure the absorbance of a 2 μL aliquot at 280 nm against a W-Buffer blank. <input type="checkbox"/> 43 Centrifuge the 100 kDa filter at 12,000 \times g for 5 min to remove the W-Buffer.
 (5 min)	
5:40	<p>Recover and store the metal-conjugated antibody.</p> <ul style="list-style-type: none"> <input type="checkbox"/> 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 which yields a solution that is at least 50% HRP Protector™ peroxidase stabilizer by volume. <input type="checkbox"/> 45 Add the calculated volume of HRP-Protector minus the residual volume to the 100 kDa filter to obtain a final concentration of 0.5 mg/mL of conjugated antibody. Pipette to mix and carefully rinse the walls of the filter, ensuring that the pipette tip does not touch the delicate filter membrane. <input type="checkbox"/> 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 \times g for 2 min. <input type="checkbox"/> 47 Transfer the conjugated antibody into a new labeled Eppendorf® Protein LoBind Tube, seal tightly, and store at 4 °C until ready to titrate. <input type="checkbox"/> 48 Titrate the antibody on the suspension mass cytometry system you will use. <p>IMPORTANT Metal-conjugated antibodies produced using the Maxpar MCP9 Antibody Labeling protocol are intended for use in Fluidigm’s suspension mass cytometry only. For more titration guidelines and information on how to use Cd-labeled antibodies in your experimental system, see the Maxpar Antibody Labeling User Guide (PRD002).</p> <input type="checkbox"/> 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.
 (2 min) 	

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