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Maxpar MCP9 cadmium labeling

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SUBMIT TO PLOS ONE

ABSTRACT

Antibody labeling with cadmium isotopes

EXTERNAL LINK

https://www.fluidigm.com/binaries/content/documents/fluidigm/resources/maxpar-antibody-labeling-user-guide-prd002rev14/maxpar-antibody-labeling-user-guide-prd002rev14/fluidigm%3Afile

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PROTOCOL CITATION

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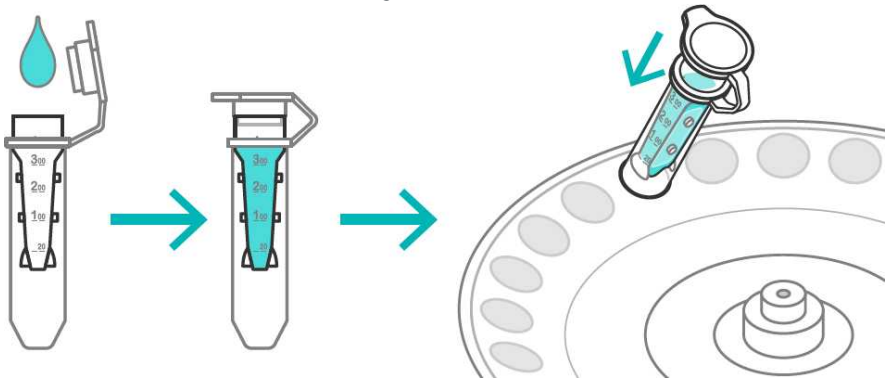
GUIDELINES

- Due to the ion optics of the mass cytometer, these lower-mass cadmium 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 Maxpar MCP9 labeling should consist of antibody clones with high antigen expression and high antibody sensitivity.
- Ensure that samples used with ^{116}Cd-labeled antibodies do not contain high levels of tin (Sn).
- When using ^{127}IdU, oxides from ^{111}Cd staining may spill over into the ^{127}I channel.
- The expected yield from Maxpar MCP9 antibody labeling is typically lower than the expected yield from Maxpar X8 antibody labeling. The average expected recovery with the MCP9 kit is approximately 50%, as compared to the average expected recovery of 60% with the Maxpar X8 kit.
- The use of saponin-based reagents with Cd-labeled antibodies generated by this kit may result in high background and/or nonspecific staining.
- Store Maxpar MCP9 polymer at -20° C with desiccant and thaw only once.
- Store TCEP in 10 µL aliquots at -20° C and thaw only once.
- Use filter tips for all steps.
- For every wash step, wash buffers down the inside wall of the filter device and **do not touch** the delicate filter membrane.
- For antibody wash step, minimize contact of pipet tips with antibody solution in order to increase yield.
- For centrifugation steps, 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



Orientation of the Amicon Ultra 0.5 mL centrifugal filter unit.

MATERIALS TEXT

- 100-200 µg carrier-free antibody
- Tris-carboxyethylphosphine (TCEP), neutral pH, 0.5 M in 10 µL aliquots stored at -20° C (Pierce 77720)
- Maxpar MCP9 antibody labeling kit (Fluidigm 201111A, 201112A, 201114A, 201116A)
  - Maxpar MCP9 antibody labeling reagent (one tube per 0.1 mg antibody)
  - L buffer (20 mM ammonium acetate, pH 6)
  - R buffer (0.1 M phosphate buffer with 2.5 mM EDTA, pH 7.2)
  - C buffer (Tris-buffered salt with 1 mM EDTA, pH 7.5)
  - W buffer (Tris-buffered salt, pH 7.5)
- Maxpar cadmium nitrate (50 mM stock)
- HRP protector peroxidase stabilizer (Boca 222 050, 222 125)
  - Do **NOT** add sodium azide
- ProClin 150 (Millipore Sigma 49376-U)
- Amicon Ultra 500 µL V bottom centrifugal filter unit, 50 kDa (Millipore UFC505008)
- Amicon Ultra 500 µL V bottom centrifugal filter unit, 3 kDa (Millipore UFC500308)
- Amicon Ultra 500 µL V bottom centrifugal filter unit, 100 kDa (Millipore UFC510008)
- microcentrifuges, at least 12,000 x g, room temperature
- heat bath, 37° C

Preparation

1 Bring all buffer solutions to room temperature.

2 Centrifuge the stock antibody at 12,000 × g for 5 minutes to sediment antibody aggregates, and then verify the stock antibody concentration by spectrophotometry.

3 Ensure that the water bath and dry heat block are equilibrated to **37 °C**.

Polymer loading and wash 1

4 Thaw Maxpar MCP9 polymer to room temperature before opening to avoid moisture condensation.

5 Centrifuge the MCP9 polymer tube and the tube containing 50 mM cadmium nitrate for 10 seconds in a minicentrifuge.

6 Add 87 µL of **L** buffer to the MCP9 polymer tube to resuspend the polymer. Mix thoroughly by pipetting until the polymer is completely dissolved (approximately 1 min).

7 Add 13 µL of 50 mM cadmium metal solution to the MCP9 polymer tube. Mix thoroughly by pipetting.

8  1h

Incubate at **37° C** for 60 minutes in a water bath or dry heat block. Note that the temperature is different from Maxpar X8 metal labeling. **01:00:00**

9 Label a 3 kDa filter and add 100 µL **L** buffer.

10 Retrieve the metal-loaded polymer mixture and then transfer all contents (approximately 100 µL) to the 3 kDa filter containing L buffer.

11 Add 100 µL **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. The filter should now contain approximately 300 µL of L buffer-metal-loaded polymer solution.

12 Use a P100 pipette to mix thoroughly, being careful not to touch the delicate filter.

13  25m

Centrifuge at 12,000 × g for 25 minutes at room temperature. **00:25:00**

Antibody wash 1

14 Roughly 10 minutes before MCP9 polymer centrifugation is complete, label a 50 kDa filter and add 100 µg antibody and

R buffer such that the total volume is 400 µL. If the required antibody volume is >400 µL, concentrate it 15 minutes earlier by centrifuging in the filter at 12000 × g for 10 minutes at room temperature.

15 Centrifuge at 12000 × g for 10 minutes at room temperature. **00:10:00** 10m

Polymer wash 2

16 Aspirate the flow-through from the bottom of the tube containing the 3 kDa filter with the metal-loaded MCP9 polymer.

17  30m

Add 300 µL of **L** buffer and centrifuge at 12000 × g for 30 minutes at room temperature. **00:30:00**

Antibody washes 2 and 3 and reduction

18 Aspirate the flow-through from the bottom of the tube containing the 50 kDa filter with the antibody.

19 Add 400 µL of **R** buffer and centrifuge at 12000 × g for 10 minutes at room temperature. **00:10:00** 10m

20 Aspirate the flow-through from the bottom of the tube.

21 Add 400 µL of **R** buffer and centrifuge at 12000 × g for 10 minutes at room temperature. **00:10:00** 10m

22 Prepare fresh 4 mM TCEP solution by diluting 8 µL of 0.5 M TCEP stock with 992 µL of **R** buffer.

23 Add 100 µL of the 4 mM TCEP solution and mix quickly and thoroughly by pipetting.

24  30m

Immediately incubate at **37° C** for **30 minutes** in a water bath. **00:30:00**

Polymer wash 3

25 Aspirate the flow-through from the bottom of the tube containing the 3 kDa filter with the metal-loaded MCP9 polymer.

26  45m

Add 400 µL of **C** buffer and centrifuge at 12000 × g for 45 minutes at room temperature. **00:45:00**

Reduced antibody washes 1 and 2

27 After the 30 minute antibody reduction is complete, retrieve the 50 kDa filter containing the partially reduced antibody from the 37° C water bath.

28 Add 300 µL of **C** buffer and gently mix by pipetting.

10m

29 Centrifuge at 12000 × g for 10 minutes at room temperature. **00:10:00**

30 Aspirate the flow-through.

10m

31 Add 400 µL of **C** buffer and centrifuge at 12000 × g for 10 minutes at room temperature. Centrifugation should finish slightly after polymer wash 3. **00:10:00**

Conjugation 1h 30m

32 Retrieve the 3 kDa filter unit containing the purified metal-loaded polymer.

33 Retrieve the 50 kDa filter unit containing the purified partially reduced antibody and aspirate the flow-through.

34 Resuspend the metal-loaded polymer (residual volume approximately 20 µL) in 60 µL of **C** buffer (total volume approximately 80 µL).

35 Transfer the resuspended contents to the corresponding partially reduced antibody in the 50 kDa filter (final conjugation volume approximately 100 µL).

36 Mix gently by pipetting.

1h 30m

37 

Incubate at 37° C for 90 minutes in the water bath. During the incubation, label a new 100 kDa filter unit. **01:30:00**

Conjugated antibody washing 40m

38 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).

39 Mix gently by pipetting, and then transfer contents to the 100 kDa filter unit.

40 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.

41  10m

Centrifuge the 100 kDa filter unit at 5000 × g for 10 minutes. If fluid fails to flow through the filter, verify the correct orientation of the filter in the centrifuge and try again, or centrifuge the mixture at 8000 × g for all wash steps.

00:10:00

42 Aspirate the flow-through.

43 Add 400 µL of **W** buffer, centrifuge at 5000 × g for 10 minutes, and then discard the flow-through. **00:10:00** 10m

44 Add 400 µL of **W** buffer, centrifuge at 5000 × g for 10 minutes, and then discard the flow-through. **00:10:00** 10m

45 Add 400 µL of **W** buffer, centrifuge at 5000 × g for 10 minutes, and then discard the flow-through. **00:10:00** 10m

Quantitation

46 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.

47 Quantify the conjugated antibody by using a spectrophotometer to measure the absorbance of a 2 µL aliquot at 280 nm against a W buffer blank.

48 Centrifuge the 100 kDa filter at 12,000 × g for 5 minutes to remove the W buffer.

Recovery and storage

49 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.

50 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.

51 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 1000 × g for 2 minutes.

52 Transfer the conjugated antibody to a labeled storage tube and store at 4° C until ready to titrate.