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Maxpar Antibody Labeling for Imaging Mass Cytometry

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We use this protocol and it's working

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Abstract

Version 11 of the antibody labelling protocol published by Fluidigm.

Guidelines

Buffers and lanthanides: 4 °C
Polymers: –20 °C with provided desiccant in a sealed container

Materials

MATERIALS

- Purified IgG or polyclonal: glycerol-free and carrier-free (no BSA hydrolyzed protein or gelatin
- Centrifugal Filter Unit: 3 kDa Amicon® Ultra 500 µL V bottom **Merck Millipore (EMD Millipore) Catalog #**UFC500396
- Centrifugal Filter Unit: 50 kDa Amicon Ultra 500 µL V bottom **Merck Millipore (EMD Millipore) Catalog #**UFC505096
- Microcentrifuge
- 37 °C water bath or heat block (for 2 mL Eppendorf® tubes)
- 0.5 M TCEP: Pierce™ Bond-Breaker® TCEP Solution **Thermo Scientific Catalog #**77720
- Aerosol barrier (filter) pipette tips (200uL)
- Antibody Stabilizer (supplemented with 0.05% sodium azide after purchase) **Candor Bioscience GmbH Catalog #**131050

Safety warnings

Before handling chemicals, refer to the safety data sheet (SDS) provided by the manufacturer and observe all relevant precautions.

Before start

Antibody clones selected for conjugation are first validated for adequate sensittivity and expected staining patterns using immunofluorescence microscopy.

Maxpar Polymer is moisture-sensitive. Equilibrate polymer (stored at –20 °C) to room temperature before opening to avoid moisture condensation.
• Use filter tips in all pipetting steps to prevent cross-contamination between metal stocks and reagents.
• Before starting the Maxpar protocol, verify the purified, carrier-free antibody concentration by NanoDrop™ after blanking against **the buffer they are suspended in**. The composition of the buffer can be found on the technical data sheet supplied by the antibody vendor.
• Loading of the polymer and partial reduction of the antibody should be performed simultaneously (see Figure 1). 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.

Preload the polymer with lanthanide.

1 **1** Spin the polymer tube for 10 seconds in a microfuge to ensure that the reagent is at the bottom of the tube.
2 Resuspend the polymer with 95 µL of L-Buffer.
3 Mix thoroughly by pipetting.
4 Add 5 µL of lanthanide metal solution to the tube (final concentration: 2.5 mM in 100 µL).
5 Mix thoroughly by pipetting.
6 Incubate at 37 °C for 30–40 minutes in a water bath or heat block. During incubation, proceed immediately to Step 7 and begin buffer exchange and partial reduction of antibody.

Perform buffer exchange and partially reduce the antibody.

2 **7** Add 100 µg of stock antibody in up to 400 µL R-Buffer to a 50 kDa filter.
8 Centrifuge at 12,000 x *g* for 10 minutes at room temperature (RT).
9 During centrifugation, dilute 0.5 M TCEP stock to 4 mM in R-Buffer by mixing 8 µL of 0.5 M TCEP stock with 992 µL of R-Buffer. For each antibody being labeled, 100 µL of 4 mM TCEP-R-Buffer is required.
10 Discard column flow-through from centrifugation.
11 Add 100 µL of the 4 mM TCEP-R-Buffer to each antibody and mix by pipetting.
12 Incubate at 37 °C in a water bath or heat block for 30 minutes. Proceed to Step 13 during the 30-minute incubation.

Note

If the stock antibody concentration is too dilute to add the desired amount of antibody, pre-concentrate it in the same filter before adding the R-Buffer.

Note

IMPORTANT Do not exceed 30 minutes! Proceed immediately to Step 17 after 30 minutes and begin purifying the partially reduced antibody.

Purigy the lanthanide-loaded polymer.

3 **13** Add 200 µL of L-Buffer to a 3 kDa filter.
14 Add the 100 µL metal-loaded polymer mixture to the filter containing the 200 µL L-Buffer to the wash.
15 Centrifuge at 12,000 x *g* for 25 minutes at RT.
16 Repeat the wash by adding 400 µL of C-Buffer to the filter and centrifuge at 12,000 x *g* for 30 minutes at RT.

Note

Purify the lanthanide-loaded polymer at the same time that the antibody is being reduced (Step 12).

Purify the partially reduced antibody.

4 **17** Retrieve the 50 kDa filter containing the partially reduced antibody from the 37 °C water bath or heat block.
18 Add 300 µL of C-Buffer to the 50 kDa filter to wash the antibody.
19 Centrifuge at 12,000 x *g* for 10 minutes at RT. (A second microcentrifuge could be used at this step to avoid timing conflict with the polymer wash.)
20 Discard flow-through.
21 Repeat the wash by adding 400 µL of C-Buffer to the filter and centrifuge at 12,000 x *g* for 10 minutes at RT.

Retrieve the purified partially reduced antibody and lanthanide-loaded polymer.

5 **22** Retrieve 3 kDa filter containing the purified lanthanide-loaded polymer from the centrifuge and discard column flow-through.
23 Retrieve 50 kDa filter containing the purified partially reduced antibody from the centrifuge and discard column flow-through.

Conjugate the antibody with lanthanide-loaded polymer.

6 **24** Using a pipette, resuspend the lanthanide-loaded polymer in 60 µL of C-Buffer (total volume ~80 µL).
25 Transfer the resuspended contents to the corresponding partially reduced antibody in the 50 kDa filter (final conjugation volume ~100 µL).
26 Mix gently by pipetting.
27 Incubate at 37 °C for 90 minutes.

Wash the metal-conjugated antibody.

7

28 Add 200 uL of W-buffer to the 100 uL antibody conjugation mixture.
29 Centrifuge at 12,000 x g for 10 minutes.
30 Discard flow-through.
31 Repeat wash three more times with W-Buffer to a toal volume of 400 uL (for a total of four washes with W-Buffer)

Determine yield.

8

32 After the final wash with W-buffer, add ~80 µL of W-buffer to the 50 kDa filter to dilute the conjugate (in ~20 µL) to a volume of 100 µL. Pipette to mix and rinse the walls of the filter.
33 Quantify the conjugated antibody by measuring the absorbance at 280 nm against a W-Buffer blank (expected recovery is 60%).
34 Calculate the volume of antibody stabilization buffer (supplemented with 0.05% sodium azide after purchase) required to obtain a final concentration of 0.5 mg/mL.
35 Centrifuge the 50 kDa filter at 12,000 x *g* for 10 minutes to remove the W-Buffer.

Recover and store the metal-conjugated antibody.

9

36 Add the calculated volume of antibody stabilization buffer (supplemented with 0.05% sodium azide after purchase) minus the residual volume (~20 µL) to the 50 kDa filter to obtain a final concentration of 0.5 mg/mL of conjugated antibody.
37 Invert the 50 kDa filter over to a new collection tube (see Figure 2).
38 Centrifuge the inverted filter/collection tube assembly at 1,000 x *g* for 2 minutes.
39 Store at 4 °C until ready to titrate.
40 Titrate the antibody. 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 titration as follows: 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, 0.62 µg/mL, 0.31 µg/mL, 0.16 µg/mL, 0 µg/mL.
41 After the conjugated antibody has been titrated on the CyTOF® instrument, if necessary dilute it to the optimum working concentration in stabilization buffer and store it at 4 °C.

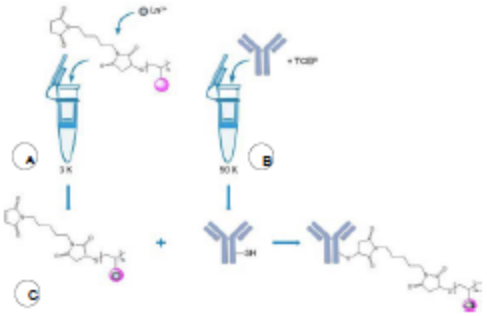

Figure 1. This procedure involves first loading the polymer with lanthanide (A) and partially reducing the antibody (B), then conjugating the antibody with the lanthanide-loaded polymer (C).

Figure 2. Invert the filter into a clean collection tube.