Fluidigm Metal Antibody Labelling Protocol

Reagent	Storage
MaxPar Metal (50 mM)	4 °C
MaxPar Polymer	-20 °C
C Buffer	4 °C
L Buffer	4 °C
R Buffer	4 °C
W Buffer	4 °C
0.5 M Bond-Breaker™ TCEP Solution 77720	RT
3 kDa Amicon Ultra UFC500396	RT
50 kDa Amicon Ultra UFC505096	RT
Antibody Stabilizer in PBS 131 050	4 °C
1% Sodium Azide Solution 786-299	RT
100-200 µg BSA-free Antibody	-20 °C / 4 °C

Procedure (100-200 µg Antibody)

Always measure the starting concentration of antibodies via NanoDrop – concentrations listed by vendors are often wrong. Use filter tips for all steps. Avoid scratching filter surfaces with pipette tips. All centrifugations are carried out at room temperature. Keep purified antibodies at 4 °C until step 5 and after finishing. The polymers are fine if stored in fridge for 2-3 days.

Before starting, check the integrity of each 50 kDa filter column:

- *Add 400 uL MilliQ water and centrifuge at 12,000 g for 30 secs.*
- *~150 µL liquid should remain above the filter. If the level is lower than this, discard the filter. If above, spin for 5 mins and begin the conjugation protocol.*

Polymer-Metal Tube

1. Retrieve 1x PCR tube of **MaxPar Polymer** per 100 µg of mAb and bring to RT. Briefly centrifuge (by placing into an eppendorf tube) before opening.
2. For each mAb, add 95 µL of **L Buffer** to one **MaxPar Polymer** tube. Pipette up and down to mix well. Check by eye to see polymer dissolve. Vortex and spin to ensure well mixed if unsure.
3. Retrieve the specific Metal solution and short centrifuge for approximately 5 seconds. Then add 5 µL **Metal Solution**

Antibody (Ab) Tube

to **MaxPar Polymer** and vortex.

4. Incubate at 38 °C, 300 rpm on ThermoMixer for 30 - 45 mins. (Vortex every 10 mins.)

Measure the (1) concentration of the antibody during this time and (2) the exact volume of the Metal Solution used in the current conjugation and update on 'Master Metals Tab' Google Sheet.

5. Then approximately 20 - 25 minutes after starting the metal-polymer incubation, add 100-200 µg of Ab into a labelled **50 kDa Amicon** filtration unit.

6. Bring total volume to 400 µL with **R Buffer**.

7. Centrifuge Ab filter units at 12,000 x *g* for 8 mins. Discard flow-through.

8. After Polymer-Metal incubation, briefly centrifuge, add 100 µL of **L Buffer**, and transfer everything to a **3 kDa Amicon** filter unit. Add another 100 µL **L Buffer** to the empty tube, pipette to rinse, and transfer to the same 3 kDa filter.

9. Centrifuge Polymer-Metal containing filter units at 12,000 x *g* for 25 mins. Discard flow-through.

10. Add 400 µL of **R Buffer**.

11. Centrifuge Ab filter units at 12,000 x *g* for 8 mins. Discard flow-through.

12. In a new tube, add 992 µL of **R Buffer** to 8 µL of **0.5 M TCEP**.

13. Add 100 µL of diluted **TCEP** to each Ab filter unit. Gently vortex (try not to get in lid), pipette to mix, and wash sides. Incubate on ThermoMixer Comfort at 38 °C for 20 - 25 mins, without agitation. (Do not invert as heat will cause liquid to pass the seal.)

14. Add 400 µL of **C Buffer** to each Polymer-Metal filter unit.

15. Centrifuge Polymer-Metal filter units at 12,000 x *g* for 25 mins. Discard flow-through.

16. After Ab reduction, add 200 µL of **C Buffer.**

17. Centrifuge at 12,000 x *g* for 8 mins. Discard flow-through.

18. Add 400 µL of **C Buffer** to the Ab filter unit (use buffer to rinse lid) and centrifuge at 12,000 x *g* for 8 minutes. Discard flow-through.

19. Re-suspend metal-loaded polymer in 200 µL of **C Buffer**. Vortex gently. Pipette to wash sides.

20. Transfer re-suspended metal-loaded polymer to corresponding Ab tube. Mix by pipetting.

21. Incubate on ThermoMixer Comfort at 37 °C, lid on, for 1.5 - 1.75 hours. (Do not vortex or invert the tube as this can cause liquid to pass the seal.)

22. Add 200 µL of **W Buffer**. Centrifuge at 12,000 x *g* for 8 mins. Discard flow-through.

23. Add 400 µL of **W Buffer**. Centrifuge at 12,000 x *g* for 8 mins. Discard flow-through.

24. Add 400 µL of **W Buffer**. Centrifuge at 12,000 x *g* for 8 mins. Discard flow-through.

25. Add 400 µL of **W Buffer**. Centrifuge at 12,000 x *g* for 8 mins. Discard flow-through.

26. Add 400 µL of **W Buffer**. Centrifuge at 12,000 x *g* for 8 mins.

27. Add 50 µL of **W Buffer**, pipette to mix and rinse the walls of the filter. Invert the column into a new collection tube.

28. Centrifuge at **1,000 x *g*** for **2 mins** (note change in speed and time) to elute the labelled mAb. Transfer supernatant (containing Ab) to a labelled low-bind 1.5 mL tube.

29. Add another 50 µL of **W Buffer** to the filter, pipette to mix, and rinse the walls of the filter. Invert the column into the same collection tube and gently vortex.

30. Centrifuge at 1,000 x *g* for 2 mins and transfer to same low-bind 1.5 mL tube.

31. Use NanoDrop (set to IgG mode) to measure Ab concentration (blank against W-Buffer).

32. Add 96 µL **Candor Antibody Stabilization Solution** and 4 µL **1 % Sodium Azide** (final Sodium Azide concentration = 0.02 %). (Total Ab solution volume = 200 µL (1:1 W-Buffer : Candor-Azide).)

33. Label low-bind 1.5 mL tube. (Label should include antibody target, clone, mass-tag, antibody concentration, and date conjugated) (e.g. see right).

34. Store metal labelled antibody at 4 °C.