MAXPAR® Antibody Labeling Kit

Lanthanide Labeling of Antibodies: *Pre-Load Method*

CHEMICAL HAZARD: Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

Note:

- ***This protocol has been optimized for a multitude of IgG isotypes and also works well for affinity purified polyclonal preparations. This protocol will NOT work with IgM antibodies.***
- ***Each reaction is optimized for labeling 100 µg of antibody with a lanthanide metal.***

Reagents and Materials

Kit Contents (4 rxn):

- R-Buffer (6 mL)
- C-Buffer (5.5 mL)
- L-Buffer (1.4 mL)
- W-Buffer (8 mL)
- MAXPAR® Polymer (4 tubes)
- Lanthanide solution (20 µL)

Kit Contents (40 rxn):

- R-Buffer (60 mL)
- C-Buffer (55 mL)
- L-Buffer (14 mL)
- W-Buffer (80 mL)
- MAXPAR® Polymer (40 tubes)
- Lanthanide solution (200 µL)

Storage:

- Buffers and Lanthanides: 4°C
- Polymers: -20°C with provided desiccant in a sealed container

Additional materials required:

- Antibodies to be labeled:
  Purified IgG or polyclonal: carrier-free (no BSA, Hydrolyzed protein, gelatin etc. for stabilization)
- Centrifugal Filter Unit: 3 kDa Amicon Ultra- 500 µL V bottom, Millipore Cat # UFC500396
- Centrifugal Filter Unit: 50 kDa Amicon Ultra- 500 µL V bottom, Millipore Cat # UFC505096
- Micro-centrifuge *(preferably 2 units to avoid overlap of certain steps requiring centrifugation)*
- Heat block Incubator or water bath (37°C)
- 0.5M TCEP: Bond-Breaker™ TCEP solution, Pierce Cat#77720
- Aerosol Barrier (Filter) Pipette Tips
- PBS – based antibody stabilization solution: Antibody Stabilizer, Candor Biosciences Cat#131050, supplemented with 0.05% azide after purchase

Important Notes Before Starting

- The 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.
- Prior to starting the MAXPAR protocol, purified, carrier free antibody concentration should be verified 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 at end of protocol). It is imperative, however, to not exceed recommended reduction time, or allow the partially reduced antibody to remain free of the loaded polymer.

Timing hh:mm (Approximate)	Outline of Protocol Steps
0:00	***Pre-Load the Polymer with Lanthanide***
	1. Spin polymer tube for 10 seconds in a microfuge to ensure the reagent is at the bottom of the tube
	2. Resuspend polymer with 95 µL of L-Buffer
	3. Mix thoroughly by pipetting
	4. Add 5 µL of Lanthanide metal solution to tube (final concentration: 2.5mM)
	5. Mix thoroughly by pipetting
	6. Incubate at 37°C for 30-40 minutes. During incubation, begin Buffer Exchange and Partial Reduction of Antibody
0:30	***Buffer Exchange and Partially Reduce the Antibody***
	7. Add 300 µL R-Buffer to a 50kDa filter
	8. Add up to 100 µg in up to 200 µL of stock antibody to the R-Buffer in the filter (Note: if the stock antibody concentration is too dilute to add the desired amount of antibody, pre-concentrate it in the same filter prior to addition of the R-Buffer)
	9. Centrifuge 12,000 xg for 10 minutes at RT
	10. During centrifugation, dilute 0.5M TCEP stock to 4mM in R-Buffer by mixing 8 µL of 0.5M TCEP stock with 992 µL of R-Buffer. For each antibody being labeled 100 µL of 4mM TCEP-R-Buffer is required.
	11. Discard column flow-through from centrifugation
	12. Add 100 µL of the 4mM TCEP-R-Buffer to each antibody and mix by pipetting
	13. Incubate at 37°C for 30 minutes (do not exceed)
0:45	***Purify Lanthanide-loaded Polymer***
	14. Add 200 µL of L-Buffer to a 3kDa filter
	15. Add the metal-loaded polymer mixture to the filter containing the L-Buffer
	16. Centrifuge 12,000 xg for 25 minutes at RT
	17. Add 300 µL of C-Buffer to the filter and centrifuge 12,000 xg for 30 minutes at RT
1:15	***Purify the Partially Reduced Antibody***
	18. Retrieve the partially reduced antibody from the 37°C
	19. Add 300 µL of C-Buffer to each 50 kDa filter
	20. Centrifuge 12,000 xg for 10 minutes at RT. A second microcentrifuge could be used at this step to avoid timing conflict with the polymer wash.
	21. Discard flow through
	22. Add 400 µL of C-Buffer to the filter
	23. Centrifuge 12,000 xg for 10 minutes at RT
	Retrieve the Partially Reduced Antibody and Lanthanide-loaded Polymer
1:40	24. Retrieve 3 kDa filter containing the Lanthanide-loaded Polymer from the centrifuge and discard column flow through
1:55	25. Retrieve 50 kDa filter containing the Partially Reduced Antibody from the centrifuge and discard column flow through

Timing hh:mm (Approximate)	Outline of Protocol Steps
1:55	***Conjugate Antibody with Lanthanide-loaded Polymer***
	26. Using a pipette, re-suspend the lanthanide-loaded polymer in 60 µL of C-Buffer
	27. Transfer the re-suspended contents to the corresponding partially reduced antibody in the 50 kDa filter
	28. Mix briefly by pipetting
	29. Incubate at 37°C for at least 60 minutes (up to 2 hours)
2:55	***Wash metal conjugated antibody***
	30. Add 300 µL of W-Buffer to the antibody conjugation mixture
	31. Centrifuge 12,000 xg for 10 minutes
	32. Discard flow through
	33. Repeat 3 more times with 400 µL of W-Buffer (for a total of 4 washes with W-Buffer)
3:35	***Recover Metal Conjugated Antibody***
	34. Add 50 µL of W-buffer to the 50 kDa filter, pipette to mix and rinse the walls of the filter
	35. Invert the 50 kDa filter over to a new collection tube (see Figure 2)
	36. Centrifuge the inverted filter/collection tube assembly at 1000 xg for 2 minutes
	37. Remove the inverted filter from the collection tube, rinse walls of the filter with an additional 50 µL of W-Buffer and replace it, inverted, back to the collection tube
	38. Centrifuge the inverted filter/collection tube assembly at 1000 xg for 2 minutes
3:40	***Yield Determination and Storage of Metal Conjugated Antibody***
	39. Quantify the conjugated antibody by measuring the absorbance at 280 nm against a W-Buffer blank (expected recovery is 60%)
	40. For storage, dilute the antibody to a final concentration of 0.5 mg/mL in a commercially available antibody stabilization buffer (supplemented with 0.05% sodium azide after purchase) and store at 4°C until ready to titrate
	41. Once the conjugated antibody has been titrated on the CyTOF it can be diluted to the optimum working concentration in stabilization buffer and stored at 4°C.

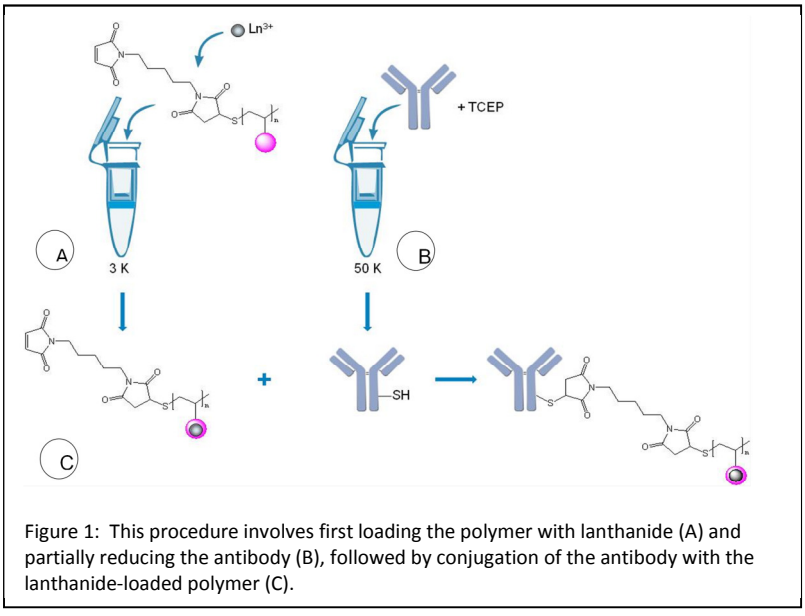

Figure 1: This procedure involves first loading the polymer with lanthanide (A) and partially reducing the antibody (B), followed by conjugation of the antibody with the lanthanide-loaded polymer (C).

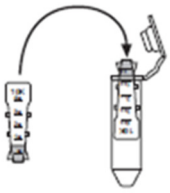

Figure 2: Invert filter into clean collection tube