

Maxpar Antibody Labeling Kit



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

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

Kit Contents

For 4 Reactions

Product Name	Volume
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

For 40 Reactions

Product Name	Volume
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 Required Materials

Product Name	Manufacturer	Part Number	Quantity
Purified IgG or polyclonal: carrier-free (no BSA, hydrolyzed protein, or gelatin, for stabilization)			
Centrifugal Filter Unit: 3 kDa Amicon® Ultra 500 µL V bottom	Millipore®	UFC500396	
Centrifugal Filter Unit: 50 kDa Amicon Ultra 500 µL V bottom	Millipore	UFC505096	
Microcentrifuge			2
37 °C water bath or heat block (for 2 mL Eppendorf® tubes)			
0.5 M TCEP: Pierce™ Bond-Breaker® TCEP Solution	Thermo Scientific™	77720	
Aerosol barrier (filter) pipette tips			200 µL

Product Name	Manufacturer	Part Number	Quantity
Antibody Stabilizer (supplemented with 0.05% sodium azide after purchase)	CANDOR® Bioscience	131050	
	or Boca Scientific	131 000	

Important Notes before Starting

- Maxpar Polymer is moisture-sensitive. Equilibrate polymer (stored at $-20\text{ }^{\circ}\text{C}$) 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.

Protocol Steps

Approximate Time (hr:min)	Steps
0:00	<p>Preload the polymer with lanthanide.</p> <ol style="list-style-type: none"> 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 $37\text{ }^{\circ}\text{C}$ 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.

Approximate Time (hr:min)	Steps
0:30	<p>Perform buffer exchange and partially reduce the antibody.</p> <p>7 Add 100 µg of stock antibody in up to 400 µL R-Buffer to a 50 kDa filter.</p> <p>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.</p> <p>8 Centrifuge at 12,000 x g for 10 minutes at room temperature (RT).</p> <p>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.</p> <p>10 Discard column flow-through from centrifugation.</p> <p>11 Add 100 µL of the 4 mM TCEP-R-Buffer to each antibody and mix by pipetting.</p> <p>12 Incubate at 37 °C in a water bath or heat block for 30 minutes. Proceed to Step 13 during the 30-minute incubation.</p> <p>IMPORTANT Do not exceed 30 minutes! Proceed immediately to Step 17 after 30 minutes and begin purifying the partially reduced antibody.</p>
0:45	<p>Purify the lanthanide-loaded polymer.</p> <p>NOTE Purify the lanthanide-loaded polymer at the same time that the antibody is being reduced (Step 12).</p> <p>13 Add 200 µL of L-Buffer to a 3 kDa filter.</p> <p>14 Add the 100 µL metal-loaded polymer mixture to the filter containing the 200 µL L-Buffer to the wash.</p> <p>15 Centrifuge at 12,000 x g for 25 minutes at RT.</p> <p>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.</p>
1:15	<p>Purify the partially reduced antibody.</p> <p>17 Retrieve the 50 kDa filter containing the partially reduced antibody from the 37 °C water bath or heat block.</p> <p>18 Add 300 µL of C-Buffer to the 50 kDa filter to wash the antibody.</p> <p>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.)</p> <p>20 Discard flow-through.</p> <p>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.</p>
1:40	<p>Retrieve the purified partially reduced antibody and lanthanide-loaded polymer.</p> <p>22 Retrieve 3 kDa filter containing the purified lanthanide-loaded polymer from the centrifuge and discard column flow-through.</p> <p>23 Retrieve 50 kDa filter containing the purified partially reduced antibody from the centrifuge and discard column flow-through.</p>

Approximate Time (hr:min)	Steps
1:45	Conjugate the antibody with lanthanide-loaded polymer.
	24 Using a pipette, resuspend the lanthanide-loaded polymer in 60 μL of C-Buffer (total volume $\sim 80 \mu\text{L}$).
	25 Transfer the resuspended contents to the corresponding partially reduced antibody in the 50 kDa filter (final conjugation volume $\sim 100 \mu\text{L}$).
	26 Mix gently by pipetting.
	27 Incubate at 37 $^{\circ}\text{C}$ for 90 minutes.
3:15	Wash the metal-conjugated antibody.
	28 Add 200 μL of W-Buffer to the 100 μL antibody conjugation mixture.
	29 Centrifuge at 12,000 $\times g$ for 10 minutes.
	30 Discard flow-through.
	31 Repeat wash three more times with W-Buffer up to a total volume of 400 μL (for a total of four washes with W-Buffer).
4:00	Determine yield.
	32 After the final wash with W-buffer, add $\sim 80 \mu\text{L}$ of W-buffer to the 50 kDa filter to dilute the conjugate (in $\sim 20 \mu\text{L}$) 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 $\times g$ for 10 minutes to remove the W-Buffer.
4:20	Recover and store the metal-conjugated antibody.
	36 Add the calculated volume of antibody stabilization buffer (supplemented with 0.05% sodium azide after purchase) minus the residual volume ($\sim 20 \mu\text{L}$) 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 $\times g$ for 2 minutes.
	39 Store at 4 $^{\circ}\text{C}$ 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 $\mu\text{g}/\text{mL}$, 2.5 $\mu\text{g}/\text{mL}$, 1.25 $\mu\text{g}/\text{mL}$, 0.62 $\mu\text{g}/\text{mL}$, 0.31 $\mu\text{g}/\text{mL}$, 0.16 $\mu\text{g}/\text{mL}$, 0 $\mu\text{g}/\text{mL}$.
	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 $^{\circ}\text{C}$.

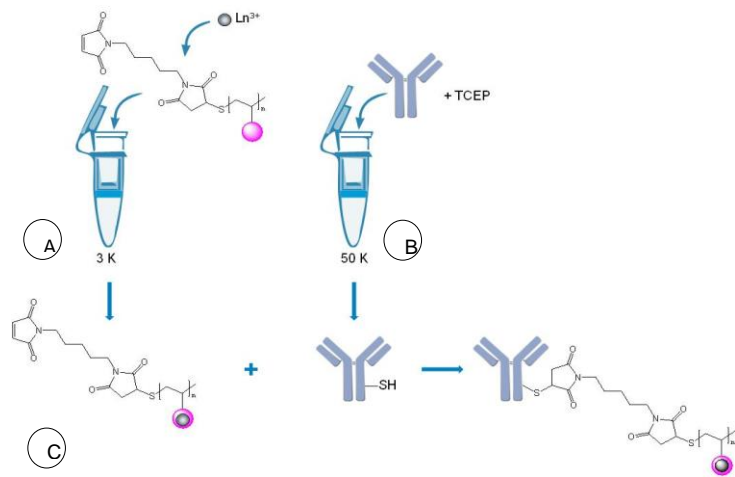


Figure 1. This procedure involves first loading the polymer with lanthanide (A) and partially reducing the antibody (B), then conjugating the antibody with the lanthanide-loaded polymer (C).

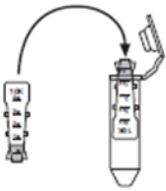


Figure 2. Invert the filter into a clean collection tube.

For technical support visit fluidigm.com/support.

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