

MAXPAR[®]-PL KIT

Lanthanide Labeling of Antibodies: *Pre-Load Method*

Store buffers at 4°C. Store polymer at -20°C with provided desiccant in a sealed container.



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

Kit Contents

- | | | | |
|------------|------|-------------------------------|--------------------|
| • R-buffer | 10mL | • MAXPAR [®] polymer | 4 (in strip tubes) |
| • C-buffer | 10mL | • Lanthanide | >20μL |
| • L-buffer | 10mL | | |
| • W-buffer | 10mL | | |

Materials Required

- Antibodies to be labeled (100μg each, BSA- and gelatin-free)
- Centrifugal Device (Pall Nanosep 3K Omega; Catalogue OD003C33 – or – Amicon Ultra 0.5mL 3K; Catalogue UFC500324, Fisher Scientific)
- Centrifugal Device (Amicon Ultra 0.5mL 50K; Catalogue UFC505024, Fisher Scientific)
- Microcentrifuge (refrigerated and capable of 14,000g)
- Heat block incubator (37°C)
- TCEP (Pierce Bond-Breaker™ TCEP Solution; Catalogue 77720)

Protocol

NOTE: Loading of the polymer (Section A) and partial reduction of the antibody (Section B) should be performed simultaneously (See Figure 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.

A. Lanthanide Loading of Polymer

1. Add 100μL L-buffer to each tube of polymer to be used, and pipette thoroughly to dissolve.
2. Transfer to 3K centrifugal device.
3. Add 5μL lanthanide solution to polymer solution in 3K centrifugal device and mix well.
4. Incubate for 30 minutes at 37°C.
5. Centrifuge at 11,000g until hold-up volume is reached (approximately 10 minutes).
6. Add 400μL L-Buffer, and centrifuge at 11,000g until hold-up volume is reached (can be greater than 20 minutes). Repeat wash step.
7. Add 100μL W-Buffer to 3K centrifugal device and vortex briefly to resuspend loaded polymer (see **Note 1**).

B. Partial Reduction of Antibody

1. While the polymer is incubating with the lanthanide, proceed with the partial reduction of the antibody.
2. Add 300μL R-Buffer to 50K centrifugal device, followed by 100μg antibody (total volume of ~400μL).
3. Centrifuge at 11,000g until hold-up volume is reached (approximately 10 minutes), then discard filtrate.

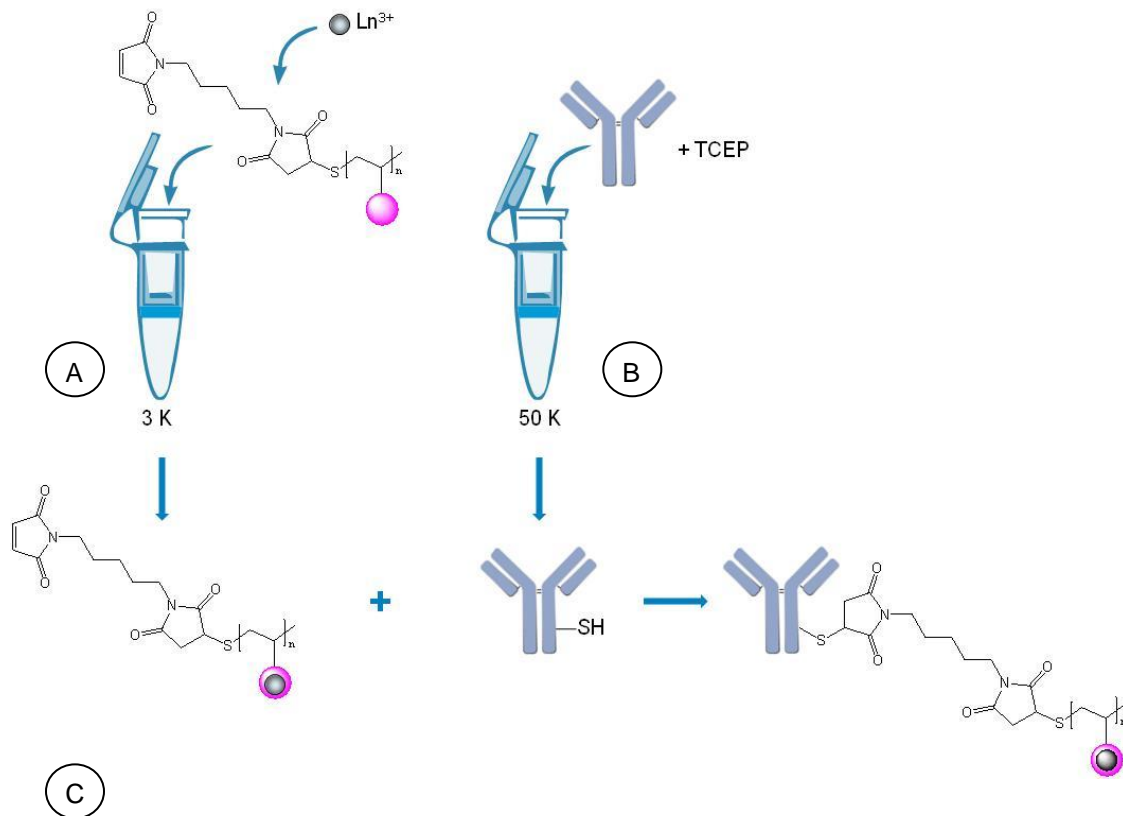
4. Prepare working concentration of 4mM TCEP: add 8 μ L of 0.5M TCEP to 1mL R-Buffer (sufficient to reduce ten antibodies).
5. Add 100 μ L of 4mM TCEP solution to centrifugal device with antibody, and vortex briefly to mix.
6. Incubate for 30 minutes at 37°C (do not exceed 60 minutes).
7. Add 300 μ L C-Buffer and centrifuge at 11,000g until minimal volume remains (approximately 10 minutes).
8. Repeat wash with 400 μ L W-Buffer and centrifuge again until hold-up volume is reached.

C. Conjugation of Lanthanide Bearing Polymer to Reduced Antibody

1. From Section A, add the 100 μ L of lanthanide loaded polymer to the 50K device containing the partially reduced antibody.
2. Mix gently by pipetting, and incubate at 37°C for 30-45 minutes.
3. Bring to 400 μ L with W-buffer and centrifuge. Repeat 3-4 times and resuspend in 100 μ L W-buffer (see **Note 2**).

Note 1: If starting with 50 μ g antibody, the lanthanide loaded polymer should be diluted in 50 μ L of W-Buffer in order for the conjugation to proceed at 1mg/mL concentration of antibody.

Note 2: The final conjugate is washed and resuspended in W-Buffer. For long term storage, the antibody should be resuspended in W-Buffer, supplemented with 1% BSA and sodium azide. Protein concentration should be determined prior to adding BSA.



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