

PROTOCOL v1.5

MAXPAR™ KIT

Lanthanide Labelling of Antibodies

Store protected from moisture at 4°C. This kit is stable for 6 months when properly stored.

Important Considerations

- The maleimide moiety of the Polymer Tag is moisture-sensitive. Store product in the original container at 4°C with supplied desiccant. Equilibrate to room temperature before opening to avoid moisture condensation onto the reagent.
- Prepare the polymer tag immediately before use. When in solution, the maleimide moiety may hydrolyze and become non-reactive; therefore, stock solutions cannot be prepared for storage. Discard any unused reconstituted reagent.

Kit Contents

R-Buffer	10mL
C-Buffer	10mL
L-Buffer	10mL
W-Buffer	10mL
MAXPAR™	4 x 0.2mg
Lanthanide	400µL

Protocol

A. Additional Materials Required

- Antibodies to be labelled (100µg each, 0.5 – 10 mg/mL, BSA- and gelatin-free)
- Centrifugal Device (Pall Nanosep 30K Centrifugal Devices, Catalogue OD030C33)
- Microcentrifuge (capable of 10,000 g)
- Heat block Incubator (37°C)
- TCEP (Pierce Bond-Breaker™ TCEP Solution, Catalogue 77720)
- Aerosol barrier pipette tips (recommended)

B. Partial Reduction of Antibody Disulfide Bonds

1. Add 200-300µL R-Buffer and 100µg antibody solution to Centrifugal Device membrane.
2. Centrifuge 10,000 g for 10 minutes. Discard flow-through.
3. Dilute 10µL 0.5M TCEP to 250µL with R-Buffer (20 mM TCEP). Add 80µL R-Buffer and 20µL of the diluted (20mM) TCEP to the Centrifugal Device membrane and mix gently (4mM TCEP final concentration). Do not vortex.
4. Incubate 30 minutes at 37°C
5. Add 300µL C-Buffer. Centrifuge 10,000 g for 10 minutes. Discard flow-through, and repeat with 400µL C-Buffer.

Note: If desired, measure sulfhydryl content with Ellman's Reagent.

Note: All spin times are approximate. Occasionally, spin times may need to be increased to allow complete flow - through.

Proceed immediately to *Section C* to minimize sulfhydryl oxidation.

C. Labelling of Reduced Antibody

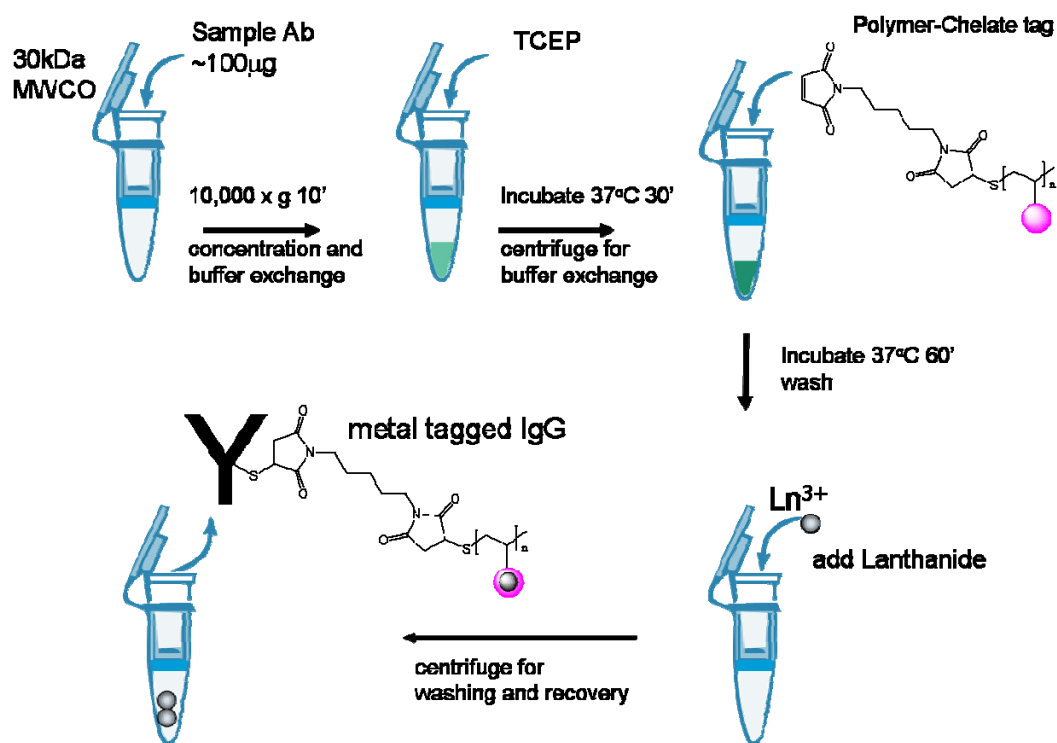
1. Add 100 μ L C-Buffer to membrane containing partially reduced antibody.
2. Add 100 μ L C-Buffer to the vial containing the MAXPAR™ polymer tag, and fully dissolve with pipetting.
3. Transfer the 100 μ L of the prepared polymer tag to the tube containing 100 μ L of the reduced antibody solution and gently mix well. Do not vortex.
4. Allow the reaction to proceed at least 1 hour at 37°C.
5. Add 200 μ L L-Buffer to Membrane. Centrifuge 10,000 g for 10 minutes. Discard flow-through.
6. Add 400 μ L L-Buffer to Membrane. Centrifuge 10,000 g for 10 minutes. Discard flow-through.
7. Add 100 μ L of lanthanide solution to the labelled antibody. Mix well. Do not vortex.
8. Incubate 30 minutes at 37°C.

D. Washing and Recovery of Lanthanide Labelled Antibody

1. Add 300 μ L W-Buffer. Centrifuge 10,000 g for 10 minutes. Discard flow-through. Repeat three to four times.
2. Add 100 μ L W-Buffer. Gently pipette several times to recover the conjugate and transfer to eppendorf tube.

Note: The labelled antibody is stable for ~8 months at 4°C.

Note: At Step 1 and 2 (Section D), the labelled antibody is washed and recovered in W-Buffer. This may be substituted with another appropriate buffer.



Warranty: DVS Sciences Inc. products are warranted to meet stated product specifications and to conform to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to 6 months from date of sales for products used, handled and stored according to DVS Sciences' instructions. DVS Sciences' sole liability for the product is limited to replacement of the product or refund of the purchase price. DVS Sciences products are supplied for laboratory applications only. They are not intended for medicinal, diagnostic, or therapeutic use. DVS Sciences products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from DVS Sciences Inc. DVS Sciences strives for complete customer satisfaction. If you are not satisfied with the performance of a DVS Sciences product, please contact DVS Sciences.