

ANTIBODY LABELING KIT V3

This protocol is optimized for the conjugation of 100 µg of antibody with one tube of metal-loaded MIBItag.

SAFETY: The solutions listed in this SOP can be corrosive to tissues and can cause skin damage. They are harmful if swallowed or inhaled. Avoid contact with eyes, skin, or clothing. Wear eye protection, gloves and protective clothing when handling. Be aware of safety precautions relating to the handling and use of all solutions. Consult the product labeling or Safety Data Sheet (SDS), as necessary.

Kit contents:

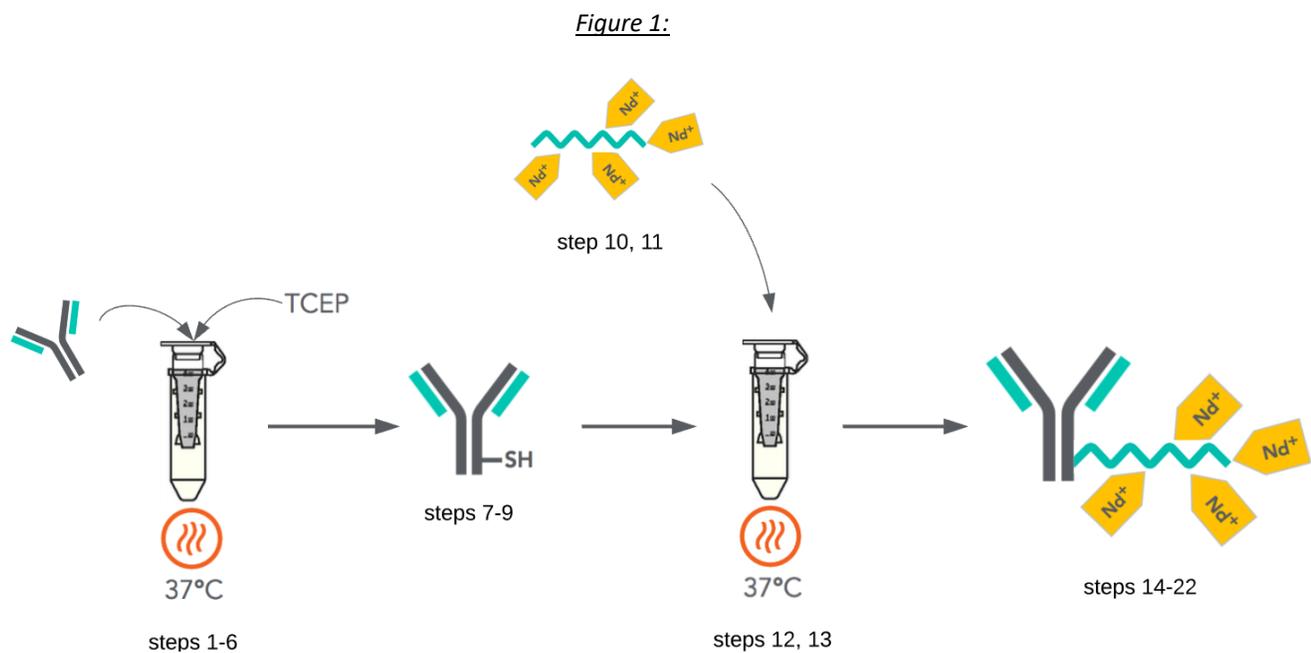
Store at 4°C	Buffer 2 Buffer 3 Buffer 4 Buffer 5
Store at room temperature	50-kDa Amicon Ultra Filter Antibody collection tube and label
Store at -20°C	Metal-loaded MIBItag *

* Always store hygroscopic MIBItag in an airtight container or bag with desiccant at -20°C.

Additional material not included:

- Purified antibody to be labeled
- Micro-centrifuge
- Heating block or water bath set to 37°C
- 0.5 M TCEP Bond-Breaker™

Conjugation workflow illustrated in *Figure 1*.



Antibody Preparation and Reduction	<ol style="list-style-type: none"> Add 100 µg of antibody to a 50-kDa filter device, do not exceed 400 µL. If antibody volume is less than 400 µL, make up the difference with Buffer 2. (See Appendix I.I for calculation examples) $\text{Antibody Needed } (\mu\text{L}) = \frac{\text{Conjugation Size } (\mu\text{g})}{\text{Antibody Conc. } (\mu\text{g}/\mu\text{L})}$ <ol style="list-style-type: none"> Centrifuge at 12,000 xg for 10 min at room temperature. Discard flow-through. Add 400 µL of Buffer 2 to the 50-kDa filter device and repeat step #2. Mix 8 µL of TCEP stock with 992 µL of Buffer 2 (final concentration: 4 mM TCEP). Add 100 µL of the 4 mM TCEP to the concentrated antibody in the 50-kDa filter device. Mix by pipetting slowly. Incubate antibody in TCEP for 30 min at 37°C. [DO NOT exceed 30min]
Antibody Wash	<ol style="list-style-type: none"> Add 300 µL of Buffer 3 to the partially reduced antibody in the 50-kDa filter device from step #6. Briefly vortex to mix. Centrifuge at 12,000 xg for 10 min. Discard the flow-through. Add 400 µL of Buffer 3 to the 50-kDa filter device and repeat step #8.
Conjugation	<ol style="list-style-type: none"> Remove the metal-loaded polymer from -20°C and allow it to come to room temperature. Perform a quick spin-down to collect the metal-loaded polymer to the bottom of the tube and then reconstitute the metal-loaded polymer in 200 µL of Buffer 3. Transfer the reconstituted metal-loaded polymer into the corresponding 50-kDa filter device containing the partially reduced antibody. Thoroughly mix by pipetting. Incubate at 37°C for 60-90min.
Post-Conjugation Washes	<ol style="list-style-type: none"> Add 200 µL of Buffer 4 to the antibody conjugation mixture in the 50-kDa filter device. Centrifuge at 12,000 xg for 10 min at RT. Discard the flow-through. Repeat steps #14-15 with 400 µL of Buffer 4 two more times for a total of three washes.
Antibody Quantification and Storage	<ol style="list-style-type: none"> Add 100 µL of Buffer 4 to the 50-kDa filter device and mix thoroughly by pipetting. Determine the concentration of conjugated antibody by loading 2 µL of Antibody in Buffer 4 and measuring the IgG absorbance at 280 nm on NanoDrop using Buffer 4 as blank. Centrifuge at 12,000 xg for 10 min at RT to remove Buffer 4.
Conjugated Antibody Elution	<ol style="list-style-type: none"> Calculate and add Buffer 5 to the 50-kDa filter device to obtain final antibody concentration of 0.5 mg/mL, making sure to thoroughly rinse the walls of the column. (See Appendix I.II for calculation examples) $\text{Buffer 5 Volume to Add} = \frac{\text{NanoDrop Concentration} \times \text{Volume}}{\text{Desired Concentration}}$ <ol style="list-style-type: none"> Invert the micro-filter device into a new collection tube and centrifuge at 1,000 xg for 2 min at RT. <u>Optional antibody filtration step:</u> Pre-wet 0.1 µM filter (not provided) with 100µL of Buffer 5 and spin 12,000xg for 2 min. Discard the flow-through. Load the filter with conjugated antibody and filter by spinning at 12,000xg for 2 min. Collect the flow-through. Label and store conjugated antibody at 4°C.

Appendix I

I.I Antibody Preparation and Reduction Step 1 Calculation Example

To calculate the amount of purified antibody to use for conjugation:

$$\text{Antibody Needed } (\mu\text{L}) = \frac{\text{Conjugation Size } (\mu\text{g})}{\text{Antibody Conc. } (\mu\text{g}/\mu\text{L})}$$

Conjugation Size	Antibody Concentration	Calculation Example
100 μg	0.5 mg/mL (equivalent to 0.5 $\mu\text{g}/\mu\text{L}$)	Antibody Needed (μL) = $\frac{100 \mu\text{g}}{0.5 \mu\text{g}/\mu\text{L}} = 200 \mu\text{L}$

I.II Conjugated Antibody Elution Step 20 Calculation Example

To calculate the amount of Buffer 5 needed to dilute the conjugated antibody to a desired concentration:

$$\text{Buffer 5 Volume to Add} = \frac{\text{NanoDrop Concentration} \times \text{Volume}}{\text{Desired Concentration}}$$

Volume is the amount of conjugated antibody in buffer 4 that the NanoDrop sample was taken from. Most often this would be 120 μL ; this is calculated by adding the residual volume in the filter, about 20 μL , and 100 μL of Buffer 4 (Step 17).

NanoDrop Reading	Desired Concentration	Volume	Calculation Example
0.630 mg/mL (equivalent to 0.63 $\mu\text{g}/\mu\text{L}$)	0.5 mg/mL	120 μL	Buffer 5 Volume to Add = $\frac{0.630 \text{ mg/mL} \times 120 \mu\text{L}}{0.5 \text{ mg/mL}} = 151.2 \mu\text{L}$