MIBItag Conjugation Protcol

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

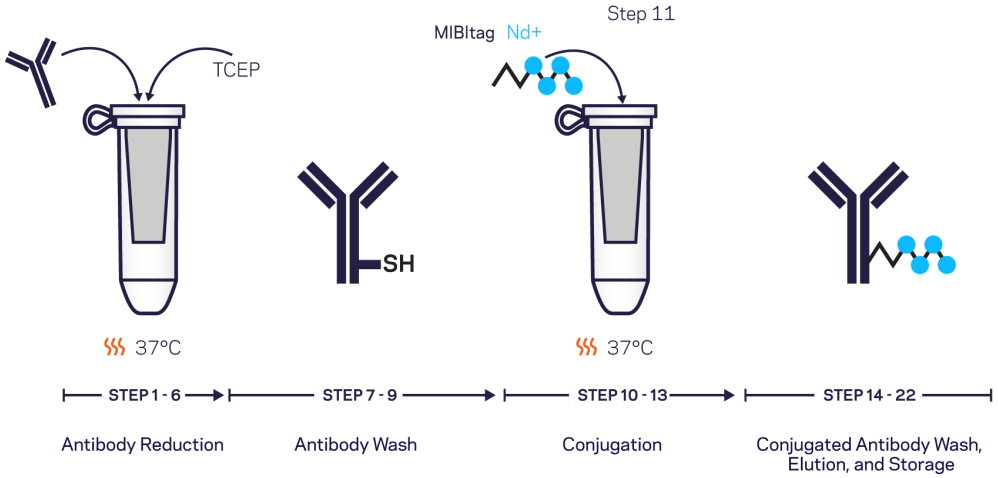
Store at 4°C	Buffer 2 Buffer 3 Buffer 4 Buffer 5
Store at room temperature	50-kDa Amicon Ultra Filter (Fisher Cat. # UFC505096) ** Antibody collection tube and label **
Store at –20°C	Metal-loaded MIBItag *

*** Always store hygroscopic MIBItag in an airtight container or bag with desiccant at –20°C.**
****These are provided only in the smaller 4-reaction conjugation kits.**

Additional material and equipment required

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

Figure 1. Conjugation workflow



Background

Below is a summary of key points.

- The MIBItag is moisture-sensitive. Store desiccated at –20℃. To avoid moisture condensation in the product, fully equilibrate the vial to room temperature before opening. MIBItags are supplied in 4 connected tubes with each tube sufficient for labeling 100 µg of antibody. Cut the required tubes of MIBItag needed for the current conjugation, being careful to avoid compromising the structural integrity of remaining tubes which could allow moisture to degrade the MIBItags.
- It is critical to use antibodies free of carrier protein (e.g. BSA, cell culture supernatant). Tris-based and phosphate-based buffers, as well as sugars such as trehalose and sucrose, are compatible with this protocol. Antibodies stored in a high concentration of glycerol can be used but the glycerol concentration needs to be reduced to 5% or less with Buffer 2 to allow filtration to proceed.
- The antibody concentration should be confirmed using a spectrometer such as a Nanodrop to ensure the appropriate amount of antibody is used for conjugation and to provide accurate determination of antibody recovery following conjugation.
- Antibody recovery can be reduced if the white filter membrane of the 50-kDa filter device is damaged, such as by contact with a pipet tip.
- Do not allow the filter to dry out at any point during the protocol.
- Ionpath recommends storing 0.5 M TCEP Bond-Breaker™as 10 µL aliquots at –20℃.
- Antibody recovery generally increases with higher amounts of antibody labeled. A 400 µg antibody conjugation will usually result in recovery above 80%, higher than the expected 60% and above for a 100 µg conjugation. This protocol can accommodate between 100 and 400 µg of antibody. For example, to conjugate 400 µg of antibody, simply reconstitute 4 tubes of the metal-loaded polymer in 200 µL of Buffer 3 in Step 11; all other steps should use the same volume of buffers.
- Some antibodies lose activity when conjugated and this can only be determined empirically. In these cases it is recommended to test conjugating alternative clones or to try our companion protocol, Cust-0006 (SATP with MIBItag Conjugation Protocol), that labels primary amines of the antibody as opposed to sulfhydryl.

Antibody Preparation and Reduction

1. 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)

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

2. Centrifuge at 12,000 xg for 10 min at room temperature. Discard flow-through.
3. Add 400 µL of Buffer 2 to the 50-kDa filter device and repeat step #2.
4. Mix 8 µL of TCEP stock with 992 µL of Buffer 2 (final concentration: 4 mM TCEP).
5. Add 100 µL of the 4 mM TCEP to the concentrated antibody in the 50-kDa filter device. Mix by pipetting slowly, being careful to avoid contact of the filter by the pipet tip.
6. Incubate antibody in TCEP for 30 min at 37˚C. [DO NOT exceed 30 min]

Antibody Wash

7. Add 300 µL of Buffer 3 to the partially reduced antibody in the 50-kDa filter device from step #6. Briefly vortex to mix.
8. Centrifuge at 12,000 xg for 10 min. Discard the flow-through.
9. Add 400 µL of Buffer 3 to the 50-kDa filter device, briefly vortex to mix, and repeat step #8.

Conjugation

10. Remove the metal-loaded polymer from –20˚C and allow it to come to room temperature.
11. 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.
12. Transfer the reconstituted metal-loaded polymer into the corresponding 50-kDa filter device containing the partially reduced antibody. Thoroughly mix by pipetting, avoiding contact with the filter
13. Incubate at 37˚C for 60-90 min.

Post-Conjugation Washes

14. Add 200 µL of Buffer 4 to the antibody conjugation mixture in the 50-kDa filter device and briefly vortex to mix.
15. Centrifuge at 12,000 xg for 10 min at RT. Discard the flow-through.
16. Repeat steps #14-15 with 400 µL of Buffer 4 two more times for a total of three washes.

Antibody Quantification and Storage

17. Add 100 µL of Buffer 4 to the 50-kDa filter device and mix thoroughly by pipetting, avoiding contact with the filter.
18. 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.
19. Centrifuge at 12,000 xg for 10 min at RT to remove Buffer 4.

Conjugated Antibody Elution

20. Following Step 19, there will be about 20 µL residual volume remaining in the filter. Calculate and add Buffer 5 to this residual volume in 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 while being careful to avoid contact of the filter by the pipet tip. (See Appendix I.II for calculation examples)

$$Buffer\ 5\ Volume\ to\ Add = \frac{NanoDrop\ Concentration\ x\ Volume}{Desired\ Concentration} - Residual\ Volume$$

21. Invert the micro-filter device into a new collection tube and centrifuge at 1,000 xg for 2 min at RT.

Optional antibody filtration step: Pre-wet 0.1 µm filter (not provided) with 100 µL of Buffer 5 and spin 12,000 xg for 2 min. Discard the flow-through. Load the filter with conjugated antibody and filter by spinning at 12,000 xg for 2 min. Collect the flow-through.

22. 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:

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

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

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:

$$Buffer\ 5\ \ Volume\ to\ Add = \frac{NanoDrop\ Concentration\ x\ Volume}{Desired\ Concentration} - Residual\ Volume$$

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 µg/µL)	0.5 mg/mL	120 µL	$Buffer\ 5\ Volume\ to\ Add = \frac{0.630\ mg/mL \times 120\ \mu L}{0.5\ mg/mL} - 20\ \mu L = 131.2\ \mu L$