

HyNic-Antibody Modification and 4FB-Bead Immobilization Protocol



Step 1: Antibody Preparation

Depending on the initial form of your antibody (lyophilized or solubilized), proceed as follows:

1.1 Lyophilized Antibody:

1.1.1 Reconstitute the lyophilized antibody in appropriately size tube with **1X Modification Buffer** and mix well to obtain a 3 - 5 mg/mL solution.

1.1.2 Solubilized Antibody: If the antibody concentration is less than 3 mg/mL, concentrate the sample at least to 3 - 5 mg/mL.

1.2 Exchange protein into **1X Modification Buffer** and determine protein concentration by measuring A_{280} , BCA or Bradford assays.

Step 2: Antibody Modification

2.1 Using HyNic-Antibody Modification Calculator, input name, antibody concentration (mg/mL), amount of antibody (mg) to be modified and amount of S-HyNic weighed. For a pre-weighed S-HyNic vial insert 1.0 mg.

2.2 Dissolve S-HyNic in 200 μ L anhydrous DMF.

2.3 From the calculator output add indicated volume of S-HyNic/DMF solution to the buffer exchanged antibody solution (from step **1.2**). Mix well. Incubate the reaction for 2-3 hours at room temperature.

2.4 Desalt reaction mixture into **1X Conjugation Buffer**.

2.5 Determine concentration of HyNic-modified antibody by BCA or Bradford Assay. Do NOT use A_{280} to determine protein concentration.

2.6 If desired, the molar substitution ratio (MSR), *i.e.* number of HyNic groups incorporated on antibody can be determined using the 2-sulfobenzaldehyde colorimetric assay- see attached document.

Step 3: Antibody-4FB Beads Immobilization

3.1 Prepare desired amount of 4FB-beads for immobilization with HyNic-antibody by addition of 1/10 volume 10X **Conjugation Buffer**.

3.2 From calculator, add required amount of HyNic-antibody to buffer exchanged 4FB-beads.
Recommended antibody/bead ratio is 1:10.

3.3 Add 1/10 volume **TurboLink Buffer**.

3.4 Incubate reaction mixture on a rotary shaker at RT for 4 hours or overnight at 4°C.

NOTE: If beads clump following antibody immobilization follow steps 3.5-3.7. Otherwise, proceed to step 3.8.

3.5 Exchange beads into **1x Conjugation Buffer** by dialysis.

3.6 Prepare a 10 mg/mL stock solution of 2-sulfo-benzaldehyde in water

3.7 From calculator add calculated volume of 2-sulfobenzaldehyde/water stock solution to reaction mixture. Mix the beads by vortexing, and sonication for 1 minute, then place on a rotary shaker at RT for 2 hours.

3.8 Exchange antibody-immobilized beads into desired buffer by dialysis.

3.9 Add desired concentration of sodium azide or other preservative if required.