

Small Scale Antibody Conjugation with DSB-X**A. Prepare buffer and DSB-X biotin**

1. Fresh 1 M sodium bicarbonate (8,4 g NaHCO₃ in 100 mL dH₂O, pH 8.3 – 8.5)
2. Fresh DSB-X biotin; One vial DSB-X biotin succinimidyl ester (kit component A) and 40 µl DMSO (kit component B), mix by pipetting

B. Conjugate the Antibody

1. Start with 1 mg/mL purified antibody in 10-20 mM PBS or equal (max 3 mM NaN₃).
Optional: buffer exchange to remove buffers containing NH₂- or NH₄⁺ by spin column (section C)
2. Transfer 200 µl antibody to reaction tub (kit component C)
3. Add 20 µl of freshly made 1 M sodium bicarbonate
4. While steering, add 3 µl freshly made DSB-X biotin
5. Incubate with steering 1 – 1.5 hours at room temperature

C. Prepare Spin Column and Purify Conjugated Antibody

1. Wet filter in spin column (kit component E) with PBS
2. Add 2 ml resin (kit component D), drain buffer by gravity (Figure 1a - b)
3. Place in collection tube (kit component F) and centrifuge 3 minutes at 1100 x g using a swing out rotor (Figure 1c)
4. Apply carefully antibody conjugation mixture (up to 250 µl) and let the liquid adsorb into the gel (Figure 2a)
5. Place column in new collection tube (or empty the used one) and centrifuge 5 minutes at 1100 x g using a swing out rotor (Figure 2b)
6. DSB-X-conjugated Antibody is collected in the collection tube. Free DSB-X biotin is left behind in column (Figure 2b)

Table 1: Conversion antibody cons. to volume DSB-X-biotin

Antibody conc. (mg/ml)	DSB-X biotin (micro liter)
0,5	2
1	3
1,5	4
3	7

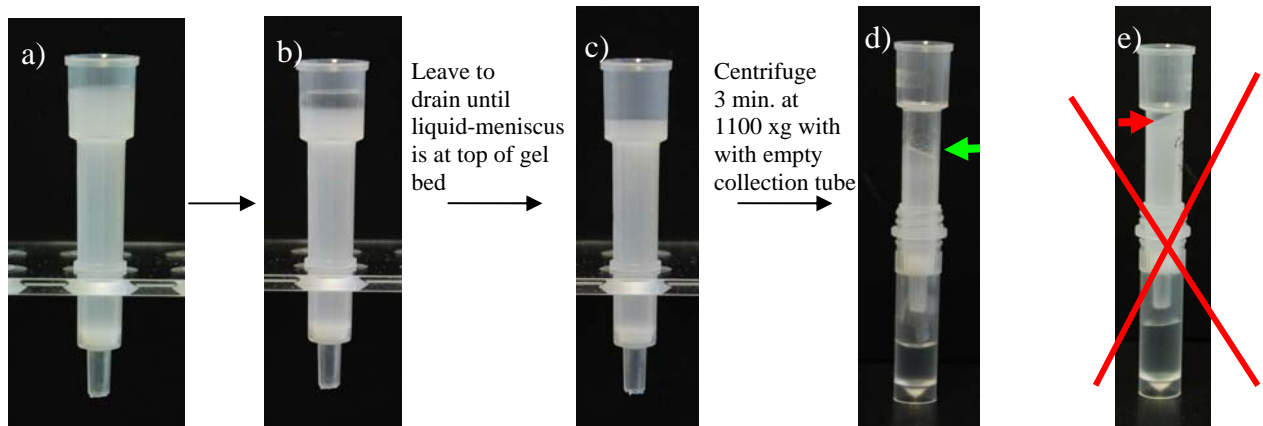


Figure 1: Preparation of spin column

Column with 2 ml resin (a), leave to sediment (b), drain liquid before centrifugation (c). After centrifugation for 3 min. at 1100 xg on swing out rotor (d) gel bed should be as indicated by green arrow. If liquid is not drained prior to centrifugation the gel bed will be too high, red arrow (e).

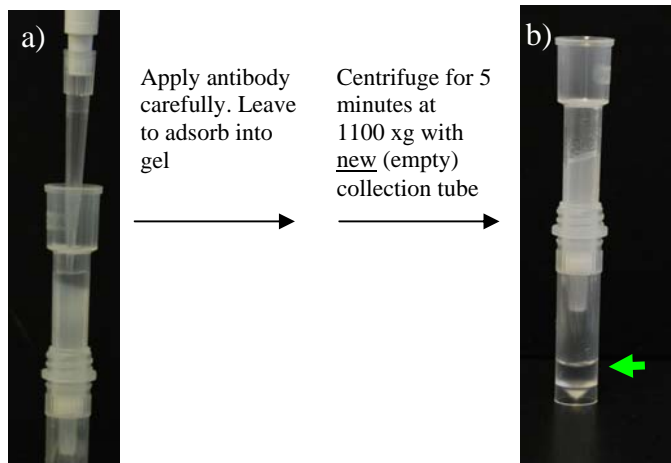


Figure 2: Purification of conjugated antibody

Add carefully up to 250 µl conjugated antibody (a), leave to adsorb into gel, change to new (empty) collection tube, centrifuge 5 min. at 1100 xg on swing out rotor (b). Conjugated antibody purified for free DSB-X-biotin is collected (green arrow).