

Coupling Antibodies to Protein A/G

This protocol can be used to generate an affinity matrix consisting of an antibody covalently linked to Protein A/G-coated beads. Target proteins can be immunoprecipitated from a sample, and eluted from the matrix with low or high pH. The matrix can be re-used several times in this manner, depending on the antibody, to purify large amounts of the target protein.

To elute bound proteins, add to the beads a similar volume of either 100 mM ammonium hydroxide, pH 11-12 or 100 mM glycine, pH 2.5. The pH extremes will serve to dislodge the protein of interest from the antibody, and it is best to empirically determine which elution conditions will work best. Several serial applications of the elution buffer will be required to liberate a maximal amount of the target protein. After elution, neutralize the ammonium hydroxide with 1% formic acid; or elute the glycine with 0.5M Tris base. Eluted samples can be probed by Western to determine the best elution conditions and the number of serial elutions required.

1. Wash 1 mL PGA (or PAS) slurry (50:50 beads to PBS) twice in Conjugation Buffer (50 mM Tris pH 7.6, 150 mM NaCl, 2% BSA) in a 1.5 mL tube.
2. Bring beads to a 1 mL volume in Conjugation Buffer and add the antibody. Incubate \geq 1 hr at RT with rocking to couple antibody to beads. Spin 5 min at 3,000g. Save the supernatant.
3. Transfer beads to a 15 mL tube with 10 mL Na Borate buffer, pH 9.0. Wash beads twice, spinning 3,500 rpm for 4 min at RT.
4. Covalently couple antibody to beads by adding 50 mg DMP (dimethyl pimelimidate, SIGMA D8388) to 10 mL Na Borate Buffer. Ensure pH > 8.3. Incubate 30 min at RT with rocking.
5. Stop reaction by pelleting beads and adding 10 mL 0.2 M ethanolamine, pH 8.0. Wash once, then incubate 2 hr at RT with rocking.
6. Pellet beads and transfer to a 2 mL tube with PBS. Wash to remove ethanolamine.
7. Wash beads with glycine for as little time as possible; Add 1 mL 100 mM glycine, pH 3.0, flick to mix and spin 12,000g x 30 sec. Remove supernatant and wash once with 1 mL 100 mM Tris pH 8.0.
8. Wash beads in PBS, ensuring pH \sim 7.4. Resuspend the beads in 1x bed volume in PBS and add Na Azide to 0.02%. Matrix is stable at 4°C for > 1 year.

Conjugation Buffer:

50 mM Tris, pH 7.6 - 4,626 μ L
150 mM NaCl (2M stock) - 375 μ L
2% BSA - $\frac{100 \text{ mg}}{5 \text{ mL}}$

0.2 M Na Borate Buffer:

Na Tetraborate decahydrate (FW 381.37) - 3.051 g per 40 mL H₂O.
-Heat-dissolve prior to use. Adjust pH to 9.0.

0.2 M Ethanolamine:

Ethanolamine (FW 61.08 g/mol, ρ 1.015 g/mL, >98% is 16.62 M)
Add 301 μ L to 20 mL H₂O. Adjust pH to 8.0 (caution, pH drops quickly from pH ~ 8.0).
Bring up to 25 mL final volume.