

Covalent Coupling of Antibodies to Protein A Beads

1. Wash desired amount of Bio-Rad Affi-prep Protein A support (Catalog #153-6153) with 1X PBS three times
2. Add antibody (1 mg antibody per 1 mL solid beads) in 1X PBS (~3-5 mL total)
3. Incubate gently rotating at room temperature for 1 hr
4. Wash beads twice with 0.2 M sodium borate pH 9.0
5. Resuspend the beads in 10 volumes of 0.2 M sodium borate pH 9.0
 - a. Take equivalent of 10 μ L beads as uncoupled sample #1 and add to 4X SDS-PAGE sample buffer
6. Add enough dimethyl pimelimidate powder (Sigma catalog #D8388) to bring the final concentration to 20 mM (51.8 mg into 10 mL)
7. Incubate gently rotating at room temperature for 30 min
 - a. Take equivalent of 10 μ L beads as coupled sample #2 and add to 4X SDS-PAGE sample buffer
8. Stop the reaction by washing once in 0.2 M ethanolamine pH 8.0
9. Incubate in 0.2 M ethanolamine pH 8.0 for 2 hr at room temperature gently rotating
10. Wash beads three times in 1X PBS
11. Resuspend the beads in 1X PBS containing 0.01% sodium azide
12. Store at 4 °C

Check the efficiency of coupling by boiling the collected samples, SDS-PAGE, and coomassie staining. You should observe antibody (heavy chain at ~50 kDa) in sample #1, but not #2.