Covalently Conjugate Antibody to Beads
A Lamond Lab protocol (www.lamondlab.com; 2007)

Bind antibody to beads at desired concentration.

__Note:__ Different matrices have different binding capacities (e.g. protein G sepharose can bind antibodies at concentrations of up to 6 mg/ml). It is not essential to saturate the beads with antibody for most applications (it can be very expensive, and a waste of antibody). We first test antibodies bound to beads non-covalently, to determine what concentration we want to bind to the beads and how many beads we will need to use per IP, to deplete our protein.

__Very Important Note:__ Until recently, we were binding anti-GFP antibodies to protein G sepharose at 0.2 mg/ml for our SILAC-based proteomics experiments. This protocol has been working fine, but we have now altered it to try to minimize contaminants that we know are sticking mostly to the beads rather than coming down with the tag alone (our built-in negative control). Although the quantitation step allows us to easily identify these contaminants, we would prefer to not have them in our sample at all! For that reason, we now bind the antibody at a higher concentration (2 mg/ml) for all proteomics experiments, to minimize contaminants.

e.g. for anti-GFP (Roche), I bind 500 ul (200 ug) to 0.1 ml of washed protein G sepharose beads 4 h or overnight in a 1 ml Eppendorf tube. The anti-GFP was resuspended in 0.5 ml dH2O and left on ice for 30 min first, and the protein G sepharose beads were washed several times in PBS to remove the 20% ethanol in which they're stored.

Wash beads well in buffer of choice (PBS, Tris, etc.) to remove unbound antibody.

Wash beads 2X with 10 volumes of 0.1 M sodium borate, pH 9 (i.e. 1 ml of borate buffer for the 0.1 ml of anti-GFP beads).

Make up 10 volumes of borate buffer containing 20 mM DMP (dimethyl pimelimidate from Sigma…D8388-250 mg…store vial in freezer and use only once).

** What I do is weigh out the amount of DMP that I need for that volume (e.g. 5.2 mg in 1 ml borate buffer makes a 20 mM DMP solution), and then add the borate buffer right before I'm ready to use it. Weigh the same amount into another tube, but don't resuspend in buffer yet (put lid on tube tightly)…you'll need it for the second DMP step).

Spin down beads and resuspend in 1 ml DMP/borate solution.

Shake or mix end-over-end for 30 min at room temperature.

Spin down, remove buffer and resuspend beads with 1 ml fresh DMP/borate solution (i.e. add the 1 ml borate buffer to the extra tube of 5.2 mg DMP you weighed out and use this as the fresh buffer).

Shake or mix end-over-end for 30 min at room temperature.

Spin down, remove buffer and wash beads 2x with 10x 50 mM glycine, pH2.5 (i.e. 1 ml of 50 mM glycine, twice). I keep a stock of 1 M glycine, pH2.5 at 4C and make up this 50 mM wash buffer fresh every time.

Wash several times in final buffer of choice (PBS, Tris, etc.) and store at 4C in a 50% slurry (e.g. 0.1 ml beads plus 0.1 ml PBS).