Coupling Py antibody to agarose beads

Use Pharmacia GammaBind Plus Sepharose (cat # 17-0886-02). Binding capacity is 18 mg/ml but I have never tried to bind antibody to this extent. The binding capacity of Pharmacia Protein G Sepharose is > 20 mg/ml but GammaBind is supposed to bind tighter to antibodies and the linkage is protease resistant.

Wash 5 ml (50% slurry) with 3 X 25 ml PBS in 50 ml conical tube. Spin to pellet beads in clinical centrifuge 3 K for 2 minutes.

Add 2.5 ml 5 mg/ml purified Py antibody and allow to bind for 2 hours by rotating end over end at room temperature.

Pellet beads in clinical centrifuge; save flow-through.

Wash beads 2 X 35 ml with 0.2 M Na Borate pH 9; save each wash.

Resuspend beads in 25 ml 0.2 M Na Borate pH 9 and remove 100 μl suspension for gel.

Add dimethyl pimelimidate (Sigma D-8388) to 20 mM (129.5 mg/25 ml).

Mix 30 minutes at room temperature; remove 100 μl suspension for gel.

Wash beads with 25 ml 0.2 M ethanolamine pH 8.

Incubate beads for 2 hours in 25 ml 0.2 M ethanolamine pH 8.

Wash beads 3 X with PBS and store at 4 C in 2.5 ml PBS + 0.01% merthiolate.

Check coupling on 12% polyacrylamide protein gel by coomassie staining; load 20 μl samples from each step in coupling procedure.

PBS, 1 liter
8.0 g NaCl
0.2 g KCl
1.44 g Na2HPO4
0.24 g KH2PO4
adjust pH to 7.2 with 1 M HCl, autoclave

0.2 M Na Borate pH 9, 0.2 liter
2.47 g boric acid (USB 1748B-3)/200 ml water, adjust pH with NaOH
(will not dissolve until pH is adjusted)

0.2 M ethanolamine pH 8, 0.1 liter
1.95 g ethanolamine (Sigma E-6133)/100 ml water, adjust pH with 1 M NaOH