ANTIBODY PURIFICATION ON THE PROTEIN G COLUMN

Protein G rather than Protein A is the column of choice for purifying mouse IgGs from ascites, because mouse IgG1 binds much (much!) better to Protein G, and most mouse mAbs turn out to be IgG1. However, Protein G cannot be used to purify from tissue culture supernatants containing fetal calf serum, because it has a very high affinity for the bovine IgGs in the serum. (Rat IgGs don't bind well to either Protein G or Protein A, and should probably be purified on an FcRn column.)

Column we currently have: Pharmacia Hitrap Protein G (5 ml gel volume). It is convenient but not in any way necessary to run this on the FPLC.

Capacity: I have never gotten more than about 7.5 mg mouse IgG off this column, even when overloading with ascites (i.e. antibody left in the flow-through), but I've only tried a few different antibodies. 2 or 3 ml ascites is usually a good amount to try first.

Flow rate: 1 ml per minute seems to work fine; I haven't researched if higher is OK.

Buffers: **Binding (A): 50 mM NaPhosphate, pH 6**
500 mM NaCl
0.05% NaAzide

(Per Luis, including the NaCl and lowering the pH to 6 improves binding.)

Elution (B): 0.1 M glycine, pH 2.7

Yes, I know Pharmacia recommends 0.1 M citric acid, pH 3, **but there's a good chance it won't elute your antibody!** 1G3 (anti-FcRn) for example barely comes off at all at pH 3, but elutes perfectly nicely at 2.7 (and I have the trace to prove it). So don't let anyone talk you into using pH 3 with a new antibody - or at least run some pH 2.7 buffer over the column after the pH 3 the first time to make sure no more is eluted.

There is no azide in the elution buffer because even 0.05% causes a significant absorbance (> 0.1) at 280 at low pH. It's very weird, but I also have the data to prove this.

Column run:

1. Clean the column with your elution buffer (30 - 50 ml). This is very important to make sure you don't elute any leftoever antibody from someone else's previous run along with your sample.
2. Equilibrate with binding buffer (15-30 ml).
3. Load your sample.
4. Wash with binding buffer until the trace returns to baseline (15-30 ml).
5. Elute - 20 1 ml fractions is usually enough. Peak usually starts around fraction 8. Use 50 ul 1M dibasic NaPhosphate/1 ml fraction to neutralize the pH. Tris can also be used if the antibody will not be coupled to CNBr beads.
6. Re-equilibrate column with binding buffer.
7. When done, wash column with 20% ethanol (15 ml) for storage.

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