Purification of Rabbit/Sheep bleeds on Protein A/Protein G columns

POLYCLONAL SERUM ~ 10 mg/mL total IgG (Maximum 10% of specific antibody)

COLUMN CAPACITY ~ 10-20 mg total serum/mL wet beads

1. Pre-wash 0.5 mL of beads 3x with PBS in a 15 mL falcon tube to remove the ethanol solvent (wash, centrifuge and remove supernatant, repeat, etc).
2. Incubate the beads with 1 mL of serum for 1-2 hours at RT with fluent agitation.
3. Remove the unbound serum from the beads and wash 2x with PBS.
4. Wash 1x with PBS + 0.5 M NaCl (high salt wash) and then 1x with PBS.
5. Put the beads in a column and let the PBS elute by gravity.
6. Add 0.5 mL fractions of 100 mM Glycine pH 2.5 (fresh!!!) to elute bound antibodies.
7. Neutralize each fraction with 0.1V of 1 M Tris-HCl pH 8.0 that is already in the recovery tube (final pH ~8).
8. Check the protein concentration of the eluted sample with Bio-Rad Bradford reagent:

   Dilute 10 μL of sample in 200 μL of Bradford reagent and water to 1 mL

   OD (595nm) ~ 0.45 means that the original sample is ~1mg/mL

   If starting from different amount of sample just have to adjust the dilution factor.

   Typically, the first 3 fractions contain most of the eluted antibody.

9. Put the eluted antibodies in a Millipore filter and concentrate by centrifuging at maximum speed (4 oC).
10. Repeat the concentration 3x after addition of PBS (or injection buffer) to replace the solvent and get the antibodies in a physiological buffer. Pool the fractions to achieve desirable concentration
11. Store at –80 oC or at –20 oC in 50% glycerol.