IgG purification using Protein A or G Column Chromatography

Please note – this protocol has been optimised for certain conditions and is meant as a guide only, you may need to alter some parameters to suit your own experiment

This is a general method applicable for purification of IgG from tissue culture supernatant or serum, using either Protein A or Protein G coupled to Sepharose. Note that human IgG3, mouse IgG1 and all isotypes of rat, chicken, horse and sheep IgG do not bind well to Protein A and these antibodies should be purified using Protein G. This protocol is for a 5ml column volume, but can be scaled up for larger purifications, providing that the volume of wash buffers etc is scaled up accordingly.

Note*: Ensure that the Protein A or G, and all buffers, are at room temperature before beginning*

1. Assemble a suitably-sized column with a tap on the bottom and a feeder line going in to the top. Ensure there is a filter disc at the bottom of the column so that the beads don't pass straight out of the column
2. Rinse the empty column through with about 5 ml of 100 mM Tris-HCl, pH 8.0, to evacuate air bubbles from the filter disc. Close the tap with a 2ml of Tris still in the column.
3. Mix the Protein A- or G-coupled Sepharose into a slurry, then slowly and gently pipette into the column, allowing the slurry to run down the side of the column to prevent air bubbles forming
4. Allow the buffer to run out, then apply approx 20 ml of 100 mM Tris-HCl, pH 8.0, to equilibrate. If buffer contained a preservative such as ethanol, enough Tris must be run through to remove all traces
5. Equilibrate serum or supernatant with a 1/20 volume of 2 M Tris-HCl, pH 8.0. It is important that the pH of the sample is raised to 8.0, as some IgGs will not bind well at pH 7.4
6. Allow the level of buffer to drop to the top of the gel bed, then apply serum or supernatant to the column through a 45 μm filter. Filtering the sample in this way will prevent the column from becoming clogged up with particulate matter
7. Collect the unbound fraction in 20 ml bottles and retain in case something goes wrong with the purification
8. Allow the level of liquid to drop to the top of the gel bed, then wash with 25 ml of 100 mM Tris-HCl, pH 8.0, then with 25 ml of 10 mM Tris-HCl, pH 8.0. Collect the waste in 20 ml bottles, except for the last 2 ml, which should be collected separately and tested for absorbance at 280 nm. If absorbance is 0, then proceed with elution. If not, then wash further.
9. During washing, prepare approx 20 eppendorfs with 30 μl of 2M Tris-HCl, pH 8.0, for neutralisation of antibodies as they are eluted of the column in acid
10. Allow the level of Tris to drop to the top of the gel bed, then add 10 ml of 0.1 M glycine, pH 3.0, to elute bound antibodies from the column

11. Allow the first 2 ml to run off, then begin collecting 500 µl fractions into the pre-prepared eppendorfs. Check the pH of a few of the fractions by spotting 15 µl onto a pH test strip, to ensure the acid has been effectively neutralised. If not, add more 2 M Tris.
12. Wash the column with 15 ml of 0.1 M glycine, pH 2.6, then immediately re-equilibrate with 15 ml of 100 mM Tris-HCl, pH 8.0.
13. To prevent contamination, store the column at 4°C in 100 mM Tris-HCl, pH 8.0 containing 20% ethanol
14. Determine the OD^{280} of the fractions. Start out by testing the earliest fractions neat, then as soon as one fraction gives an absorbance reading above background, test subsequent fractions at a 1:5 dilution. Return to testing fractions neat when the absorbance readings drop below about 0.3, and continue only until the readings have returned to baseline.
15. Pool protein-containing fractions and dialyse against PBS, or an appropriate labelling buffer if the IgG is to be immediately labelled

Notes: Strongly bound proteins, lipoproteins and lipids can be removed by washing the column with 5ml of 0.1% Triton X-100 at 37°C. Ensure that the time the gel is in contact with the detergent is kept to a minimum

Although the column can be used multiple times without re-packing, the flow rate will get significantly slower as the gel packs down, therefore to speed up the process, the column should be re-packed each time. To remove the beads from the column, close the tap with about 5 ml of Tris at the top of the column, then use a plastic pasteur pipette to resuspend the gel into a slurry. Transfer the slurry to a tube, then add more Tris and repeat the process until all beads are removed. The column can then be re-packed as from Step 1