Purification of IgG Using Protein A- or Protein G-Agarose

The following protocol is a simple, reliable method for purifying total IgG from crude protein mixtures such as serum or ascites fluid. Protein A binds to the Fc portion of IgG. Protein G binds preferentially to the Fc portion of IgG, but can also bind to the Fab region, making it useful for purification fo $F(ab')_2$ fragments of IgG_1. Some species and IgG subtypes bind differentially to Protein A or Protein G. Refer to Table 2 for recommendations on which resin to use.

Procedure: Purification of IgG Molecules

1. **Buffer Preparation:**
   Prepare all buffers necessary for this procedure prior to starting.
   a. **Wash/Binding Buffer:** Dilute the wash/binding buffer 1:5 in dH_2O in a clean container (e.g. 20 mL buffer concentrate + 80 mL dH_2O). Prepare enough diluted buffer based on the sample quantity to be dialyzed and ~30X the quantity of agarose used for purification. Store prepared buffer up to 1 week at 2 - 8°C. Final 1X concentration of buffer is 0.1M Sodium Phosphate, 0.15M NaCl, pH 7.4.
   b. **Elution Buffer:** Dilute the elution buffer 1/10 in dH_2O in a clean container (e.g. 10 mL buffer concentrate + 90 mL dH_2O). Prepare enough diluted buffer based on 4X the quantity of agarose used. Store prepared buffer up to 1 week at 2 - 8°C. Final 1X buffer concentration is 0.2M Glycine, pH 3 ± 1.85.
   c. **Storage Buffer:** Ready to use.

2. **Sample Preparation**
   To ensure that proper ionic strength and pH are maintained for optimal binding, it is necessary to dilute serum samples, ascites fluid or tissue culture supernatant at least 1/1 with binding buffer. Alternatively, the sample may be dialyzed overnight against wash/binding buffer. KPL recommends using a 12,000 MW cut-off dialysis tubing with at least two buffer exchanges. Remove any particulate matter from the sample by centrifugation or filtration through a 0.8 μm filter.

2. **Column and Resin Preparation**
   a. Pour 20% Ethanol in the bottom of a petri dish or in a flat bottom container. Float the frit on top of the ethanol. Using the large round end of a 1 mL pipette tip, press the frit firmly into the ethanol to force air out. Repeat this step until the frit is completely wet.
   b. Push the frit into the barrel of the column until it rests firmly on the bottom.
   c. With the cap removed, clip the end of the column to create a hole to allow liquid to flow through.
   d. Wash the frit with 5 column volumes of 1X Wash/Binding Buffer.
   e. Prepare a 1/1 suspension of resin in 1X Wash/Binding buffer. The required amount of agarose per mg immunoglobulin to be purified can be estimated by the binding capacity.

Recommended Column Volumes

Antibody Source	Recommended Bed Volume (mL) per mL sample
Immune Serum	2 mL
Tissue Culture Supernatant (with 10% fetal bovine serum)	0.2 mL
Tissue Culture Supernatant (serum-free)	0.01 mL
Ascites fluid	2 mL

   f. Pour slurry into column. Allow column to flow by gravity to pack the column bed.
   g. Equilibrate the packed affinity resin with 10 column volumes (CV) of the wash/binding buffer. For example, if the packed bed is 1 mL, equilibrate with 10 mL Wash/Binding Buffer.

4. **Sample Purification**

   a. Gently apply sample to the column by layering onto the top of the resin. Be careful not to disturb the bed surface.

   b. Wash column with 10 CV of the 1X Wash/Binding buffer, or until the absorbance of eluate at 280 nm approaches the background level.

   c. Before beginning the elution step, set up enough tubes to collect the entire elution volume as 1 mL fractions (4 CV will be used to elute the antibody). To each collection tube add 240 mL 5X Wash/Binding Buffer. To elute the antibody, gently add 1 mL of 1X Elution Buffer to the top of the resin, collecting the eluate in a prepared collection tube. Repeat until the entire volume has been collected, up to 4 column volumes. **Note:** If the eluate is to be collected in a single bulk volume, add 240 mL 5X Wash/Binding Buffer per mL Elution Buffer to the collection vessel before starting the elution. Elution of bound immunoglobulin can be monitored by absorbance at 280 nm, if desired.

5. **Column Regeneration**

   Once the sample has been eluted, wash the affinity matrix with 2 CV of elution buffer. Re-equilibrate the column with at least 10 CV of 1X Wash/Binding Buffer. When column is equilibrated, pH of eluate will be the same as that of the wash/binding buffer.

6. **Clean-in-Place**

   With certain applications, substances which contain denatured proteins or lipids do not elute in the regeneration procedure. The following steps can be taken to clean the column:

   a. To remove strongly bound hydrophobic proteins, lipoproteins and lipids, wash the column with a non-ionic detergent (e.g. 0.1% Triton X-100) at 37°C, with a contact time of ~1 minute.

   b. Immediately re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer.

   c. As an alternative, wash the column with 70% ethanol. Allow the column to stand for 12 hours. Re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer.

   d. To remove precipitated or denatured substances, wash the column with 2 CV of 6M guanidine hydrochloride. Immediately re-equilibrate the column with 5 - 10 CV of 1X Wash/Binding Buffer (see step 5).

7. **Resin Storage**

   Store affinity matrix in Storage Buffer at 2 - 8°C. **Do not** store the matrix frozen or at room temperature. The matrix can be stored in the column by sealing the outlets or removed from the column and stored as a slurry.

Table 1. **Relative Affinity of Immobilized Protein A and Protein G for Various Antibody Species and Subclasses of Polyclonal and Monoclonal IgG's**[2].

Species/ Subclass	Protein A	Protein G
Monoclonal		
Human		
IgG_1	++++	++++
IgG_2	++++	++++
IgG_3	—-	++++
IgG_4	++++	++++
Mouse		
IgG_1	+	++++
IgG_{2a}	++++	++++
IgG_{2b}	+++	+++
IgG_3	++	+++
Rat		
IgG_1	—-	+
IgG_{2a}	—-	++++
IgG_{2b}	—-	++
IgG_{2c}	+	++
Polyclonal		
Rabbit	++++	+++
Cow	++	++++
Horse	++	++++
Goat	-	++
Guinea pig	++++	++
Sheep	+/-	++
Pig	+++	+++
Rat	+/-	++
Mouse	++	++
Chicken	—-	+
Human IgG	++++	++++
Human IgM	—-	+
Human IgD	—-	+
Human IgA	—-	+

— (weak or no binding) ++++ (Strong binding)

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