9. *Do I need to control the salt concentrations during Protein A and G chromatography?*
Use 0.1-0.5 M salt to reduce non-specific adsorption. When working with Protein A, use high salt (2-3 M NaCl) with high pH to promote the binding of mouse IgG1.

10. *Is pH an important parameter to control during Protein A and G chromatography?*
The elution pH is the most critical variable. Protein G usually requires more acidic pH conditions to desorb the target immunoglobulins. For Protein A, elution by pH steps (starting at pH 6) may fractionate different species (weaker binding bovine IgG from target antibodies) or subclasses. High pH (pH 8-9), in conjunction with high salt may promote binding of mouse IgG1 to Protein A. The binding buffer pH should normally be higher than pH 6.0-7.0.

11. *Can I elute antibodies from a Protein A and G column using divalent cations?*
Concentrations of divalent cations (particularly Mg^{2+}) up to 1 M can sometimes replace acidic pH if there is concern about loss of activity of acid-labile immunoglobulins.

Troubleshooting Assistance:

Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. The resin should be warmed slowly to room temperature before use.

The sample does not flow easily through the column

- The resin is clogged with particulates. Pre-filter the sample just before loading it on to the resin. . Ascites must be delipidated before use (see earlier procedure).
- If the resin is not stored at 2-8 ˚C, or they have been used more than once and stored in the absence of a bacteriostat, microbial growth may restrict flow through the resin.

No elution of the target protein is observed from the column

- The pH of the elution buffer may be incorrect. It is advisable to prepare new solutions.
- The elution conditions are too mild to desorb the target protein.

The recovery of target protein is low

- The binding of antibodies to Protein A or G is attributed in part to hydrophobic forces. Use chaotropic salts to reduce the strength of all hydrophobic interactions.

Poor resolution of the target protein

- The sample volume or concentration may be too large for the capacity of the resin. In this case, reduce the sample load or sample volume.
- The sample may also need to be filtered carefully.

The target protein elutes at an unexpected position

- There may be an ionic interaction between the protein and resin. You should maintain the ionic strength above 50 mM.
- There may be hydrophobic interactions between the sample and resin. In this instance, reduce the salt concentration and add suitable detergents or organic solvents.
- The column may be dirty.

The elution profile cannot be reproduced

- The nature of the sample may have altered and so it may be important to prepare a fresh sample.
- The sample load may be different from the original sample load. It is advisable to keep all these parameters constant.
- Proteins or lipids may have precipitated in the resin bed. Use elution conditions which stabilize the sample.
- The buffer pH and ionic strength is incorrect and new buffers will need to be prepared.

Glossary:

affinity chromatography - chromatographic separation based on a specific interaction between an immobilized ligand and a binding site on a macromolecule.

antibody - an immunoglobulin produced by the immune system of vertebrates in response to exposure to a foreign substance.

antigen - a molecule which can bind specifically to an antibody.

antiserum - the serum fraction from an animal that has been immunized or exposed to an immunogen and contains antibodies to a particular antigen.

ascites - a liquid tumour formed by injection of a hybridoma cell line into the peritoneal cavity. It is a common source of monoclonal antibodies from mice.

bed volume - the total volume occupied by the chromatographic packed bed. It is also referred to as the column volume or CV.

cell culture supernatant - the fluid made during cell culture (either roller bottle, suspension or perfusion) containing tissue media components and the secreted target.

chaotropic agent - a molecule which interferes with hydro-phobic interactions by disrupting the ordered structure of water molecules. Examples include urea and guanidine.

hybridoma - a hybrid cell line produced by fusing antibody producing cells with myeloma cells to generate immortal reproducing cells that produce specific monoclonal antibodies indefinitely in cell culture.

immobilized - bound to a surface, usually through covalent linkages.

immunoglobulin (Ig) - comprising 5 distinct classes in most higher animals. Classes called IgG (the most common), IgM, IgA, lgD and IgE. They differ from each other in size, charge, amino acid composition and carbohydrate content.

ion exchange chromatography - chromatographic separation based on different charge properties of macromolecules.

isoelectric point - the pH at which the protein has no net charge.

monoclonal antibody - an antibody derived from a single clone of immune cells. They are usually formed from a hybridoma cell line.

polyclonal antibodies - antibodies produced to the same immunogen by different cell types. Antibodies from antiserum are almost always polyclonal.

protein A/protein G - cell wall proteins of certain pathogenic bacteria which specifically bind to the Fc region of immunoglobulins.

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