

## Questions and Answers:

*1. What is the preferred rotor for the Proteus Mini and Midi spin columns?*

**Mini spin columns:** The preferred rotor is a fixed angle rotor. There is no need to orientate the Mini spin column in the fixed angle rotor.

**Midi spin columns:** The preferred rotor is a swing bucket rotors. For optimal performance with a fixed angle rotor, ensure that the orientation of the spin column in the rotor is the same for sample binding, washing and elution steps

*2. Do I need to filter the buffers prepared in my laboratory?*

It is good laboratory practice to filter all buffers. However, buffers supplied with the kit are pre-filtered for immediate use.

*3. Do I need to pre-filter my sample before loading it on to a Proteus spin column?*

All samples must be filtered through a 0.2  $\mu\text{m}$  pre-filter **immediately** before loading the samples on to the spin column.

*4. What are the typical binding capacities of Proteus Mini and Midi spin columns?*

**Mini spin columns:** Protein A and G resin plugs have typical capacities of 1 mg rabbit IgG from serum.

**Midi spin columns:** Protein A and G resin plugs have minimum capacities of 20 mg rabbit IgG from serum.

*5. How should I prepare my sample for the Proteus spin column?*

Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. Protein A affinity separations usually require the sample to be diluted 1:1 (v/v) in 1 x binding buffer. We recommend that all samples are diluted 1:1 (v/v) in the binding buffer supplied with the Protein A or Protein G kit.

*6. How can I process a large volume sample?*

The Mini and Midi spin columns have a finite maximum volume capacity. If you have a volume of sample (>200 ml), we recommend that you use the established procedure of ammonium sulphate precipitation to concentrate your target antibody. Although many IgGs ( $\gamma$ -globulins) precipitate at a lower concentration of ammonium sulphate than most other proteins, 50% ammonium sulphate is sufficient. Please visit the Pro-Chem website for the ammonium sulphate protocol.

*7. What is the maximum volume of solution I can load on to a Mini or Midi spin column?*

**Mini spin columns:** You can load a maximum volume of 0.65 ml.

**Midi spin columns:** You can load up to 20 ml in a swing bucket rotor and up to 10 ml in a fixed angle rotor.

*8. What is the highest speed that I can spin the Proteus Mini and Midi spin columns?*

**Mini spin columns:** Although the spin columns have been tested at 11,960 g (13,000 rpm in a fixed angle rotor with an average radius of 49 mm), we do not recommend spin speeds greater than 5,000 g. At very high speed, you may observe gel shrinkage away from the side walls. This will not affect the performance of the spin columns as the gel will rehydrate rapidly in subsequent spin steps.

**Midi spin columns:** There is no need to spin the devices at speeds greater than 1,250 g. No performance data is available at centrifugal speeds greater than 1,500 g.

*9. Is there a minimum spin speed for the Mini and Midi spin columns?*

There is no minimum spin speed for either then Mini or Midi spin columns. The devices can be spun at speeds as low as 50 g.

10. *What are the minimum elution volumes from the Proteus spin columns?*

**Mini spin columns:** The minimum elution volume is 0.5 ml.

**Midi spin columns:** The minimum elution volume is 5 ml.

11. *How many times can I re-use the Proteus Mini and Midi spin columns?*

**Mini spin columns:** Assuming that all samples are correctly filtered, sufficient buffer is provided in the kit for 3 re-uses of each Mini spin column.

**Midi spin columns:** Each Midi plug can be re-used typically up to 5 times. There is sufficient buffer volume in the kit for one complete use of each Midi spin column.

12. *How can I regenerate the Proteus Protein G plug?*

**Mini spin columns:** We recommend that you wash the plugs with 2 x 0.65 ml elution buffer B2 (pH 2.5) by centrifuging the spin columns at 1,800 g for 1 min. Then, re-equilibrate the plugs with 2 x 0.65 ml binding buffer A by centrifuging

the spin columns at 1,800 g for 1 min. Proceed to the pre-equilibration step if plugs are to be re-used immediately. Do note that spin times of used plugs may be longer. After regeneration, plugs can also be stored, without their end caps, in a beaker containing 0.1 % sodium azide (made up in distilled water) at 2-8 °C until further use.

**Midi spin columns:** We recommend that you wash the plugs with 10 ml elution buffer B2 (pH 2.5) by centrifuging the spin columns at 500 g for 3 min. Then, re-equilibrate the plugs with 10 ml binding buffer A by centrifuging the spin columns at 500 g for 3 min. Proceed to the pre-equilibration step if plugs are to be re-used immediately. Do note that spin times of used plugs may be longer. After regeneration, plugs can also be stored, without their end caps, in a beaker containing 0.1 % sodium azide (made up in distilled water) at 2-8 °C until further use.

*13. Do I need to perform regeneration of the spin column immediately after the elution step?*

We do not recommend storing the resin columns in elution buffer pH 2.5. Prolonged storage in pH 2.5 buffer could harm the Protein A ligand. After the elution step, continue to the regeneration procedure. The resin plugs can then be stored for re-use.

*14. Can I autoclave the Proteus Protein A and Protein G plugs?*

The Proteus Protein A or G plugs cannot be autoclaved.

*15. If I experience significant fouling of the resin plug, do you recommend any cleaning-in-place procedures?*

Most suitable cleaning procedures tend to be determined empirically. The chosen procedure depends largely upon the nature of the previous sample loaded on the spin column. Consult Pro-Chem's website for some CiP procedures.

*16. Should I be concerned if the plugs partially dry out during the centrifugal steps?*

The plugs are robust. Partially dried plugs rehydrate rapidly. There are no adverse effect upon the performance of the plugs.

*17. Do I need to be careful with the type of chaotropic ion I use when eluting antibodies from affinity columns?*

It is recommended to use the mildest chaotropic agents at the lowest possible concentration that will ensure rapid elution and high recovery of activity. Iodination reactions employing either chloramine T or IODO-GEN (1,3,4,6-tetrachloro-3-6-diphenylglycouril) are particularly sensitive to inhibition by low concentrations of thiocyanate ions and, if antibodies are eluted from affinity columns or Protein A affinity columns, using this chaotropic ion, it is essential that they are dialyzed thoroughly after elution to remove thiocyanate ions.

*18. How do I monitor purity of the isolated antibodies?*

Purity is best measured by gel electrophoresis. When analyzed by SDS-PAGE under non-reducing conditions, IgG antibodies should give a single protein band of about 160-170 kDa. On reduction with DTT or 2-mercaptoethanol, two or more bands will be seen corresponding to the individual heavy chains (50-55 kDa) or light chains (25-30 kDa). Other protein bands that are visible only on reduction may point to proteolytic action. This can often be prevented by careful use of protease inhibitors in culture supernatants before storage.

*19. What are the critical starting conditions for Protein A and G Proteus spin columns?*

Sample pH and salt concentration are usually not critical, except that the pH should be equal to or above 5.0. Protein A or G spin columns can also serve as tools for rapid buffer exchange. However, the salt concentration is normally quite high (> 0.1 M) to prevent non-specific binding.

*20. 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.

*21. 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.

*22. Can I elute antibodies from a Protein A and G spin 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.