PROTEUS

Questions and Answers:

1. What is the shelf-life of a Proteus spin column?
The spin columns and resin plugs are guaranteed for 2 years after the date of manufacture provided they are stored at 2-8°C.

2. 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 rotor. 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.

3. 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.

4. Do I need to pre-filter my sample before loading it on to a Proteus spin column?
All samples should be filtered through a final 0.2 μm syringe filter just before the sample loading step.

5. What are the binding capacities of Proteus Mini and Midi IMAC spin columns?
Mini spin columns: Protein IMAC resin plugs have typical capacities of 1 mg His-tagged protein. **Midi spin columns:** Protein IMAC resin plugs have typical capacities of 10 mg His-tagged protein.

6. How should I prepare my sample for the Proteus spin column?
Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. We recommend that all samples are pre-filtered down to a 0.2 μm pore size. High viscosity is mostly attributed to contaminating DNA or RNA. The intrinsic viscosity of a lysate can be reduced by either drawing it through a syringe needle several times or by adding appropriate amounts of DNase and/or RNase (5-10 μg/ml) to the lysis buffer and incubating the mix on ice for 15 mins.

7. Should I add β-mercaptoethanol to the lysis buffer?
Reducing agents can reduce the resin matrix and adversely affect binding of the His-tagged protein to the spin column. Its inclusion depends upon whether the His-tagged protein elutes with contaminants as β-mercaptoethanol can reduce all disulphide bonds formed between the contaminating proteins and the target protein. Concentration less than or equal to 10 mM β-mercaptoethanol can be used with Proteus IMAC resin spin columns. Do not use strong reducing agents such as DTT or DTE as these tend to reduce the metal ion, which will lower the binding efficiency of the IMAC column.

8. 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. Multiple 0.65 ml or 20 ml loads can be performed with low experession systems.

9. What is the highest speed that I should 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 1,000 g. No performance data is available at centrifugal speeds greater than 1,250 g.

10. Is there a minimum spin speed for the Mini and Midi spin columns?
There are no minimum speeds for either the Mini or Midi spin columns. The devices can be spun at speeds as low as 50 g.

11. Why are the sample loading steps for the Mini and Midi spin columns extended to 6 min and 30 min respectively?

The metal chelate resin plug incorporates a technologically-advanced flow regulator which is designed to control the flow rate of the samples through the active column matrix. Observed yields and purities fluctuate as a direct function of the flow rate of the sample loading step. The flow regulator is pre-set by Pro-Chem to slow down the flow rate to an optimal capture speed. The concomitant increase in the residence time of the target protein with the matrix of the spin column increases substantially the yield and purity of the purified target protein. However, unlike many other chromatography systems, there are negligible hold-up volumes and His-tagged protein elution to recover the purified target protein is rapid for both Mini (1 min) and Midi (3 min) columns.

12. 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 4 ml.

13. How can I regenerate the Proteus IMAC plugs?

Mini spin columns: We recommend that you wash the plugs with 2 x 0.65 ml elution buffer 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 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. After regeneration, plugs can also be stored, without their end caps, in a screw-capped Falcon tube 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 by centrifuging the spin columns at 500 g for 3 min. Then re-equilibrate the plugs with 10 ml binding buffer 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. After regeneration, plugs can also be stored, without their end caps, in a screw-capped Falcon tube containing 0.1 % sodium azide (made up in distilled water) at 2-8 °C, until further use.

14. Can I autoclave the Proteus IMAC plugs?

The Proteus IMAC plugs cannot be autoclaved.

15. Can I immobilize the metal chelate resin with a different metal ion?

Ensure that the resin is stripped of Ni^{2+}. This is achieved by successive washing with 10 column volumes of (i) 0.2 M EDTA, 0.5 M NaCl (ii) 0.2 M NaOH (iii) distilled water and finally (iv) 0.1 M metal salt.

16. What can I do if the resin has changed colour?

The blue colour is attributed to the Ni^{2+} salt. Reductants will cause the resin to turn brown and chelating agents will cause the resin to turn white. Ensure that all solutions are compatible with the Ni-IDA resin.

17. How can I re-charge the plug with $NiSO_4$?

Wash the Midi plugs with 20 ml distilled water followed by 20 ml washes with 0.1 M $NiSO_4$ solution (made up in distilled water). Wash off any unbound $NiSO_4$ with 20 ml distilled water and equilibrate the plug with 20 ml 1 x PBS buffer, pH 7.4. Spin the Midi plug at 500 g for 3 min for all above steps. Use appropriate buffer volumes for re-charging Mini plugs and spin the Mini plug at 1,800 g for 1 min.

18. How can I ensure that levels of contaminants in the final eluate remain low?

We recommend that the binding buffer contains minimum 10 mM imidazole and the wash buffer contains minimum 20-30 mM imidazole.

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

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

20. Should I remove imidazole after the final elution step?

You should always remove imidazole if the protein is going to be stored. Otherwise, the protein may precipitate out of solution at –20 or –80 °C.

21. Can I load purified protein immediately on to an SDS-gel?

Proteins purified under native conditions can be loaded on to an SDS-polyacrylamide gel. Those proteins purified under denaturing conditions in 6-8 M urea can also be loaded directly on to a denaturing SDS-polyacrylamide gel. Proteins purified in the presence of 4-6 M guanidine HCl should be buffer exchanged in buffers lacking the denaturant prior to a denaturing SDS-PAGE.

22. Do I need to remove the His-tag from the recombinant protein after purification?

Normally, a protease cleavage site e.g. Factor Xa Protease is engineered between the His-tag and the target protein. The target protein can then be re-purified by passing it through a Proteus Ni^{2+}-IDA spin column in order to purify undigested His-tagged protein. For most applications, it is not necessary to remove the His-tag. However, it is often desirable to remove the His-tag if X-ray crystallography or NMR is to be used to determine the structure of the target protein.

23. Under what circumstances should I re-use the spin columns?

The spin columns can be re-used. Re-use does depend on the properties of your target protein. You may observe that flow rates slow down in successive bind-wash-elute cycles as more samples are progressively loaded on to the columns. In addition, if the plug is not re-charged with Ni^{2+}, binding capacity may be reduced.

24. 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 2 re-uses of each Mini spin column. **Midi spin columns:** Each Midi plug can be re-used typically 2 times without the need for Ni^{2+} re-charging. There is sufficient buffer volume in the kit for 1 complete use of each Midi spin column.