Instructions for using IMAC/Poros® MC Material

Selecting and Loading the Metal Ion:

The metal ion loaded on the imidodiacetate chelating groups on POROS MC has a critical effect on both the binding strength and selectivity for different proteins. Consider the following information as you choose a metal ion:

- The most commonly used ion, in order greatest to lowest binding strength, are: Cu^{2+}, Ni^{2+} and Co^{2+} (these last two ions are of about equal binding strength)
- You can also use other metal ions, including Fe^{3+} and Al^{3+}. Fe^{3+} is unusual in that, unlike other ions, elution can often be effected by increasing pH or increasing concentrations of salt.
- Use Cu^{2+} for initial studies when nothing is known about the characteristics of the protein of interest. Cu^{2+} tightly binds to any proteins that will work with metal chelate chromatography.

Then substitute other metal ions to increase selectivity for the protein of interest or key contaminants, or to reduce binding strength for improved recovery.

Saturating the Imidodiacetate Sites:

Make sure that the imidodiacetate sites on the column are fully saturated with the proper metal. To do this:

1. Before loading the metal, perform a stripping wash with 10 to 20 column bed volumes of 50mM EDTA in 1M NaCl.
2. Wash with 5 to 10 column bed volumes of water.
3. Depending on pH levels, do the following:
   - Load metal ions as sulfate or chloride salts in weakly acidic solutions (pH 4.5 to 5) to avoid precipitation of metal hydroxide complexes.
   - Load Fe^{3+} under more acidic conditions (pH 2 to 4) because of solubility difficulties at higher pH.

   The concentration of the metal salt is not critical (0.05 to 0.25M concentrations are generally used).
4. Load at least 0.25mM metal/mL column volume (50mL of 0.1M for a 4.6mmID x 100mmL (1.7mL) column).
   With a Cu and Ni, you can observe metal breakthrough visually or with a UV detector.
5. Wash with 5 to 10 column bed volumes of water to remove excess metal.
6. Wash the column with 5 to 10 column bed volumes of 0.1 to 0.5M NaCl to remove metals that me be bound ionically.
7. Wash with 5 to 10 column bed volumes of starting buffer.

NOTE: Wash thoroughly with salt solution between metal loading steps. Any condition with free metal ions in solution can lead to precipitation and column plugging. If plugging occurs, wash the column at a low flow rate with dilute acid to re-dissolve the metal.

Stripping and Reloading the Column:

Whether or not you should strip and reload the column with metal between runs depends upon the metal used and the elution protocol:

- Reloading is essential with weakly complexing ions such as Zn^{2+} orCo^{2+}.
- Cu^{2+} loaded columns can sometimes be used for many runs with out reloading of metal, unless stripping eluents such as EDTA are used.

In all cases, stripping and reloading after every run gives maximum reproducibility.

Selecting a Buffer and an Elution Method:

Because metal chelate chromatography is not as well developed as more traditional chromatography modes such s ion exchange or reversed-phase, it is virtually impossible to predict retention behavior. As a result, conditions of binding and elution must often be developed by trial and error.

However, very short run times on POROS MC columns make this a development process much faster than with conventional media.

Starting Buffers:

Regardless of the starting buffer and elution method you choose, it is always important to:

1. Use the buffers of the highest purity practical.
2. Degas and filter (.22 or 45µm) all buffers prior to use.
3. Maintain relatively high ionic strength (0.1 to 1.0M) throughout.

Follow these guidelines when you select a starting/wash buffer:

- Starting buffers can be in the range of pH 4 to 8.5. Generally, pH 7 to 8 gives the best results.
- Acetate and phosphate buffers often result in strong binding.

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- Buffers containing primary amines (such as Tris) often weaken binding and can strip metals, but may still be used in some cases.
- 0.1 to 1.0M NaCl is recommended to suppress secondary ionic interactions and proteins/protein interactions.
- If the column is run saturated with elution agent, the starting buffer should also contain 0.5mM of the elution agent.
- Chaotropic agents such as guanidine/HCl or urea may be used if needed.

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