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Pack beaded affinity resin into columns

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Introduction

Chromatographic separation of soluble components in liquid samples by differential affinity to ligands immobilized on a beaded porous resin is fundamental to protein and molecular biology research. We offer a variety of Thermo Scientific Pierce Protein Research Products for affinity chromatography, including complete kits that contain pre-packed columns of specific affinity supports and "stand-alone" resins that are not packed into columns of any kind.

Depending on the scale and type of experiment to be performed, an affinity resin can be used in batch or microcentrifuge spin column format (see Additional Information), or it can be packed into some sort of larger gravity-flow column. We offer several styles and sizes of empty columns (see Related Products) and this Tech Tip describes a general procedure for successfully packing these and other columns with beaded resin for use in gravity-flow and spin purification methods.

Procedure for Assembling and Packing a Column

1. Prepare water or buffer (e.g., Phosphate-buffered saline, PBS) for packing and washing the column. Ensure that excess dissolved air is removed (i.e., degassed) from the solution (see Additional Information Section).
2. Obtain or prepare a 20-50% slurry of the hydrated resin support to be packed.
3. Equilibrate the column, resin slurry, and degassed buffer to room temperature.
4. Position the empty column upright in a clamp or receiver tube with the bottom cap in place.
5. If the column does not already contain a bottom porous filter disc (on which to pack the resin bed), add the disc as follows:
   a) Add a sufficient volume of degassed buffer to the column to fill it up to the reservoir (wide-mouth) portion.
   b) Gently tap the end and side of the column to dislodge any air bubbles from the column tip and side walls. Including 0.03-0.05% detergent (e.g., Surfact-Amps™ X-100, Product No. 28314) to the buffer solution may make it easier to remove air bubbles, especially from the column tip.
   c) Float a porous disc on top of the liquid within the column, and use the reverse end of a Pasteur pipette or reverse end of a serum separator (Product No. 69710) to push the disc evenly to the bottom of the column.
6. If the column already contains a bottom porous filter disc, fill the column half full with buffer, then tap the end and side of the column to dislodge air bubbles from the below the disc. If necessary, place the bottom-capped column into a 15 ml or 50 ml centrifuge tube and centrifuge for 2 minutes at 1,000 × *g* to dislodge the air from the column tip.
7. Decant or pipette most of the liquid from the empty column, being sure to prevent air bubbles from entering the tip region of the column below the inserted disc. Place the column back in its stand with bottom cap still in place.
8. Swirl or stir bottle of resin slurry to obtain a homogeneous suspension, then add a sufficient volume of slurry to obtain the desired settled resin-bed volume. For example, 2 ml of 50% slurry will result in a 1 ml settled (i.e., packed) bed.
9. Allow the resin to settle in the column for at least 30 minutes.
10. Remove the bottom cap and allow the buffer to drain from the column only until it reaches the top of the resin bed. Do not allow the resin bed to become dry or fill with air bubbles. Gently squirt additional buffer around the inside top part of the column to remove residual resin beads that may have remained along the column walls during packing.
11. If desired, add a second porous filter disc on top of the resin bed following the procedure outlined in step 5.
12. The packed column is now ready for storage or use. Store the column upright with top and bottom caps in place and with sufficient buffer above the gel bed to prevent it from drying or becoming infiltrated with air bubbles.

Notes:

- Store the packed column upright and capped at 4°C with the resin bed submerged under 1-2 ml of buffer. Include 0.02% sodium azide in the storage buffer to prevent microbial growth in carbohydrate-containing resin supports.
- Always remove the top cap before the bottom cap to avoid drawing air bubbles down into the resin bed.
- Prevent air bubbles from forming in the resin bed by using only degassed buffer and sample solutions. Degassing involves subjecting a solution to vacuum to "boil" off excess dissolved air (see Additional Information section).

Related Products

Gravity-flow Columns (1-10 ml)

29920	**Disposable Polystyrene Columns**, 100 columns, for 0.5-2.0 ml resin beds
29922	**Disposable Polypropylene Columns**, 100 columns, for 1-5 ml resin beds
29924	**Disposable Polypropylene Columns**, 100 columns, for 2-10 ml resin beds

Centrifuge (or Gravity-flow) Columns (1-10 ml)

89896	**Pierce Centrifuge Columns, 2 ml**, 25 columns
89897	**Pierce Centrifuge Columns, 5 ml**, 25 columns
89898	**Pierce Centrifuge Columns, 10 ml**, 25 columns

Microcentrifuge Spin Cups and Columns (10-1,000 µl)

Different styles and sizes of microcentrifuge spin cups and columns are also available for use with 10-500 µl of resin. For more information, visit our web site and search for Product No. 69702 or 69725. These small spin cups and columns are frequently used for small-scale batch immunoprecipitation (IP).

Additional Information

A. Related Tech Tips:

- Tech Tip #29: Degas solutions for use in affinity columns
- Tech Tip #7: Remove air bubbles from columns
- Tech Tip #52: Pierce Spin Cup and Column dimensions and volumes
- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins

B. Chemical Compatibility and Physical Properties of Plastic Columns

Plastic (polystyrene or polypropylene) columns are compatible with dilute or weak acids, aliphatic alcohols and bases. Strong or concentrated acids can be used for brief periods. Plastic columns are not compatible with aldehydes, esters, hydrocarbons, ketones or strong oxidizing agents. Plastic columns can be use between 4°C and 50°C; they will deform > 90°C. Except for the microcentrifuge spin cups and columns, which have paper or cellulose acetate filters, Pierce Spin and Centrifuge Columns use polyethylene porous discs.

Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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