TECH TIP # 7

Thermo SCIENTIFIC

Remove air bubbles from columns to restore flow rate

TR0007.3

Introduction

Affinity purification methods often use gravity-flow to process sample and buffer solutions through a resin bed that is packed into a column. All steps in column chromatography procedures depend on efficient and uniform contact of solution with the solid support. If air bubbles form in the resin bed, they limit contact with the resin and impede flow through the column.

Degassing or removing dissolved air from all samples and buffers before applying them to a column will minimize or eliminate problems associated with the presence of air bubbles. Degas solutions by subjecting them to vacuum for several minutes (see related Tech Tip procedure). If solutions are not degassed before use, dissolve air can "outgas" from the solution as it passes through the column. Although the resulting air bubbles may be too small to see with the unaided eye, their effect will be observed as an unacceptably slow rate of column flow.

Prevent large air bubbles from being drawn down into a column resin bed by always removing the column's top cap before the bottom cap and replacing the bottom cap before the top cap. If bubbles do form in a column resin bed by outgassing from solutions or improper resin packing or handling, the following methods can be used to correct the problem.

Removing Small Bubbles from Below the Bottom Filter Disc

1. Fill the column to the very top with degassed water or buffer.
2. Stretch laboratory film over the top of the column, making sure that there is no air trapped between the top of the liquid and the laboratory film.
3. Place a thumb over the sealed column top and invert the column until the bubble is in the exit tip.
4. With your thumb, apply gentle pressure to the "diaphragm" created by the laboratory film until the trapped air is expelled from the tip.
5. Return the column to an upright position and remove the laboratory film. The column is now ready for use.

Stirring Method for Removing Air Bubbles from Column Resin Bed

1. Cap the column bottom and add water or buffer so that the resin bed is covered by a height of 1-2 cm of solution.
2. If the packed column has a filter disc on top of the resin bed, it must be removed. Push down on one edge of the disc with a narrow weighing spatula or dissecting probe, forcing up the opposite edge. Use forceps to remove the disc.
3. Gently stir the resin matrix with a clean weighing spatula or pasteur pipette, until all portions of the resin are loosely suspended in the solution.
4. With the bottom cap still in place, allow the column to stand for several minutes until the resin mostly settles (packs) itself back into a bed.
5. To replace the top porous disc (optional), place it on the liquid within the column and use an inverted serum separator or pasteur pipette to push it down to just above the resin bed (Figure 1). Leave 1-2 mm of space between the top of the resin bed and the bottom of the top disc. The column is now ready for use.

Centrifugation Method for Removing Air Bubbles from Columns

1. Cap the column bottom and add water or buffer so that the resin bed is covered by a height of 1-2 cm of solution.
2. If the packed column has a filter disc on top of the resin bed, it may have to be removed. Push down on one edge of the disc with a narrow weighing spatula, forcing up the opposite edge. Use forceps to remove the disc.
3. Place the entire bottom-capped column in a test tube or centrifuge tube and centrifuge for 10 minutes at 1,000 × *g*.

Notes:

- Make sure to place a balance opposite of the column before beginning the centrifugation.
- Some resins can be centrifuged at higher speeds, but agarose may crush at speeds exceeding 1,000 × g.
- A "clinical-type" centrifuge, i.e., one with swinging baskets, works best.
- Occasionally a column may need to be centrifuged twice to remove all of the entrapped air.

4. To replace the top porous disc, place it on the liquid within the column and use an inverted serum separator or Pasteur pipette to push it down to just above the settled resin (Figure 1). Leave 1-2 mm of space between the top of the resin bed and the bottom of the top disc. The column is now ready for use.

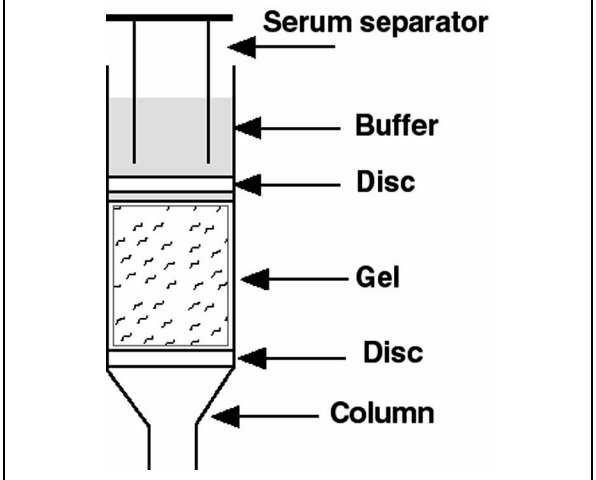

Figure 1. Placement of the top porous disc.

Additional Information

- Tech Tip #29: Degas solutions for use in affinity and gel filtration columns
- Tech Tip #13: Pack resin into Pierce Disposable Plastic Columns
- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins

Related Thermo Scientific Pierce Products

89896	**Centrifuge Columns, 2 ml**, 25/pkg, for spin or gravity-flow methods
89897	**Centrifuge Columns, 5 ml**, 25/pkg, for spin or gravity-flow methods
89898	**Centrifuge Columns, 10 ml**, 25/pkg, for spin or gravity-flow methods

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.