

Empty Columns Large / Small Instructions

Disposable columns used for gravity-flow chromatography with EndoTrap®



Product description

The following items are provided with this package (depending on the size of the columns):

800007
(previous 311068L)

- ☑ Polypropylene columns for 2 – 10 ml resin (Large Columns)
- ☑ top and bottom caps (polypropylene)
- ☑ 0.6 inch diameter, 45 µm porous polyethylene discs

800008
(previous 311068S)

- ☑ Polypropylene columns for 0.2 – 2 ml resin (Small Columns)
- ☑ top and bottom caps (polypropylene)
- ☑ 0.3 inch diameter, 30 µm porous polyethylene discs

Degas all solutions (resin, buffer) before applying to the column to prevent air bubbles from clogging the column and reducing flow!

Instruction for packing resin into columns

Please make sure that you **follow the instructions carefully and in the right order and pay attention to Figure 1.**

1. To ensure that the plastic material are not contaminated with LPS, wash the **columns**, the **caps** and the **discs** with at least **1 M of NaOH** to get them free of LPS (recommended is insertion over night).
2. Wash columns with **pyrogen-free water** and let it air-dry.
3. Allow the resin slurry and deionised water to come to room temperature.
4. Place bottom cap on the end of a column.
5. Place the column in a 16 x 125 mm test tube and add 2.75 ml of **degassed deionised water** to the small column or 12 ml to the large column. Tap the end of the column sharply to dislodge any air bubbles.
6. Push the disc to the bottom of the column (i.e. with the reverse end of a pyrogen-free Pasteur pipette), as the disc passes to the bottom, the air will be purged out through the disc.
7. Empty the columns of water and add 2.75 (small columns) or 12 ml (large columns) of **degassed resin slurry**. The volume of slurry should contain the desired quantity of settled resin (i.e. between 0.5 – 2 ml for the small column and 2 – 10 ml for the large column). If necessary, adjust the slurry concentration.
8. Allow at least 30 minutes for the resin to settle in the column.
9. Place one of the discs on the top of the liquid within the column and depress it to just above the settled resin level. **Leave approximately 1 – 2 mm of space between the top of the resin bed and the bottom of the top disc.**
10. Wash the inside top part of the column with **deionised water** to wash away traces of resin that may have remained along the sides during packaging.
11. For **storage**, keep approximately 2 ml of **Hyglos' regeneration buffer** over the top disc to prevent packed resin from drying out. A preservative, such as sodium azide (0.02%), should be included in the storage solution to prevent microbial growth. Place the top cap in the column and store upright at 4 °C.

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Using the packed column for affinity chromatography

Note: To use a column, remove the **top cap** first. This prevents bubbles from being drawn into the resin. Next, remove **bottom cap** and place the column in suitable holders. Allow storage solution to drain completely from column.

Reconditioning the resin bed

Periodically, the columns may need to be reconditioned to maintain their chromatographic properties. For instance, during use or storage, some small air bubbles may form between the two discs that bracket the resin. If this occurs, remove air bubbles by centrifugation for 10 minutes at 1000 g. A "clinical type" centrifuge equipped with swinging baskets works best. Occasionally, all of the entrapped air may have to be removed from the resin bed.

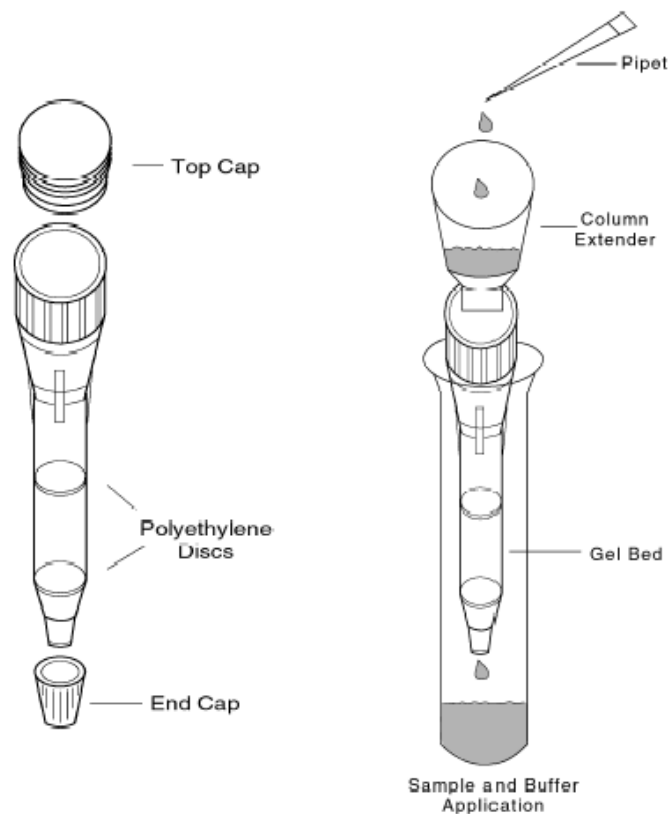


Figure 1: Assembly of Disposable Columns and use with a glass test tube.

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Technical support: Different possibilities to remove air bubbles from columns to restore flow rate

Affinity chromatography often uses gravity-flow to process sample solutions through a resin bed that is packed into a column. All steps depend here on efficient and uniform contact of solution with the resin support. If air bubbles form in the resin bed, they limit contact with the resin and thereby interfere flow-through the column.

Degassing or removing dissolved air from all samples and buffers before applying them to a column will reduce or eliminate problems associated with the presence of air bubbles.

Degas buffer by applying vacuum or sonification for ten minutes, primarily with buffer at room temperature. Excessive gas in running buffer will form bubbles in the resin bed during endotoxin removal. This is particularly important, when the applied buffer has a different temperature as the EndoTrap® column, e.g. when using cold buffer on a column at room temperature. Air bubble formation in the EndoTrap® column may lead to clogging of the column and decrease the quality of endotoxin removal.

Different possibilities to remove air bubbles from columns:

The first way is to centrifuge the closed column (filled with buffer by a height of 1-2 cm) by $\sim 1000 \times g$ for ten minutes (using a "clinical-type" centrifuge, i.e. one with swinging baskets works best). For this procedure please place the column into a suitable Falcon™ tube.

Also you can remove bubbles by stirring. Here you have to cap the column bottom and add EndoTrap® equilibration buffer so that the resin bed is covered by a height of 1-2 cm. Thereafter remove the disc atop the resin bed. Then gently stir the resin matrix with a clean Pasteur pipette until the resin is fluffily suspended in the buffer. Allow the column to stand for several minutes until the resin mainly settles itself back. Thereafter replace the top disc on the top of the liquid within the column and depress it to just above the settled resin level. Leave approximately 1 – 2 mm of space between the top of the resin bed and the bottom of the top disc.

Another possibility to remove bubbles that are below the bottom disc is to put a tube onto the end of the column and to connect it with a 5-ml syringe. Make sure that the column is open and that the column is completely filled with buffer. With the help of the syringe you can pull out the bubbles from the column.

Do not forget to degas all solutions before applying to the column to prevent air bubbles from clogging the column and reducing flow!

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Related products

- 800005
(previous 311068F-1) Disposable Polypropylene Funnels (package of one piece)
Column extender (30 ml) to fit the top of disposable plastic columns
(800007 or 800008)
- 800006
(previous 311068F-5) Disposable Polypropylene Funnels (package of five pieces)
Column extender (30 ml) to fit the top of disposable plastic columns
(800007 or 800008)

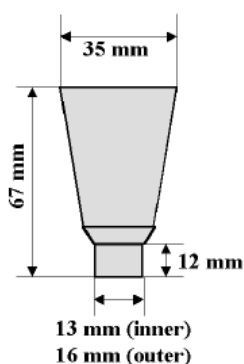


Figure 2: Funnels parameters.

Additional Information: Chemical compatibility and physical properties of plastic columns

Polypropylene Columns are compatible with diluted or weak acids, aliphatic alcohols and bases. Strong or concentrated acids can be used for brief periods. The columns are not compatible with aldehydes, esters, hydrocarbons, ketones or strong oxidizing agents. They are resistant to organic solvents for a brief period of exposure. Disposable plastic columns are intended for use between 4 °C and 50 °C, and they will deform above 90 °C.

The polyethylene porous discs used in the columns are more resistant to chemicals of all kinds than the column plastic!

For inquiries and technical support please contact

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