

QIAGEN Supplementary Protocol

Filling the chromatography column with QIAGEN[®] Anion-Exchange Resin (dry powder or slurry) and recycling the column

The procedures described below provide guidelines for the cleaning, refilling, and recycling of a used Vantage L Laboratory Column VL 44 x 25, (cat. no. 96440250) purchased from Millipore (www.millipore.com).

IMPORTANT: When working with hazardous chemicals, always wear a suitable lab coat, disposable gloves, protective face mask, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs), available from the product supplier.

The products described here are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

This supplementary protocol describes the following procedures:

- Filling the column with dry resin powder
- Filling the column with resin slurry
- Dismantling and cleaning the column

Equipment and reagents to be supplied by user

- 1-liter glass beaker (autoclaved), pyrogen-free
- 400 ml Buffer QN. To prepare 1 liter Buffer QN, dissolve 93.50 g NaCl and 10.46 g MOPS in 800 ml distilled water. Adjust the pH to 7.0 with NaOH, add 150 ml pure isopropanol and adjust the volume to 1 liter with distilled water.
- Ultrapure 500 (cat. no. 11500, contains 0.675 kg QIAGEN Anion-Exchange Resin in a 2-liter wide mouth bottle)
- 10 ml sterile disposable pipet
- 1 M NaOH
- Distilled water
- 0.2 M HCl
- 0.2% SDS



Filling the column with dry resin powder

IMPORTANT: Always wear a protective face mask to avoid inhalation of fine resin dust particles.

Procedure

1. **Assemble the lower adaptor unit (see 2–9 in figure) and screw onto the glass column tube (see 1 in figure).**
2. **Fill the column with 130–135 g QIAGEN Anion-Exchange Resin, then shake and tap the column until the resin is completely settled in the column tube (see 1 in figure).**
3. **Carefully remove the resin particles that are attached electrostatically on the upper interior part of the glass column where the upper adaptor is assembled. Resin particles between the seal of the bed support could lead to leakage (see 2–9, 11, and 13 in figure).**
4. **Assemble upper adaptor unit (see 2–9, 11, and 13 in figure).**

Filling the column with resin slurry

IMPORTANT: A chromatography column filled with the resin slurry should ideally be used on the same day, or within 24 hours of packing the column.

Procedure

1. **Assemble the lower adaptor unit (see 2–9 in figure) and screw onto the glass column tube (see 1 in figure).**
2. **Pour 0.4 liters Buffer QN into a 1-liter glass beaker.**
3. **Add 130–135 g QIAGEN Anion-Exchange Resin to the 1-liter glass beaker containing Buffer QN, and immediately mix by manual stirring with a sterile 10 ml disposable pipet.**

Minimize resin dust by placing the wide mouth bottle containing the QIAGEN Anion-Exchange Resin on the edge of the 1-liter glass beaker and pouring the resin slowly. Continuously mix Buffer QN during the addition of the resin to prevent the resin from settling.

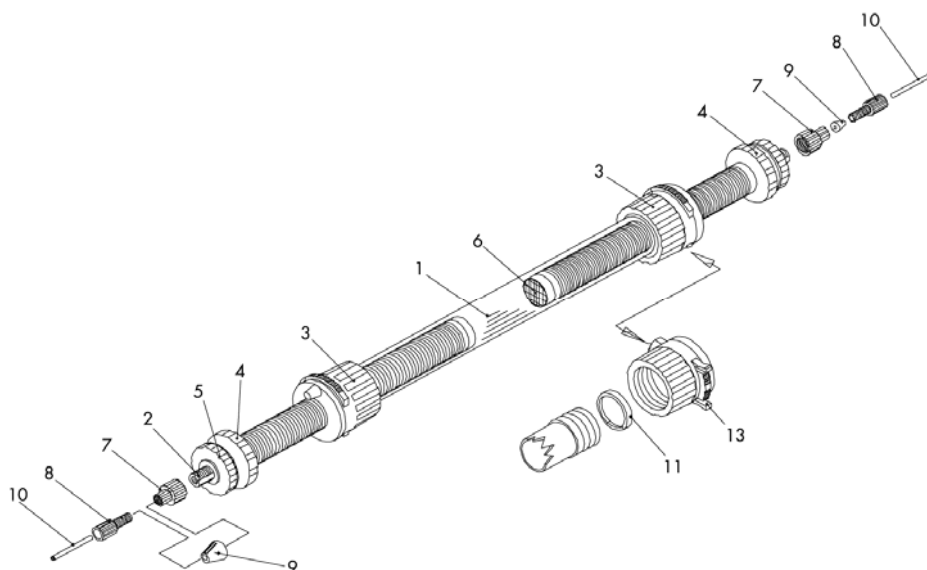
4. **After complete resuspension of the QIAGEN Anion-Exchange Resin in Buffer QN, pour the resin slurry into the chromatography column as quickly as possible.**
5. **Rinse the top interior walls of the column with an additional 10 ml Buffer QN to ensure that all of the resin is washed down. Allow the resin to settle for 10–15 min.**
6. **Enable excess Buffer QN to flow out of the column. Simultaneously, rinse the interior walls of the column with Buffer QN using a sterile 10 ml disposable pipet to wash down any remaining resin.**
7. **Replace upper adaptor unit (see 2–9, 11, and 13 in figure).**

Dismantling and cleaning the column

IMPORTANT: Note that the QIAGEN Anion-Exchange Resin is not reusable.

Procedure

1. Unscrew both outer adaptor units (see 2–9, 11, and 13 in figure) and completely dismantle them.
2. Remove the upper and lower frits and used resin from the glass column tube (see 1 in figure). The frits and used resin should be autoclaved before disposal.
3. Remove residual resin from inside the column tube by wiping with a paper towel.
4. Place all column parts in 1 M NaOH and leave to soak for at least 1 h.
5. Fill flow tube (see 2 in figure) with 1 M NaOH using a pipet and leave to soak for at least 1 h.
6. Wash all column parts thoroughly using distilled water until the pH of the water is no longer alkaline (~pH 5.5).
7. Rinse the flow tube well with distilled water.
8. Treat all column parts for at least 4 h (overnight, if more convenient) with 0.2 M HCl.
9. Fill the flow tube with 0.2 M HCl using a pipet and leave for at least 4 h (overnight, if more convenient).
10. After 4 h (or overnight treatment), wash the column parts well with distilled water until the pH of the water is no longer acidic.
11. Rinse flow tube well with distilled water.
12. Clean the inside of the column tube (see 1 in figure) with 0.2% SDS solution using a bottle-brush, and then wash thoroughly with distilled water.
13. Leave all parts to dry completely before reassembling the column.



Components of the Vantage L Laboratory Column VL 44 x 25, (cat. no. 96440250).

(Figure reprinted with permission from Millipore Corporation.)

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|---------------------|----------------------|
| 1. Column tube | 7. Flow tube adaptor |
| 2. Flow tube | 8. Flangeless nut |
| 3. Adjuster housing | 9. Ferrule |
| 4. Adjuster tube | 10. Tubing |
| 5. Seal adjust knob | 11. Extension seal |
| 6. Seal/bed support | 13. Adjuster clip |

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Selected handbooks can be downloaded from www.qiagen.com/literature. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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