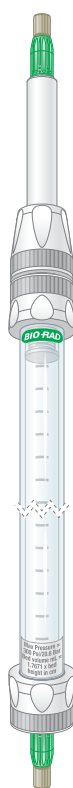

Econo Alpha Empty Glass Chromatography Columns

Instruction Manual

Catalog Numbers

12009463
12009429
12009430
12009461
12009462
12009427
12009428



Please read the instructions in this manual prior to using Econo Alpha Empty Glass Chromatography Columns. If you have any questions or require any further assistance, please contact the Bio-Rad chromatography technical support group at 1-800-424-6723.



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Section 1 Introduction

Econo Alpha Columns are empty glass columns for low- to medium-pressure chromatography. They are convenient, easy to pack with your resin of choice, and compatible with common aqueous buffers used in protein purification. They are supplied assembled and ready for use (Figure 1).

Description of column hardware parts:

- 1 Connection cap
- 2 Plunger
- 3 Adjusting nut
- 4 Retaining cap
- 5 Graduation marks every centimeter for packing guidance
- 6 Glass tube, constructed of high-quality borosilicate glass
- 7 Pressure/volume label, includes the surface area and pressure rating of the column for easy reference
- 8 Tube thread
- 9 Polytetrafluoroethylene (PTFE) frit
- 10 FKM/FPM O-ring
- 11 Adjustable end piece
- 12 Fixed end piece

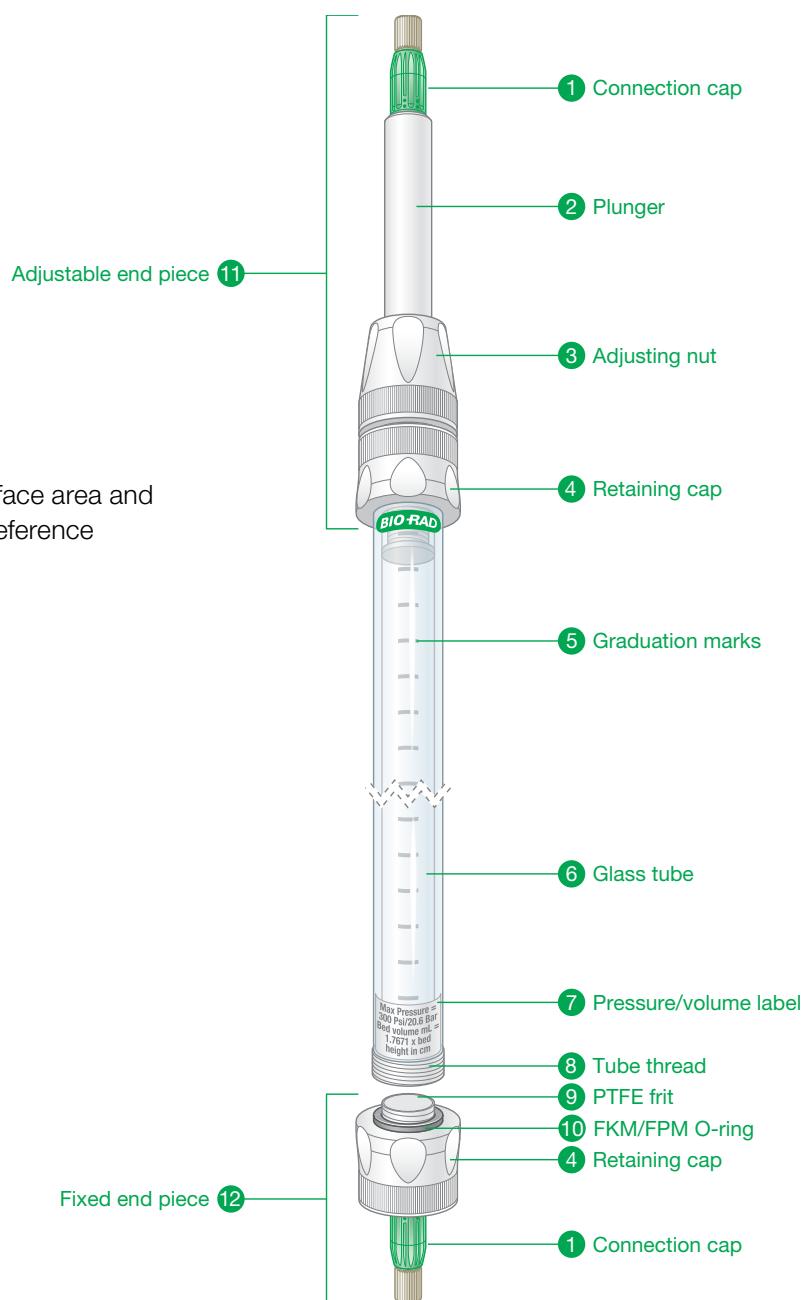


Fig. 1. Econo Alpha Column.

Section 2

Procedure for Packing Using the Column Sleeve

To pack the column with the chosen resin, use a column sleeve to connect two glass columns. The fixed end piece should be fitted to the lower column and plugged before pouring the resin slurry. See Figure 2.

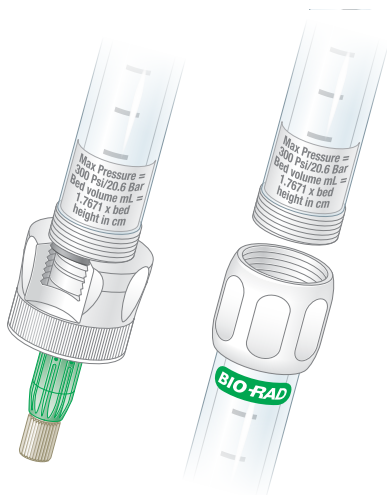


Fig. 2. Fixed end piece (left) and column sleeve (right).

1. Assembling the Column

- 1.1 Make sure all parts are clean and the seals on both ends of each column are undamaged.
- 1.2 Using 20% ethanol, rinse the seal of the fixed end piece, then apply the end piece to the bottom of the column and tighten.
- 1.3 Connect the two columns using the column sleeve.
- 1.4 Attach fluorinated ethylene propylene (FEP) tubing (minimum length, 100 cm) that has 1/4-28 fittings on either end to the bottom column with the fixed end piece. Cap the other end so that the tubing is sealed.
- 1.5 Using a ring stand or similar column holder, assemble the column so that the top of the second column is accessible.
- 1.6 Make sure the column is level and vertical. A column that is not level can result in uneven resin packing.

2. Packing the Column

The resin should be handled per the manufacturer's recommendations. The following information is provided as an example only:

- 2.1 To calculate the appropriate amount of resin to use, first determine the desired final bed height. For this example we will use a final bed height of 40 cm. Include the compression factor in calculations if the resin is compressible (refer to manufacturer's product specification sheet).
- 2.2 Assuming a compression factor of 1.15, then the bed height is effectively 40 cm x 1.15 = 46 cm.
- 2.3 Using the initial height of the bed (46 cm), the total amount of resin can be calculated.
- 2.4 Volume of resin required = $\pi \times \text{column radius}^2 \times \text{height of the bed}$.
- 2.5 There are four separate column sizes with inner diameters (IDs) of 6.6, 10, 15, and 25 mm. See Table 1 for column formats and pressure ratings.
- 2.6 Using the calculated volume of resin and percentage of slurry, calculate the total amount of resin slurry to be added to the column:

Slurry volume = total volume resin ÷ percentage slurry

For example, to achieve a 40 cm bed height for a 2.5 cm ID (radius = 1.25 cm) column:

Total bed height = 40 cm x 1.15 = 46 cm

Volume of resin = $\pi (3.14) \times 1.25 \text{ cm}^2 \times 46 \text{ cm} = 226 \text{ ml resin}$

Slurry volume = 226 ml resin ÷ 0.5 (50% slurry) = 452 ml slurry

Table 1. Column formats* and pressure ratings.

Column Dimensions (ID x L), mm	Hardware Pressure Rating
6.6 x 50	900 psi, 62.05 bar, 6.20 MPa
10 x 100	600 psi, 41.36 bar, 4.13 MPa
10 x 500	600 psi, 41.36 bar, 4.13 MPa
15 x 100	300 psi, 20.68 bar, 2.06 MPa
15 x 500	300 psi, 20.68 bar, 2.06 MPa
25 x 100	150 psi, 10.34 bar, 1.03 MPa
25 x 500	150 psi, 10.34 bar, 1.03 MPa

* Columns include one fixed end piece and one adjustable end piece with adaptors for both 10-32 and 1/4-28 fittings.

3. Fitting and Adjusting the Adjustable End Piece

- 3.1 Once the slurry has been poured and the resin has settled, allow the liquid level to drain until only 1–2 cm of liquid are above the bed.
- 3.2 Screw the adjusting nut and retaining cap fully onto the end piece.
- 3.3 Make a tubing connection into the connection cap.
- 3.4 With the frit uppermost, pump buffer through the adjustable end piece until it is primed and the frit is wetted.
- 3.5 Carefully push the PTFE seal of the end piece into the end of the glass tube until the O-ring contacts the glass and resistance is felt. The retaining cap should contact the tube thread.
- 3.6 Always keep the end piece in line with the column. Do not insert at an angle because this may damage the seal.
- 3.7 Prewetting the PTFE seal, O-rings, and entrance to the glass tube with water or buffer can assist with insertion. Turn the retaining cap counterclockwise until a click is heard; the threads inside the retaining cap and on the tube can now engage. The O-rings will give extra resistance as they enter the glass tube. The retaining caps do not need to be tightened.
- 3.8 Hold the column and slowly turn the adjusting nut clockwise to move the plunger to the desired position in the tube. Ensure no air is trapped between the bed and frit.
- 3.9 Connect the column to a pump or chromatography system and begin running the mobile phase through the column.

4. Removing the Column Extension

- 4.1 Continue to run the mobile phase through the column until all of the resin is in the bottom column and the liquid above is clear.
- 4.2 Carefully unscrew the sleeve and remove it, over a collection vessel, while ensuring that the packed bed in the bottom column is not disturbed.
- 4.3 Allow the liquid level to drain until only 1–2 cm of liquid are above the bed.
- 4.4 Screw the adjusting nut and retaining cap fully onto the end piece.
- 4.5 Make a tubing connection into the connection cap.
- 4.6 Connect the column to a pump or chromatography system and begin running the mobile phase through the column.

5. Adjusting the Column to Final Bed Height

- 5.1 Continue to run the mobile phase through the column until the bed is at a steady height.
- 5.2 Stop the pump and adjust the plunger to the desired final bed height.

6. Column Performance Test

Once packing is complete we recommend conducting a column performance test to determine height equivalent to a theoretical plate and peak symmetry.

Section 3 Ordering Information

Catalog #	Description	Frits	
Econo Alpha Columns			
12009463	Econo Alpha Column , 6.6 x 50 mm	12009479	Econo Alpha Frit , 6.6 mm, 5 µm
12009429	Econo Alpha Column , 10 x 100 mm	12009480	Econo Alpha Frit , 6.6 mm, 10 µm
12009430	Econo Alpha Column , 15 x 100 mm	12009481	Econo Alpha Frit , 6.6 mm, 20 µm
12009461	Econo Alpha Column , 25 x 100 mm	12009482	Econo Alpha Frit , 6.6 mm, 30 µm
12009462	Econo Alpha Column , 10 x 500 mm	12009483	Econo Alpha Frit , 6.6 mm, 50 µm
12009427	Econo Alpha Column , 15 x 500 mm	12009474	Econo Alpha Frit , 10 mm, 5 µm
12009428	Econo Alpha Column , 25 x 500 mm	12009475	Econo Alpha Frit , 10 mm, 10 µm
		12009476	Econo Alpha Frit , 10 mm, 20 µm
		12009477	Econo Alpha Frit , 10 mm, 30 µm
		12009478	Econo Alpha Frit , 10 mm, 50 µm
Adjustable Plungers			
12009493	Econo Alpha Adjustable Plunger , 10 mm	12009464	Econo Alpha Frit , 15 mm, 5 µm
12009492	Econo Alpha Adjustable Plunger , 15 mm	12009465	Econo Alpha Frit , 15 mm, 10 µm
12009494	Econo Alpha Adjustable Plunger , 25 mm	12009466	Econo Alpha Frit , 15 mm, 20 µm
Packing Sleeves (connector)			
12009487	Econo Alpha Packing Sleeve , 6.6 mm	12009467	Econo Alpha Frit , 15 mm, 30 µm
12009486	Econo Alpha Packing Sleeve , 10 mm	12009468	Econo Alpha Frit , 15 mm, 50 µm
12009484	Econo Alpha Packing Sleeve , 15 mm	12009469	Econo Alpha Frit , 25 mm, 5 µm
12009485	Econo Alpha Packing Sleeve , 25 mm	12009470	Econo Alpha Frit , 25 mm, 10 µm
Column Extensions			
12009501	Econo Alpha Column Extension , 6.6 x 50 mm	12009471	Econo Alpha Frit , 25 mm, 20 µm
12009497	Econo Alpha Column Extension , 10 x 100 mm	12009472	Econo Alpha Frit , 25 mm, 30 µm
12009498	Econo Alpha Column Extension , 15 x 100 mm	12009473	Econo Alpha Frit , 25 mm, 50 µm
12009499	Econo Alpha Column Extension , 25 x 100 mm	O-Rings	
12009500	Econo Alpha Column Extension , 10 x 500 mm	12009491	Econo Alpha O-Rings , 6.6 mm
12009495	Econo Alpha Column Extension , 15 x 500 mm	12009490	Econo Alpha O-Rings , 10 mm
12009496	Econo Alpha Column Extension , 25 x 500 mm	12009488	Econo Alpha O-Rings , 15 mm
		12009489	Econo Alpha O-Rings , 25 mm

Visit bio-rad.com/ResinsandColumns for the most up-to-date list of column and resin offerings.

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