Column Packing Instructions

Praesto® Jetted A50 / HipH

Suggested Columns

Omnifit®
ECO
Tricorn™
XK™
HiScale™

Purolite®
An Ecolab Company

Packed Laboratory-Scale Columns

A well-packed column is essential to achieve maximum efficiency, high product yield, and purity. It is important that a homogeneous packed bed is used every time a purification or separation is performed. Irregularities in packing can create an uneven flow within the bed, resulting in peak broadening, lower yield and it can subsequently affect the purity of the product. Essentially, a column that is poorly packed can lead to expensive process disruptions and ultimately, loss of a valuable product.

Herein we describe methods for packing common laboratory scale columns of 0.5 cm to 2.6 cm in diameter and process scale columns of 7 cm to 200 cm in diameter.

Table 1: Suggested columns for laboratory-scale purification

Column	Manufacturer	Inner Diameter (cm)	Bed Volume (mL)	Bed Height (cm)
Omnifit®	Kinesis	0.66 – 1	3.42 – 15.70	10 – 20
ECO (1 cm ID)	YMC	1	7.85 – 15.70	10 – 20
Tricorn™	Cytiva	0.5 – 1	1.96 – 15.70	10 – 20
XK™	Cytiva	1.6 – 2.6	20 – 106	10 – 20
HiScale™	Cytiva	1.6 – 2.6	20 – 106	10 – 20

Required Materials and Equipment

- Praesto Jetted A50 / HipH.
- Chromatography column.
- Column packing tube.
- Purified water or 100 - 500 mM NaCl solution (packing solution).
- A chromatography system, such as a Bio-Rad NGC™ or an ÄKTA™ system. Alternatively, a stand-alone pump, equipped with a pressure gauge can be used for packing.

Sample Preparation

- Recommended slurry percentage = 50 – 70 %.
- Recommended compression factor = 1.1.
- Wash an appropriate portion of the resin with purified water or 100 – 500 mM NaCl solution to remove the sample storage solution.
- Re-slurry the washed sample and either allow to settle by gravity or centrifuge the resin sample at 100 g for 10 minutes.
- Note the slurry percentage and add/remove packing solution to obtain the required slurry percentage for packing.
- Calculate the required slurry to add to the column using the following equation:

$$\text{Volume (ml)} = \frac{\text{Radius}^2\text{ (cm)} \times \pi \times \text{Bed Height (cm)} \times \text{Compression factor}}{\frac{\text{Slurry (\%)}}{100}}$$

N.B Compression factors for laboratory scale columns provide a guideline as to the amount of slurry volume to add. Columns are packed by flow and pressure.

Omnifit®

- Assemble the column and packing tube as per the manufacturer's instructions.
- Ensure the resin slurry is homogeneous and add to the column. Top up, if necessary, with packing buffer.
- Insert the top adaptor at a 45° angle to prevent air bubbles entering the column. Secure the top adaptor.
- Disconnect the column outlet tube from the chromatography system and direct to waste.
- Gradually increase the flow rate until a stable pre-column pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached.
- Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.
- Mark the point at which the bed has settled and stop the flow.
- Remove the packing tube. At this point, resin can be added or removed to obtain the target bed height.
- Re-insert the top adaptor and increase the volumetric flow until a stable pre-column pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached.
- Mark the bed height and stop the flow.
- Lower the top adaptor to 1 mm past the marked bed height.
- Reconnect the column outlet tube to the chromatography system.
- Performing conditioning of the column by applying 2 column volumes up flow and down flow at 70 % of the packing flow. Monitor delta pressure (pressure drop) during the conditioning, delta pressure reading should be < 3.5 bar (0.35 MPa).
- The column is now ready to be tested.

ECO

- Assemble the column as per the manufacturer's instructions.
- Ensure the resin slurry is homogeneous and add to the column. Top up if necessary, with packing buffer.
- Insert the top adaptor at a 45° angle to prevent air bubbles entering the column. Secure the top adaptor.
- Disconnect the column outlet tube from the chromatography system and direct to waste.
- Gradually increase the flow rate until a stable pre-column pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached.
- Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.
- Mark the point at which the bed has settled and stop the flow.
- At this point, resin can be added or removed to obtain the target bed height. Re-insert the top adaptor and increase the flow rate until a stable pre-column pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached. Mark the bed height and stop the flow.
- Lower the top adaptor to 1 mm past the marked bed height.
- Reconnect the column outlet tube to the chromatography system.
- Performing conditioning of the column by applying 2 column volumes up flow and down flow at 70 % of the packing flow. Monitor delta pressure (pressure drop) during the conditioning, delta pressure reading should be < 3.5 bar (0.35 MPa).
- The column is now ready to be tested.

Tricorn™

- Assemble the column and packing tube as per the manufacturer's instructions.
- Ensure the resin slurry is homogeneous and add to the column. Top-up if necessary, with packing buffer.
- Insert the top adaptor at a 45° angle to prevent air bubbles entering the column. Secure the top adaptor.
- Disconnect the column outlet tube from the chromatography system and direct to waste.
- Gradually increase the flow rate until a stable precolumn pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached.
- Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.
- Mark the point at which the bed has settled and stop the flow.
- Remove the packing tube. At this point, resin can be added or removed to obtain the target bed height.
- Re-insert the top adaptor and increase the flow rate until a stable pre-column pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached.
- Mark the bed height and stop the flow.
- Lower the top adaptor to 1 mm past the marked bed height.
- Reconnect the column outlet tube to the chromatography system.
- Perform conditioning of the column by applying 2 column volumes up flow and down flow at 70 % of the packing flow. Monitor delta pressure (pressure drop) during the conditioning, delta pressure reading should be < 3.5 bar (0.35 MPa).
- The column is now ready to be tested.

XK™

- Assemble the column as per the manufacturer's instructions.
- Ensure the resin slurry is homogeneous and add to the column. Top-up if necessary, with packing buffer.
- Insert the top adaptor at a 45° angle to prevent air bubbles entering the column. Secure the top adaptor.
- Disconnect the column outlet tube from the chromatography system and direct to waste.
- Gradually increase the flow rate until a stable precolumn pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached.
- Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.
- Mark the point at which the bed has settled and stop the flow.
- At this point, resin can be added or removed to obtain the target bed height. Re-insert the top adaptor and increase the flow rate until a stable pre-column pressure of 3 – 3.5 bar (0.3 – 0.35 MPa) is reached. Mark the bed height and stop the flow.
- Lower the top adaptor to 1 mm past the marked bed height.
- Reconnect the column outlet tube to the chromatography system.
- Perform conditioning of the column by applying 2 column volumes up flow and down flow at 70 % of the packing flow. Monitor delta pressure (pressure drop) during the conditioning, delta pressure reading should be < 3.5 bar (0.35 MPa).
- The column is now ready to be tested.

HiScale™

- Assemble the column as per the manufacturer's instructions. Ensure the resin slurry is homogeneous and add to the column. Top up if necessary, with packing buffer.
- Insert the top adaptor at a 45° angle to prevent air bubbles entering the column. Secure the top adaptor.
- Disconnect the column outlet tube from the chromatography system and direct to waste.
- Start a low flow of 100 cm/h to settle the bed. Increase the flow until a stable pre-column pressure of 2 bar is achieved. Allow the bed to form and leave to consolidate for 10 minutes.
- Gradually increase the flow rate until a stable precolumn pressure of 3 - 3.5 bar (0.3 - 0.35 MPa).
- Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.
- Mark the point at which the bed has settled and stop the flow.
- Lower the adaptor to 2 mm past the marked point.
- Reconnect the column outlet tube to the chromatography system.
- Performing conditioning of the column by applying 2 column volumes up flow and down flow at 50 % of the packing flow. Monitor delta pressure (pressure drop) during the conditioning, delta pressure reading should be < 3.5 bar (0.35 MPa).
- The column is now ready to be tested.

Packing Large-Scale Columns

Table 2: Suggested columns for large-scale purification

Column	Manufacturer	Inner Diameter (cm)	Bed Volume mL (L)	Bed Height (cm)
BPG™	Cytiva	10 – 30	0.79 – 14.1	10 – 20
AxiChrom™	Cytiva	7 – 200	0.39 – 628	10 – 20

Sample and Column Preparation

- Assemble the column as per the manufacturer's instructions.
- Prime the column and system selected with the appropriate packing solution prior to column packing.
- Recommended slurry percentage = 40 – 60 %.
- Determine the slurry concentration (several methods can be used, centrifugation of a small sample at 100 g is the recommended method).
- Calculate the required slurry to add to the column using the following equation:

$$\text{Volume (ml)} = \frac{\text{Radius}^2\text{ (cm)} \times \pi \times \text{Bed Height (cm)} \times \text{Compression factor}}{\frac{\text{Slurry (\%)}}{100}}$$

- Remove storage solution by means of column washing or decant off the liquid level after settling the resin in the selected column.
- Add packing solution 100 – 500 mM NaCl and re-suspend the resin ready for the packing procedure.
- Allow the slurry to settle (at least 2 cm from top, it may require up to 30 minutes to settle) before inserting the adaptor.

BPG™

- Consolidation flow rate = 30 cm/h.
- Packing Factor = 1.18.
- Connect the BPG column with the pump system to be used for packing.
- Close the column bottom valve.
- Insert the top adaptor once the resin slurry has settled sufficiently.
- Open the column bottom valve.
- Start a settling flow of 30 cm/h and allow the resin to settle. Once the resin has settled, mark the bed height.
- Calculate the bed height for a 1.18 compression from the marked bed height.
  - Settled bed height (cm) / Packing Factor (P.F) = Desired bed height (cm)
  - Example for a 23.6 cm settled bed height; – 23.6 cm (Settled bed height) / 1.18 (C.F) = 20 cm
- Mark the target bed height.
- Increase the flow to apply compression on the bed by flow.
- Increase the flow incrementally until a stable pressure of 2 bar is reached.
- Allow resin to settle for a minimum of 30 minutes.
- Stop the flow, close the bottom valve and disconnect the tubing from the top of the column. Manually compress the bed by adjusting the adaptor until the calculated target bed height is reached.

AxiChrom™ – (7–30 cm ID)

- Connect the selected column to the ÄKTA™ system.
- Make a packing program in Unicorn using the Wizard for packing Axichrom™ Columns.
- Use following Instructions:
  - Column Size – Axichrom™ 70 - 300
  - Mesh Size – 10/20 µm
  - Media - Custom
  - Packing Factor - 1.2
  - Adaptor Flow velocity – 30 cm/hr
  - Conditioning flow velocity - 100 cm /hr
  - Sample Volume – 1.5 %
  - Equilibration Volume – 3 CV
  - Elution Volume – 1.4 CV
  - HETP Testing Velocity – 30 cm/hr
  - HETP – Testing downflow
- Prepare packing and testing solutions as follows:
  - A1/A2 – 100 mM – 500 mM NaCl
  - Sample Inlet S1 – 1M NaCl
- Run Packing and HETP testing Method from Unicorn in ÄKTA™ System and follow instruction to pack column.
- Evaluate Result using HETP Analysis to get value of As, HETP, h etc.

AxiChrom™ – (45–200 cm ID)

- Using an appropriate slurry vessel, ensure the slurry is homogeneous and determine the slurry percentage. Recommended slurry percentage 40 - 60 %.
- Using the intelligent packing protocol in Unicorn software or an AxiChrom™ master, use the following packing parameters:
  - Column: 600/300 (10/20 µm)
  - Media: Other
  - Target Bed: 20 cm
  - Max: 21 cm
  - Min: 19 cm
  - Packing Factor: 1.2
  - Filling Speed: 300 cm/h
  - Packing Speed: 30 cm/h

Evaluation of Packed Columns

The column efficiency should be tested immediately after packing and at regular intervals during use to monitor any deterioration. The preferred method for determining the efficiency of a packed column is using the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_S). The HETP and As values are determined by applying a sample such as 2 % acetone or 1 M NaCl to the packed column. A sample volume of approximately 1.5 % of the column volume and a flow velocity of 30 cm/h will give the optimal results.

Calculating HETP and A_s

Below is the calculation by which HETP and A_s are determined. This is done by using the conductivity curve.

$$\text{Volume (ml)} = \frac{\text{Radius}^2\text{ (cm)} \times \pi \times \text{Bed Height (cm)} \times \text{Compression factor}}{\frac{\text{Slurry (\%)}}{100}}$$

$$HETP = \frac{L}{N}$$

L = bed height (cm)

N = number of theoretical plates

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)$$

V_R = volume eluted from the start of the sample application to the peak maximum.

W_h = the width of the recorded peak at half of the peak height.

V_R and W_h have the same units.

The reduced plate height is calculated by the following equation:

$$h = \frac{HETP}{d_{50v}}$$

d_{50V} = mean particle size.

The reduced plate is often taken into consideration when evaluating column packing efficiency. As a guide a value of < 4 can indicate a well packed column.

The peak corresponding to the acetone or NaCl sample should be symmetrical with an asymmetry factor as close to 1 as possible. An acceptable limit is $0.8 < A_s < 2.0$.

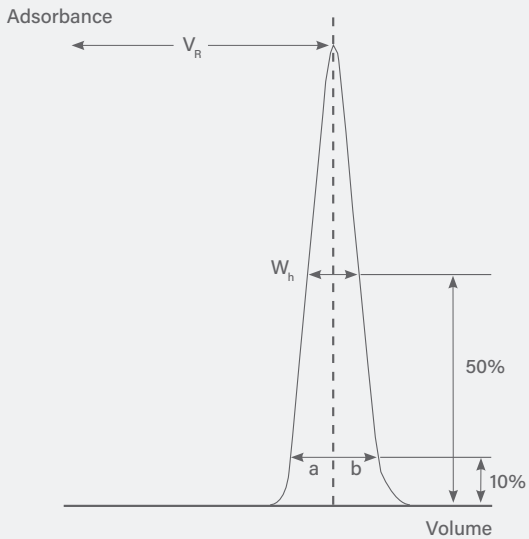
$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10 % peak height.

b = descending part of the peak width at 10 % of peak height.

A change in the shape of the peak is usually the first indication of bed deterioration.

Figure 5: An example of a HETP chromatogram.



The calculated plate number will vary according to the test conditions and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow, and temperature will all affect the results.

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Purolite, an Ecolab company, is a leading manufacturer of quality ion exchange, catalyst, adsorbent and specialty high-performance resins with global sales support.

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