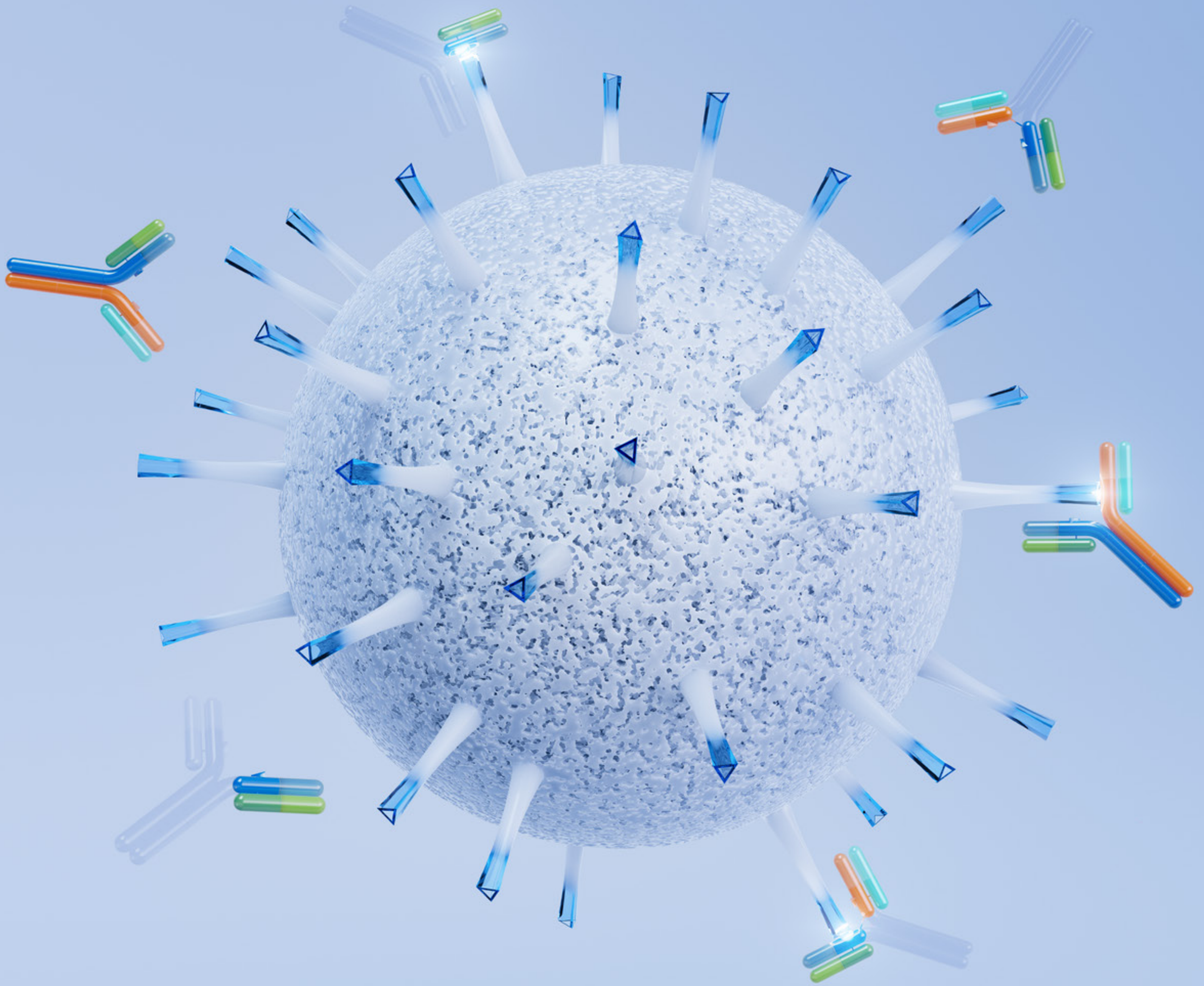
Column Packing Instructions

Praesto® 70 CH1 Laboratory Scale Column Packing

Purolite
An Ecolab Company



Praesto® 70 CH1 Laboratory Scale Column Packing

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Overview

Praesto® 70 CH1

Praesto 70 CH1 is a cross-linked agarose-based affinity resin designed for the purification of antigen binding fragments (Fabs) from human immunoglobulins (IgGs) and monoclonal antibodies (mAbs).

All Praesto chromatography resins are manufactured using our patented Jetting technology, which produces consistent agarose beads with a uniform particle size distribution.

Jetting Technology

Praesto chromatography resins are manufactured using Purolite's patented Jetting technology. Jetting offers a faster, more environmentally-friendly manufacturing process and uniform particle size distribution.

Benefits of Purolite's Jetted Resins

Narrow Bead Size Distrubution

More consistent bead size and minimal variation batch-to-batch

Sustainable Manufacturing

More environmentally friendly than alternative manufacturing methods

Increased Productivity

Faster mass transfer reduces manufacturing costs

Column Packing

Column chromatography is a well-established method for characterisation, purification, and manufacture of a wide range of products, from food to life-saving medications. In biopharmaceutical manufacture, it is critical that the purification process is robust and reproducible from lot to lot. As such, it is vital that chromatography columns are efficiently packed, and able to be qualified within a reasonable time frame.

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.

The flow and pressure properties of bioprocess chromatographic resins are of critical importance when designing a downstream purification process. Development work starts at laboratory scale using relatively small column dimensions. However, the high linear flow velocities that can be achieved at laboratory scale cannot be used at process scale. Herein, we describe packing procedures and parameters for pilot scale columns.

Abbreviations

Slurry percentage (%) = the ratio of resin to surrounding solution

Compression Factor = The level of compression required from a gravity settled bed height to the final packed bed height

Packing Factor = The level of compression required from a consolidated (under low flow) bed height to the final packed bed height

Slurry Determination

The percentage slurry is needed to calculate the required volume to be added to achieve a desired bed height.

The several techniques employed to determine slurry percentage, including centrifugation, gravity settling and a small-scale column using syringe drip force (Cytiva™ slurry concentration kit).

The accuracy of the slurry percentage measurement is not as critical at laboratory scale as volume can be added or removed during the packing process to obtain the desired bed height.

Suggested Materials and Equipment

- Praesto® 70 CH1
- Chromatography column
- Column packing tube
- Demineralized H_2O, Equilibration buffer or 0.1 M NaCl solution (Packing Solution)
- A Chromatography system, such as a BIO-RAD NGC or an AKTA system. Alternatively, a stand-alone pump, equipped with a pressure gauge can be used for packing

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 = 40–70%.
- Determine the slurry percentage.
- Calculate the required slurry to add to the column using the following equation:

$$\text{Volume (mL)} = \frac{\text{Radius}^2\ \text{(cm)} \times \pi \times \text{Bed Height (cm)} \times \text{Compression Factor}}{\left(\frac{\text{Slurry (\%)}}{100}\right)}$$

- 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 and resuspend 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.

N.B *Compression factors for laboratory scale columns are a guide on the amount of slurry volume to add, columns are packed by flow and pressure.*

Pressure/Flow Packing

0.5–2.6 cm ID Column

Step 1: Assemble the column and packing tube as per the manufacturer's instructions.

Step 2: Connect the column to the system.

Step 3: Prime the column top remove air in the tubing and nets, first up flow, until a clear liquid layer of packing solution is visible in the bottom of the column tube and subsequently prime the top adaptor.

Step 4: Ensure the resin slurry is homogeneous and add to the column. Top up, if necessary, with packing buffer.

Step 5: Allow resin to settle approximately 1–2 cm before adding top adaptor.

Step 6: Insert the top adaptor at 45° angle to prevent air bubbles entering the column. Secure the top adaptor.

Step 7: Disconnect the column outlet tube from the chromatography system and direct to waste.

Step 8: Gradually increase the flow rate until a stable pre-column pressure is reached. Use pressure flow control with the packing pressure in tables 1 and 2. If pressure flow control not possible set a pre column pressure alarm or system pressure alarm for the packing pressure in table 1 and 2 and gradually increase flow rate until the packing pressure is reached. (Refer to tables 1 and 2)

Step 9: Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.

Step 10: Mark the point at which the bed has settled and stop the flow.

Step 11: Remove the packing tube.
At this point, if the resin volume is incorrect, resin can be added or removed to obtain the target bed height. After addition or removal, resuspend the slurry and re-begin the packing process.

Step 12: Re-insert the top adaptor and increase the volumetric flow until the packing pressure is reached (Table 1 and 2)

Step 13: Mark the bed height and stop the flow.

Step 14: Cap the column outlet tube, disconnect the top of the column from the system and leave this tube uncapped.

Step 15: Lower the top adaptor to 1 mm past the marked bed height.

Step 16: Reconnect the top of the column chromatography system, uncap the bottom of the column.

Step 17: 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, refer to table 1 and 2 for details.

Step 18: The column is now ready to be tested.

TABLE 1 Packing Conditions for Laboratory Columns 0.5–1.6 cm in Diameter

	Compression Factor	Packing Pressure	Recommended Packing Flow (cm/h)*
Praesto 70 CH1	1.13	3.5 bar	1000

*Adjust flow accordingly to achieve and maintain desired packing pressure with the outlet tubing direct to waste.

TABLE 2 Packing Conditions for Laboratory Columns 2.6 cm in Diameter

	Compression Factor	Packing Pressure	Recommended Packing Flow (cm/h)*
Praesto 70 CH1	1.15	3 bar	900

*Adjust flow accordingly to achieve and maintain desired packing pressure with the outlet tubing direct to waste

Flow/Mechanical Compression Packing

2.6 cm ID Column

- Consolidation flow rate = 60 cm/h
- Compression Factor = 1.15

Step 1: Connect the HiScale column to the packing system.

Step 2: Connect the column to the system.

Step 3: Prime the column top remove air in the tubing and nets, first up flow, until a clear liquid layer of packing solution is visible in the bottom of the column tube and subsequently prime the top adaptor.

Step 4: Ensuring the resin slurry is homogeneous, add the calculated volume to the column.

Step 5: Allow resin to settle approximately 1–2 cm before adding top adaptor.

Step 6: Insert the top adaptor at 45° angle to prevent air bubbles entering the column. Secure the top adaptor.

Step 7: Disconnect the column outlet tube from the chromatography system and direct to waste.

Step 8: Start the consolidation flow and allow the resin to settle. Once the resin has settled, mark the bed height.

Step 9: Calculate the bed height using the compression factor listed in table 2 and the marked bed height.

Example Calculation

Settled Bed Height (cm)/Packing Factor (PF) = Desired bed height (cm)

Example for a 23.6 cm settled bed height;

23.0 cm (Settled Bed Height)/1.15 (C.F) = 20 cm

Step 10: Mark the target bed height

Step 11: Increase the flow to apply compression on the bed by flow
Increase the flow incrementally until a stable pressure of 2 bar is reached.

Step 12: Allow resin to settle for a minimum of 30 minutes.

Step 13: Stop the flow and disconnect the tubing from the top of the column, leave uncapped, cap the tubing from the bottom of the column. Manually compress the bed adjusting the adaptor until the calculated target bed height is reached.

Column Efficiency Testing

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 (As). 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 30–60 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 UV or conductivity curve

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

L = bed height (cm)
N = number of theoretical plates

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

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 (cm)

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 $0.8 < A_S < 2.0$

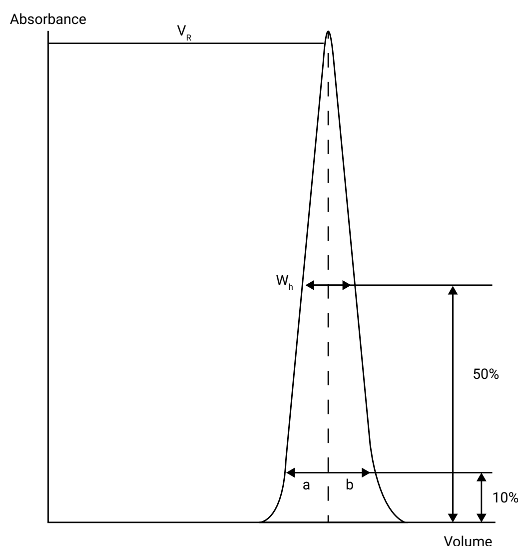
$$A_s = \frac{b}{a}$$

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 1

An example an 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 velocity, temperature will all affect the results.

Innovative Solutions for Bioprocessing

In partnership with Repligen, Purolite® develops and supplies innovative solutions for the bioprocessing industry, working with many of the top pharmaceutical companies to deliver the next-generation of healthcare. Our resins are used across the globe to deliver lifesaving medicines.

Global Support Network

No matter the location, our expert field application team members are positioned to help you solve your technical and downstream purification challenges, together. We provide the guidance necessary to develop robust, scalable, high productivity purification processes for mAbs and recombinant processes using Praesto® Jetted chromatography resins. For wherever you are in your biomanufacturing journey, we are here to help.

Purolite Affinity Resin Toolbox

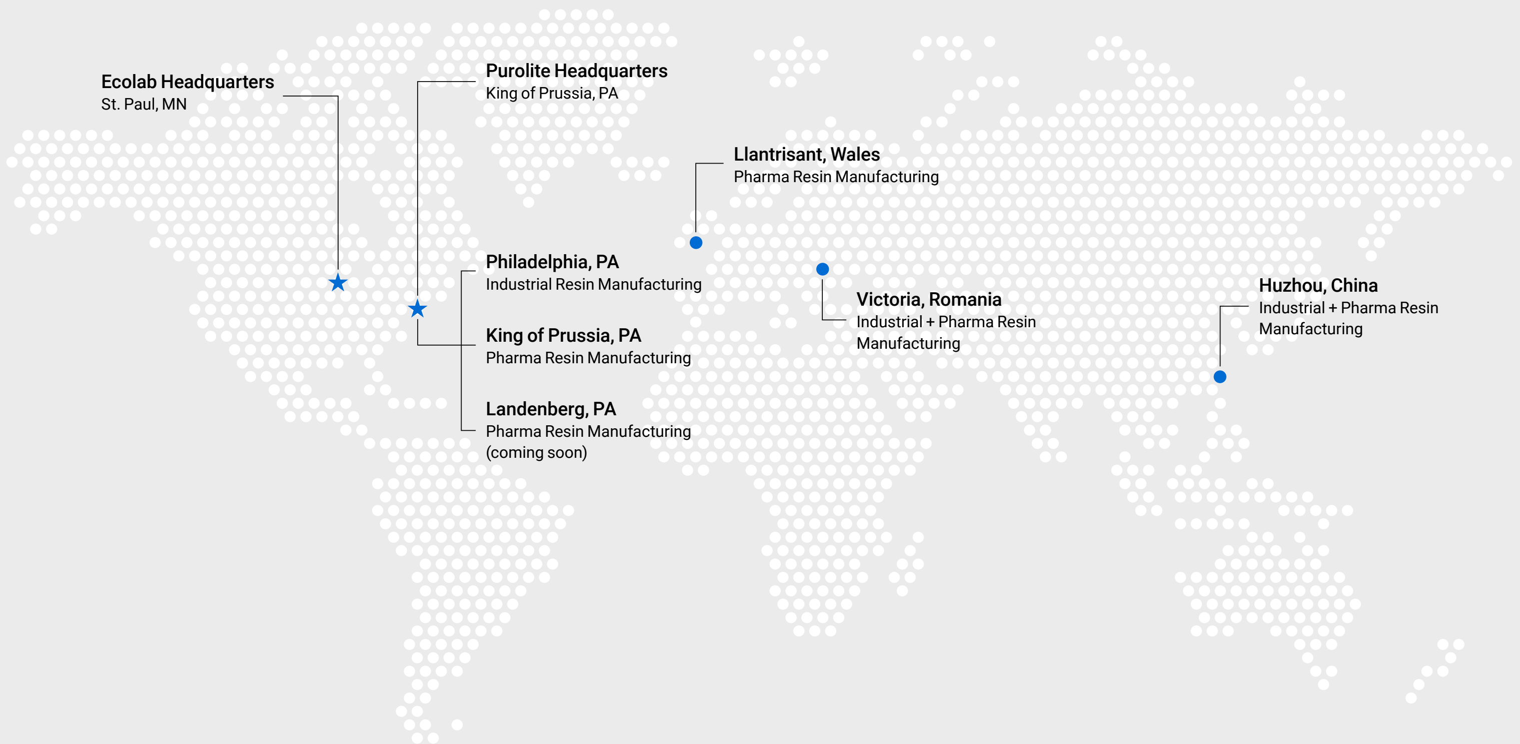
Purolite's diverse toolbox offers Protein A resins, Praesto A50 and APc+, designed for high performance and increased sustainability, as well as novel resins, Praesto A50 HipH and Praesto 70 CH1, designed to enable cost-effective and reliable purification of bispecifics and Fc fusion proteins.

Purolite Ion Exchange Toolbox

Purolite's ion exchange toolbox consists of Praesto SP and Praesto Q resins in four particle sizes to ensure predictable selectivity across particle sizes, allowing for rapid performance screening.

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

www.purolite.com



We're ready to solve your process challenges.

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