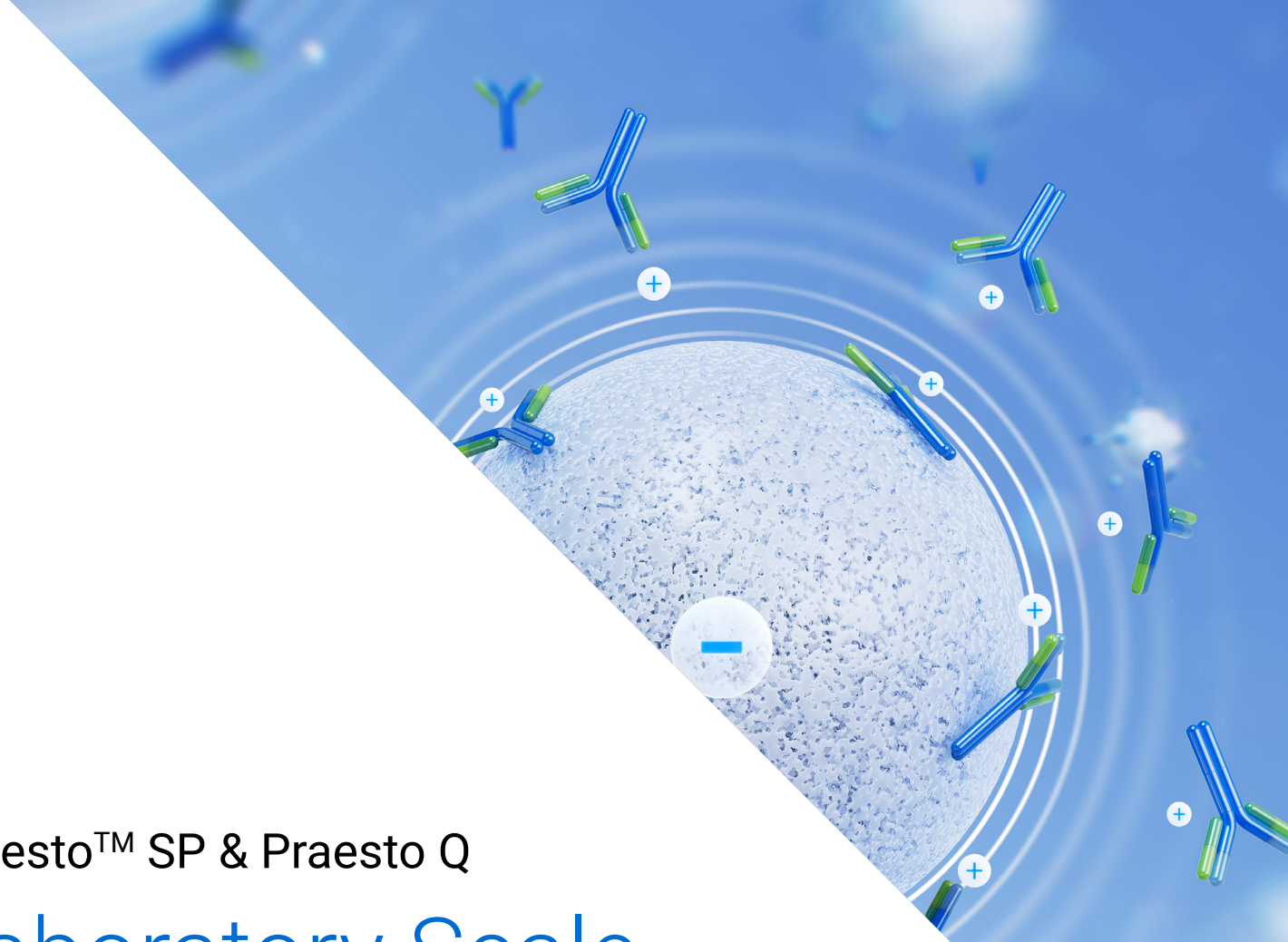
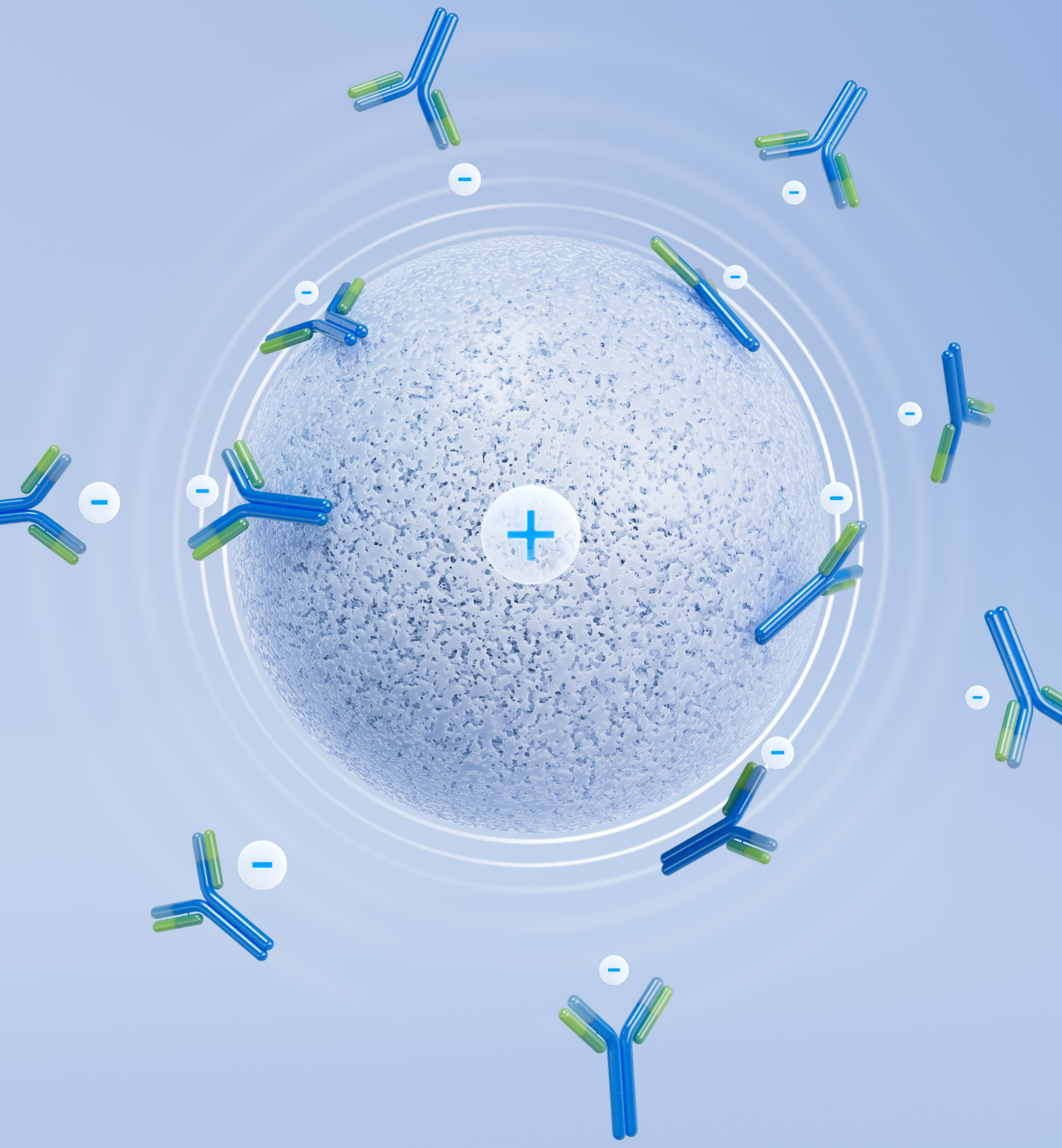


Praesto™ SP & Praesto Q

Laboratory Scale Column Packing

Purolite™
An Ecolab Company



Praesto™ SP & Praesto Q Laboratory Scale Column Packing

Contents

Overview

Praesto™ SP and Praesto Q

Praesto SP and Praesto Q are cross-linked agarose-based ion exchange chromatography resins designed for the laboratory-to-process-scale purification of recombinant proteins, monoclonal antibodies (mAbs), and other biomolecules. They are available in 90, 65, 45, and 35 µm particle sizes, covering the use of ion exchange in high-productivity capture steps as well as high-resolution polishing applications.

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 the characterization, purification, and manufacturing of a wide range of products, from food to life-saving medications. In biopharmaceutical manufacturing, 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 and lower yield, and it can subsequently affect the purity of the product. A column that is poorly packed can lead to expensive process disruptions and loss of valuable product.

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

This document provides packing procedures and parameters for laboratory scale columns.

Slurry Determination

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

Several techniques were employed to determine the 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 the laboratory scale, as volume can be added or removed during the packing process to obtain the desired bed height.

Suggested Materials and Equipment

- Praesto™ Q35, Praesto Q45, Praesto Q65, Praesto Q90
- Praesto SP35, Praesto SP45, Praesto SP65, Praesto SP90
- Chromatography column
- Column packing tube
- 100–500 mM 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

- Recommended slurry percentage = 50–70%.
- Recommended compression factor = please see tables 1 and 2.
- Wash an appropriate portion of the resin with 100–500 mM NaCl solution to remove the sample storage solution.
- Re-slurry the washed sample and either allow it to settle by gravity or centrifuge the resin sample at 100 g for 10 minutes.
- Note the slurry percentage and add/remove the packing solution to obtain the required slurry percentage for packing.
- Calculate the required slurry to add to the column using the following equation:

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

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

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

Step 2: Ensure the resin slurry is homogeneous and add to the column. Top up, if necessary, with packing buffer.

Step 3: Insert the top adaptor at a 45° angle to prevent air bubbles from entering the column. Secure the top adaptor.

Step 4: Disconnect the column outlet tube from the chromatography system and direct it to waste.

Step 5: Gradually increase the flow rate until a stable pre-column pressure is reached. (Refer to tables 1 and 2)

Step 6: Allow to run for 10 minutes at this flow. Monitor for any significant pressure changes and adjust the flow accordingly.

Step 7: Mark the point at which the bed has settled and stop the flow.

Step 8: Remove the packing tube.
At this point resin can be added or removed to obtain the target bed height.

Step 9: Re-insert the top adaptor and increase the volumetric flow until a stable pre-column pressure is reached. (Refer to tables 1 and 2)

Step 10: Mark the bed height and stop the flow.

Step 11: Lower the top adaptor to 1 mm past the marked bed height.

Step 12: Reconnect the column outlet tube to the chromatography system.

Step 13: Perform 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 tables 1 and 2 for details.

Step 14: 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 Q35	1.12	4 bar	800
Praesto SP35	1.12	4 bar	800
Praesto Q45	1.12	4 bar	800
Praesto SP45	1.12	4 bar	800
Praesto Q65	1.15	3.5 bar	600
Praesto SP65	1.15	3.5 bar	600
Praesto Q90	1.15	3.5 bar	600
Praesto SP90	1.15	3.5 bar	600

*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 Q35	1.15	4 bar	300
Praesto SP35	1.15	4 bar	300
Praesto Q45	1.15	4 bar	300
Praesto SP45	1.15	4 bar	300
Praesto Q65	1.17	3.5 bar	500
Praesto SP65	1.17	3.5 bar	500
Praesto Q90	1.17	3.5 bar	700
Praesto SP90	1.17	3.5 bar	700

*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 = 30 cm/h (Praesto™ Q/SP35 and Praesto Q/SP45).
- Consolidation flow rate = 60 cm/h (Praesto Q/SP65 and Praesto Q/SP90).
- Packing Factor = 1.15 (Praesto Q/SP35 and Praesto Q/SP45).
- Packing Factor = 1.17 (Praesto Q/SP65 and Praesto Q/SP90)

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

Step 2: Close the column bottom valve.

Step 3: Ensuring the resin slurry is homogeneous, add the calculated volume to the column.

Step 4: Insert the top adaptor once the resin slurry has settled sufficiently.

Step 5: Open the column bottom and direct it to waste.

Step 6: Start the consolidation flow and allow the resin to settle. Once the resin has settled, mark the bed height.

Step 7: Calculate the bed height using the compression factor listed in Table 1 and the marked bed height.

Example Calculation — Praesto SP45

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 8: Mark the target bed height.

Step 9: 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 10: Allow resin to settle for a minimum of 30 minutes.

Step 11: Stop the flow and disconnect the tubing from the top 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 (A_s). The HETP and A_s values are determined by applying a sample, such as 2% acetone or 1 M NaCl, to the packed column.

The optimal results will be obtained with a sample volume of approximately 1.5 % of the column volume and a flow velocity of 30–60 cm/h.

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

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

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 (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 $0.8 < A_S < 2.0$

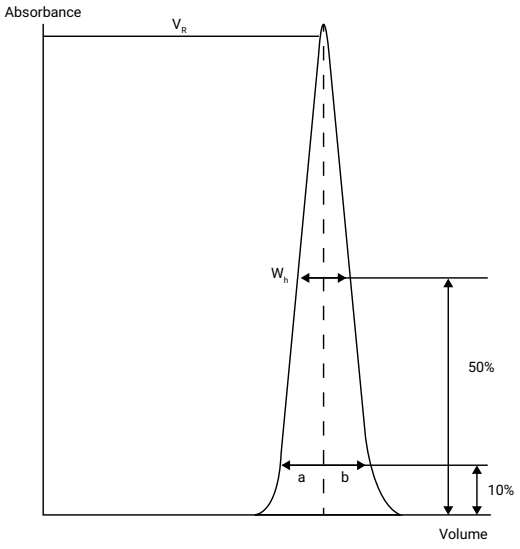
$$A_S = \frac{b}{a}$$

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 in solute, solvent, eluent, sample volume, flow velocity, and 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 Jetted A50 and Praesto AP+80, designed for high performance and increased sustainability, as well as novel resins, Praesto Jetted 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.

Notes

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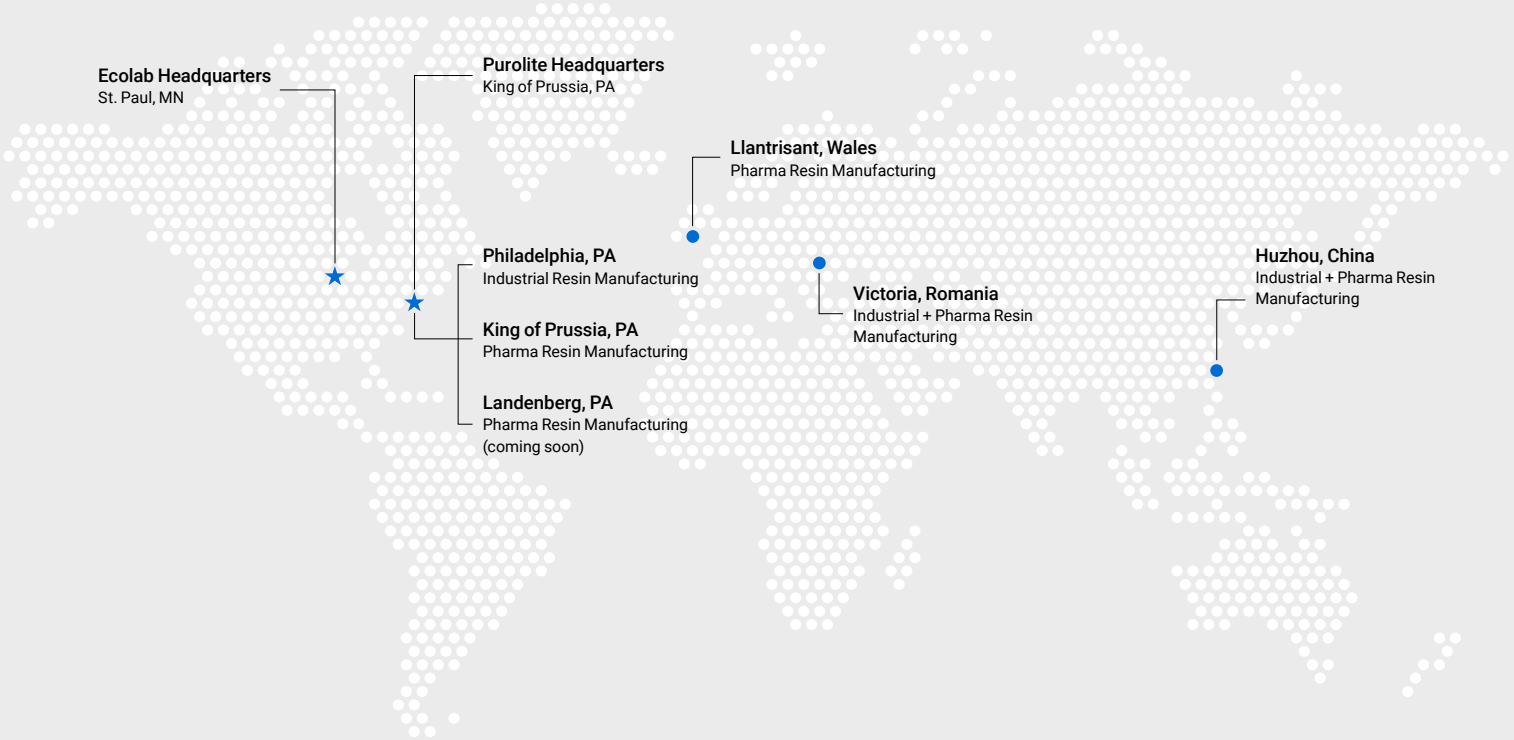
Notes

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.

For further information on Purolite products and services,
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