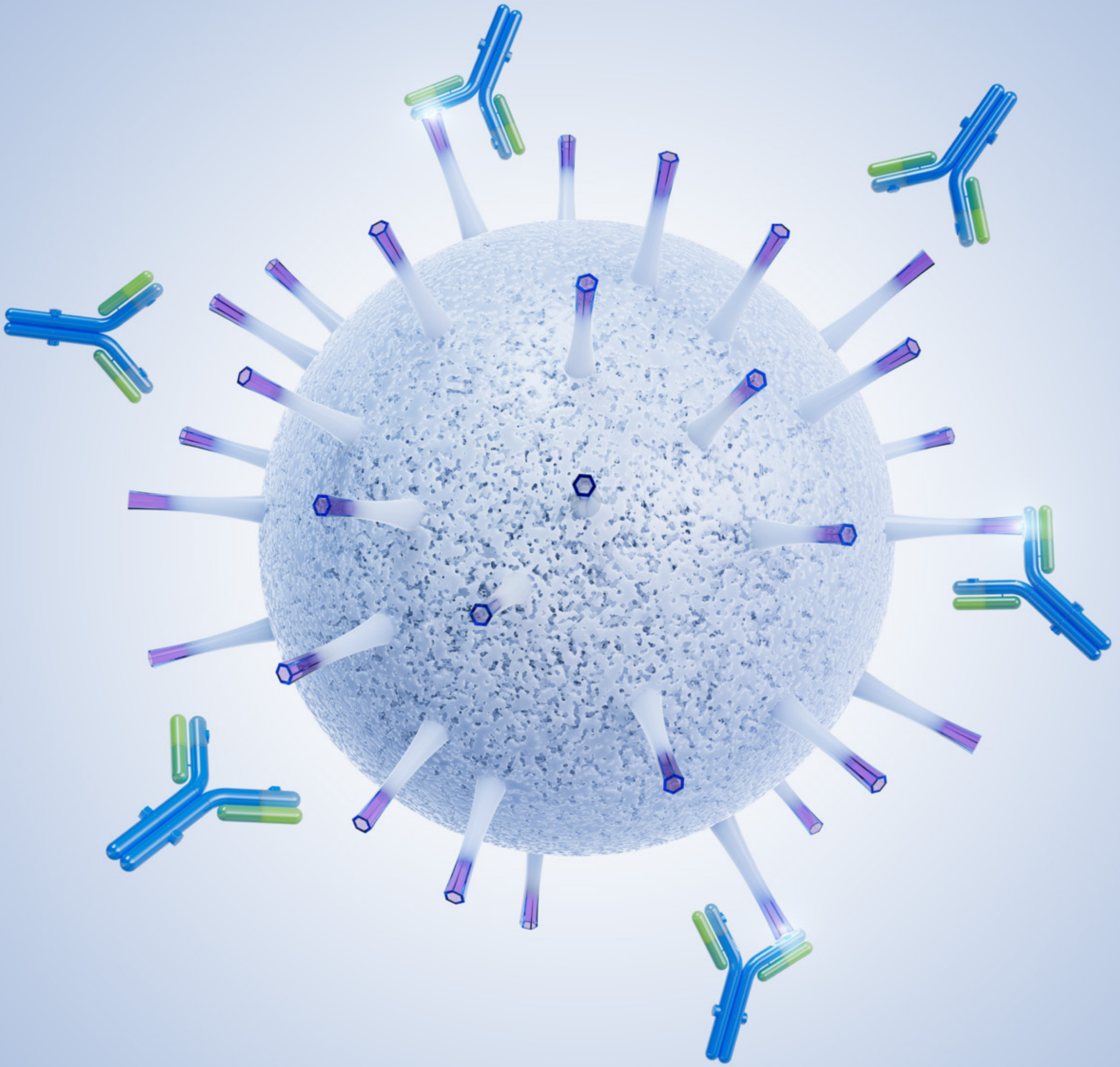
Laboratory Scale Column Packing Instructions

Purolite™
An Ecolab Company



Praesto™ AP+80 Laboratory Scale Column Packing Instructions

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Overview

Praesto AP+80

Praesto AP+80 is a high capacity, alkaline stabile protein A affinity chromatography resin, optimized for downstream processing of monoclonal antibodies and recombinant proteins.

Praesto resins are part of the Purolite bioprocessing chromatography resin product family. Based on highly cross-linked agarose, they offer good flow and pressure properties, excellent chemical and physical stability, high capacity, and are readily scalable.

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 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, 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.

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 a desired bed height.

There are 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 AP+80
- Chromatography column
- Column packing tube
- Demin H_2O 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

- Recommended slurry percentage = 50–70%
- Recommended compression factor = please see table 1.
- Wash an appropriate portion of the resin with demin H_2O or 0.1 M 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:

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

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 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 table 1)

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.
AAt this point, resin can be added or removed to obtain the target bed height. If the target bed height has not been achieved, add or remove resin. After adjustment, resuspend the slurry and restart the packing process.

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

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: Performing conditioning of the column by applying two 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)

Step 14: The column is now ready to be tested.

TABLE 1 **Packing Conditions for Laboratory Columns 0.5 to 2.6 cm in Diameter**

	Diameter	Compression Factor	Packing Pressure*	Recommended Packing Flow (cm/h)
Praesto AP+80	0.5	1.10	< 1 MPa	2457
	0.66	1.10	< 1 MPa	2457
	1.0	1.11	< 1 MPa	1682
	1.6	1.12	< 1 MPa	1493
	2.6	1.15	< 1 MPa	791

*System pressure contribution can vary dependent on system. Monitor to stay within the limits of the system and column.

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: 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 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

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. 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.

Optimal results will be obtained with a sample volume of approximately 1.5% of the column volume and a flow velocity of 30–50 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 $0.8 < A_S < 2.0$

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

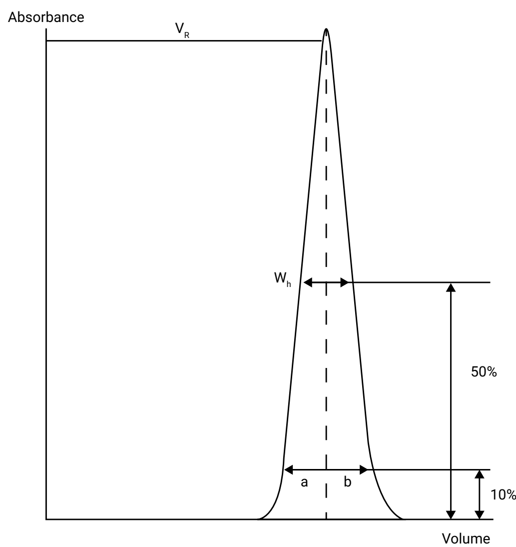
a = ascending part of the peak width at 10% peak height.

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 of solute, solvent, eluent, sample volume, flow velocity, and temperature will all affect the results.

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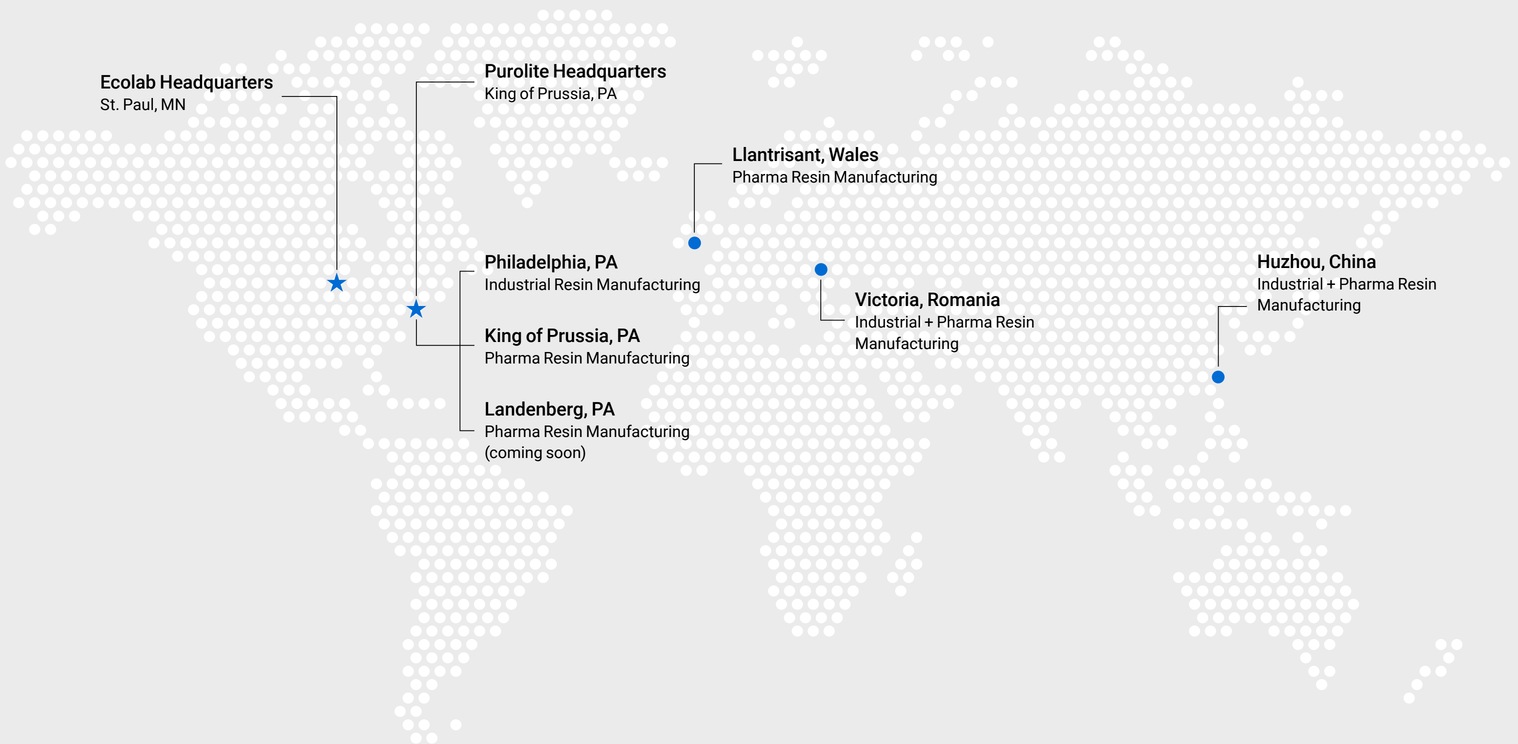

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visit www.purolite.com or contact us at the addresses below.

Americas
americas@purolite.com

Asia Pacific
asiapacific@purolite.com

EMEA
emea@purolite.com

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