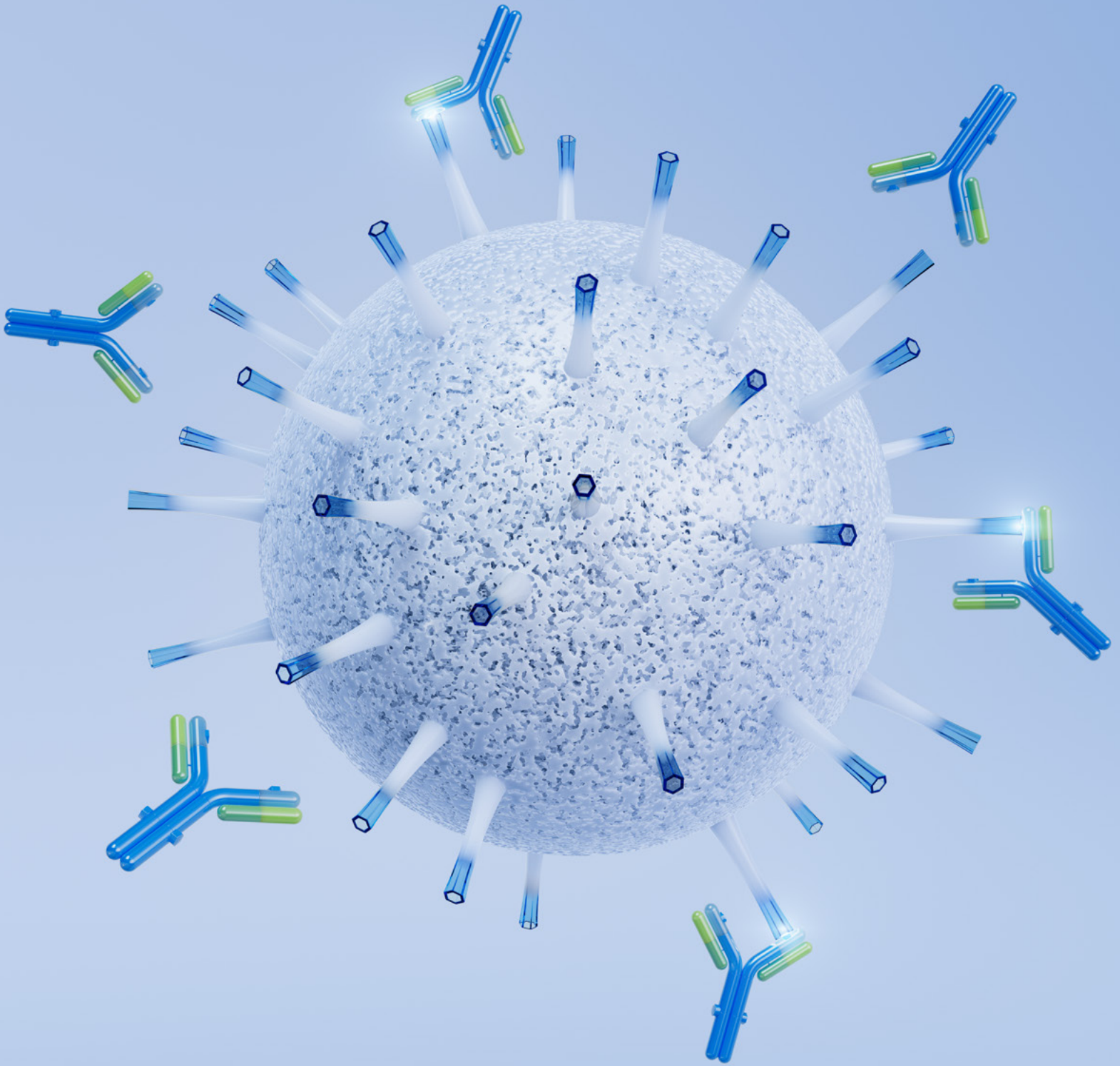




DurA Cycle A50

Laboratory Scale Column Packing Instructions



DurA Cycle A50 Laboratory Scale Column Packing Instructions

Contents

Overview	4
Column Packing	5
Pressure/Flow Packing	7
Flow/Mechanical Compression Packing	8
Column Efficiency Testing	9

Overview

DurA Cycle A50

DurA Cycle A50 is a high capacity, alkaline stable protein A affinity chromatography resin, optimized for downstream processing of monoclonal antibodies and recombinant proteins.

DurA Cycle affinity resins are part of the Purolite bioprocessing chromatography resin product family. Based on highly cross-linked agarose, they offer high-capacity resins with good flow and pressure properties 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 Distribution

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 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.

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

- DurA Cycle A50
- Chromatography column
- Column packing tube
- Demin H₂O 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 H₂O 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 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 to waste.

Step 5: Gradually increase the flow rate to the recommended 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.

If the target bed height has not been achieved, add, or remove resin at this point. After adjustment, resuspend the slurry and restart packing process.

Step 9: Re-insert the top adaptor and increase the volumetric flow until a stable pre-column pressure is reached at recommended packing flow. (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 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 for details.

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

TABLE 1 Packing conditions for laboratory columns 0.5–2.6 cm in diameter.

	Diameter	Compression Factor	Packing Pressure*	Recommended packing flow (cm/h)
DurA Cycle A50	0.5	1.10	< 1 MPa	1755
	0.66	1.10	< 1 MPa	1755
	1.0	1.11	< 1 MPa	1194
	1.6	1.12	< 1 MPa	1060
	2.6	1.15	< 1 MPa	561

*System pressure contribution can vary dependent on system, monitor and 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: Connect the column to the system.

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

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

Example for a 23.6 cm settled bed height;

23.6 cm (consolidated settled bed height) / 1.15 (PF) = 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 30–50 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

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

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;

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

d_{50v} = mean particle size (cm)

The reduced plate height 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 $0.8 < A_s < 2.0$

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

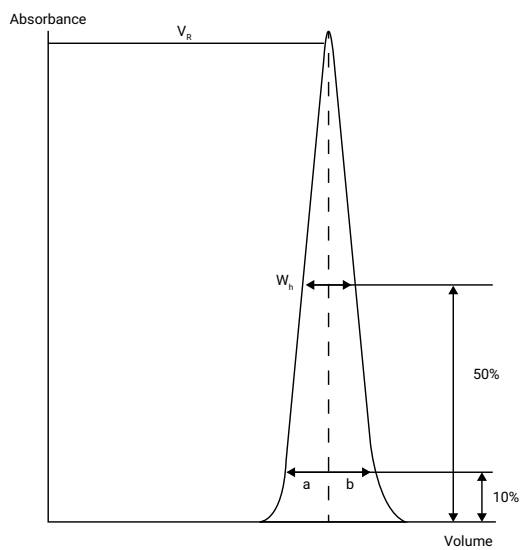
a = ascending part of the peak width at 10% peak height.

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.**

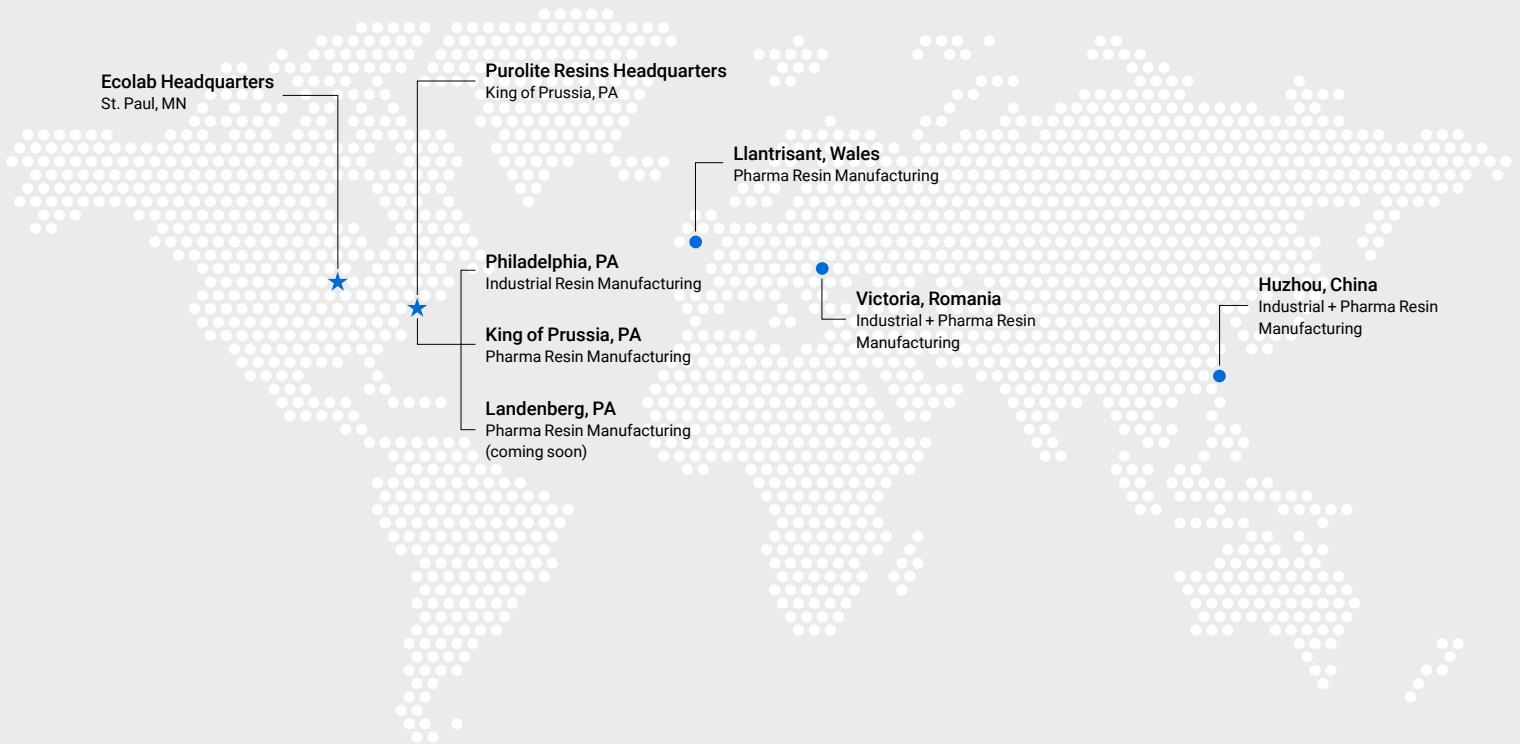


The calculated plate number will vary according to the test conditions, and it should only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, temperature will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

Ecolab is a global developer, manufacturer, and supplier of Purolite™ Resins including ion exchange, catalyst adsorbent and advanced polymers that make the world cleaner and healthier.



www.puoliteresins.com



We're ready to solve your process challenges.

For further information on Purolite products and services, visit www.puoliteresins.com or contact us at the addresses below.

Americas

americas@ecolab.com

Asia Pacific

asiapacific@ecolab.com

EMEA

emea@ecolab.com

The statements, technical information and recommendations contained herein are believed to be accurate as of the date hereof. Since the conditions and methods of use of the product and of the information referred to herein are beyond our control, Purolite expressly disclaims any and all liability as to any results obtained or arising from any use of the product or reliance on such information; NO WARRANTY OF FITNESS FOR ANY PARTICULAR PURPOSE, WARRANTY OF MERCHANTABILITY OR ANY OTHER WARRANTY, EXPRESSED OR IMPLIED, IS MADE CONCERNING THE GOODS DESCRIBED OR THE INFORMATION PROVIDED HEREIN. The information provided herein relates only to the specific product designated and may not be applicable when such product is used in combination with other materials or in any process. Nothing contained herein constitutes a license to practice under any patent and it should not be construed as an inducement to infringe any patent and the user is advised to take appropriate steps to be sure that any proposed use of the product will not result in patent infringement.



©2024 Purolite
All rights reserved.
P-000135-100PP-52024-ENG-R1-BP