Praesto® SP/Q45
Column Packing Instructions

1.0 Introduction

It is extremely important in bioprocess development and production that columns are packed efficiently and in a reasonable time frame. In this short instruction, methods for packing Praesto SP/Q45 are described. Methods for packing laboratory scale columns are detailed here.

1.1.1 Suggested Columns

Column	Inner Diameter	Bed Volume	Bed Height (cm)
Lab Scale			
Tricorn 5/50	5	0.98 ml	5
Omnifit 10/100	10	2.36 ml	3
HiScale Column	16-26	20-106 ml	10-20

2.0 Instructions - Lab Scale Columns

2.1 Tricorn 5/50: Materials and Equipment

- Praesto® SP/Q45.
- Tricorn 5/50 packing equipment
- Tricorn 5/50 column
- Glass filter funnel
- Plastic beaker
- Plastic spatula
- Measuring cylinder
- 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 (depending on the flow rate required) can be used for packing.

	Operation
2.1.1	Set up a filter funnel over an appropriate collection vessel. Pour the medium onto the filter funnel and wash the medium by pouring 500 ml of 100 - 500 mM NaCl solution. This will remove the 20% ethanol storage solution.
2.1.2	Remove the medium from the filter funnel, add to an appropriate size falcon tube and add packing solution then centrifuge at 1800 rpm for 10 minutes.
2.1.3	Calculate the slurry % and add or remove packing solution to obtain a 50 % slurry. Calculate the required slurry volume for a 5 cm packed bed. 1. Determine the slurry volume for column packing. 2. Determine the desired packed bed height. 3. Calculate the column volume (C_V) of a packed column by the following equation; a. Cross-sectional area of the column (CSA) × bed height (Bh) b. Multiply the column volume by a compression factor (C.F) ($C_V \times C.F$) (C.F = 1.2) c. Divide by the slurry concentration. d. *Example calculation;* Column: Tricorn 5/50 Desired bed height: 5 cm Slurry concentration: 50 % Compression Factor (Praesto® SP/Q45): 1.2 (CSA ×Bh ×C.F)/ (Slurry Concentration) $((0.25)^2 \times \pi) \times 5 \times 1.2)/0.5 = 2.36$ ml Required slurry volume for a 5 cm packed bed = **2.4 ml**

<table>
<tr><th></th><th>Operation</th></tr>
<tr><td>2.1.4</td><td>Unpack a Tricorn 5/50 column, assemble and connect Tricorn 5/50 packing equipment as per the manufacturer's instructions (GE Healthcare).</td></tr>
<tr><td>2.1.5</td><td>Stir the column media gently to ensure homogeneity, fill the column with the calculated slurry and top up with packing solution.</td></tr>
<tr><td>2.1.6</td><td>Insert top filter at a 45° angle to prevent air bubbles forming at the top of the column and screw the top cap on the packing column.</td></tr>
<tr><td>2.1.7</td><td>Start a flow rate of 0.5 ml/min of packing solution through column switching valve of the selected system. Once a flow is established, connect tubing from the column switching valve to the top of the packing column.</td></tr>
<tr><td>2.1.8</td><td>Remove the stop plug from the bottom of the column and replace with the outlet column tubing connected to the column switching valve. Disconnect the outlet tubing from the system and place in a collection vessel.</td></tr>
<tr><td>2.1.9</td><td>Start a packing flow at a linear velocity of 200 cm/h and leave to pack for 10 minutes or 10 column volumes.</td></tr>
<tr><td>2.1.10</td><td>Turn the flow off and attach a stop plug to the bottom of the column. Dismount the packing tube and remove excess resin using a pipette.</td></tr>
<tr><td>2.1.11</td><td>Top up the column with packing solution and attach an adaptor with a filter in place. Screw down the adaptor to 1 to 2 mm above the packed bed. Turning the adaptor down will expel any air in the adaptor tubing.</td></tr>
<tr><td>2.1.12</td><td>Reconnect the column to the system following the steps described in 2.1.7 to 2.1.8.</td></tr>
<tr><td>2.1.13</td><td>Start a packing flow at 200 cm/h and leave to run for 10 minutes or 10 column volumes. Increase the flow rate until a pressure of 0.3 MPa (3 bar is reached) and allow to run for 5 column volumes.</td></tr>
<tr><td>2.1.14</td><td>Mark the bed height after 10 minutes and stop the pump.</td></tr>
<tr><td>2.1.15</td><td>Turn the adaptor down to the mark point, and then give the adapter and extra 1/3 turn.</td></tr>
<tr><td>2.1.16</td><td>Start a conditioning flow of 200 cm/h through the column and allow to run for 10 column volumes.</td></tr>
<tr><td>2.1.17</td><td>Note: If a gap is formed between the bed and the adapter during flow conditioning, turn the adapter down to close the gap and restart the conditioning flow.</td></tr>
<tr><td>2.1.18</td><td>Note: Check for any pressure spikes during the packing procedure, any rapid increase in pressure without stabilisation would indicate filter blockage.</td></tr>
<tr><td>2.1.19</td><td>The column is now ready to be tested.
<table>
<tr><th>Phase</th><th>Buffer</th><th>Column volumes (CV)</th><th>Linear velocity (cm/h)</th></tr>
<tr><td>Equilibration</td><td>Packing Solution</td><td>3</td><td>60 cm/h</td></tr>
<tr><td>Sample Application</td><td>1% volume of CV. (2% acetone or 1M NaCl)</td><td>-</td><td>60 cm/h</td></tr>
<tr><td>Elution (Isocratic)</td><td>Packing Solution</td><td>2.5</td><td>60 cm/h</td></tr>
</table></td></tr>
</table>

2.2 Omnifit 10/100 (3 cm bed height): Materials and Equipment

- Praesto® SP/Q45.
- Omnifit 10/100 column
- Glass filter funnel
- Plastic beaker
- Plastic spatula
- Measuring cylinder
- 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 (depending on the flow rate required) can be used for packing.

<table>
<tr><th></th><th>Operation</th></tr>
<tr><td>2.2.1</td><td>Set up a filter funnel over an appropriate collection vessel. Pour the medium onto the filter funnel and wash the medium by pouring 500 ml of 100 – 500 mM NaCl solution. This will remove the 20% ethanol storage solution.</td></tr>
<tr><td>2.2.2</td><td>Remove the medium from the filter funnel, add to an appropriate size falcon tube and add packing solution, then centrifuge at 1800 rpm for 10 minutes.</td></tr>
<tr><td>2.2.3</td><td>Calculate the slurry after the last centrifugation and top up with 100 – 500 mM NaCl to obtain a 50% slurry.</td></tr>
<tr><td>2.2.4</td><td>The volume of slurry required to pack a given bed height can be estimated using the following formula:
Volume slurry = Volume packed bed x (100 / Slurry%) x 1.2 (compression factor)
Volume slurry = 2.36 x 2 x 1.2 = 5.66 ml
Bed height (50% slurry) = 7.2 cm</td></tr>
<tr><td>2.2.5</td><td>Assemble the bottom end piece to the column and mark the given bed height (7.2 cm) on the column tube.</td></tr>
<tr><td>2.2.6</td><td>Assemble the top adapter and connect it to the designated system. Start the flow at 2.6 ml/min (200 cm/h), allow to run for 5 minutes to allow any air to pass through the top adaptor.</td></tr>
<tr><td>2.2.7</td><td>Stir the slurry to ensure homogeneity and add the required volume to the column. (Up to the 7.2 cm marked point)</td></tr>
<tr><td>2.2.8</td><td>Fill the column with packing buffer and insert the top adaptor at a 45° angle to prevent air bubbles forming. Stop the flow and bring the adaptor to approximately 1 mm above the bed formation. Restart the flow and connect the bottom tubing to the system.</td></tr>
<tr><td>2.2.9</td><td>Bring the adaptor down until the gap above the bed is closed, compress until the bed has reached 3 cm.</td></tr>
<tr><td>2.2.10</td><td>Condition the packed column at 2.6 ml/min (200 cm/h) by flowing 3 column volumes of packing buffer upflow, followed by 3 column volumes downflow. Repeat this step 3 times. (check the pressure; usually it is less than 4bar = 0.4MPa).
Note: If a gap is formed, compress further and repeat the conditioning step.</td></tr>
<tr><td>2.2.11</td><td>The column is now ready to be tested
<table>
<tr><th>Phase</th><th>Buffer</th><th>Column volume (CV)</th><th>Linear Velocity (cm/h)</th></tr>
<tr><td>Equilibration</td><td>Packing Solution</td><td>3</td><td>60 cm/h</td></tr>
<tr><td>Sample Application</td><td>1% volume of CV. (2% acetone or 1M NaCl)</td><td>-</td><td>60 cm/h</td></tr>
<tr><td>Elution (Isocratic)</td><td>Packing Solution</td><td>2.5</td><td>60 cm/h</td></tr>
</table></td></tr>
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2.3 HiScale (Flow & Manual Compression Packing): Materials and Equipment

- Praesto® SP/Q45.
- HiScale 16 or 26 mm diameter column
- Glass filter funnel
- Plastic beaker
- Plastic spatula
- Measuring cylinder
- 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 (depending on the flow rate required) can be used for packing.

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<tr><th></th><th>Operation</th></tr>
<tr><td>2.3.1</td><td>Set up a filter funnel over an appropriate collection vessel. Pour the medium onto the filter funnel and wash the medium by pouring 500 ml of 100 – 500 mM NaCl solution. This will remove the 20% ethanol storage solution.</td></tr>
<tr><td>2.3.2</td><td>Remove the medium from the filter funnel, add to an appropriate size falcon tube and add 100 – 500 mM NaCl then centrifuge at 1800 rpm for 10 minutes.</td></tr>
<tr><td>2.3.3</td><td>Calculate the slurry % and adjust the volume to obtain a 50% slurry.</td></tr>
<tr><td>2.3.4</td><td>The volume of slurry required to pack a given bed height can be estimated using the following formula:
Volume slurry = Volume packed bed x (100 / Slurry%) x 1.2 (compression factor)</td></tr>
<tr><td>2.3.5</td><td>Wet the filters with water and assemble the column according to the manufacturer’s instructions.</td></tr>
<tr><td>2.3.6</td><td>Fill the column with the calculated slurry and top up with packing solution. (If necessary).</td></tr>
<tr><td>2.3.7</td><td>Once the resin has settled approximately 1 cm from the top, place the top adaptor into column and secure as per the manufacturer’s instructions.</td></tr>
<tr><td>2.3.8</td><td>Calculate the bed height for a 1.2 compression from the marked bed height.
Mark the target bed height.</td></tr>
<tr><td>2.3.9</td><td>Increase the flow until a pressure of 0.3 MPa (3 bar) is reached to apply compression on the bed by flow and let the flow run for approximately 20 minutes.</td></tr>
<tr><td>2.3.10</td><td>Stop the flow and disconnect the tubing from the top of the column. Manually compress the bed by adjusting the adaptor until the target bed height is reached. (Determined in part 2.3.8)</td></tr>
<tr><td>2.3.11</td><td>The column is now ready to be tested
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<tr><th>Phase</th><th>Buffer</th><th>Column volume (CV)</th><th>Linear Velocity (cm/h)</th></tr>
<tr><td>Equilibration</td><td>Packing Solution</td><td>3</td><td>60 cm/h</td></tr>
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3.0 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 through the use of 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 in demineralised water to the packed column.

A sample of 0.4 to 0.8 M NaCl in demineralised water can also be used.

A sample volume of approximately 1.5 % of the column volume and a flow velocity of between 30 to 50 cm/h will give the optimal results.

4.0 Calculating HETP and A_s

Below is the calculation by which HETP and AS are determined. This is done using the UV curve (or if using a NaCl sample, the conductivity curve is used).

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

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;

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

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 well packed 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 < AS < 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 as a result of excessive use.

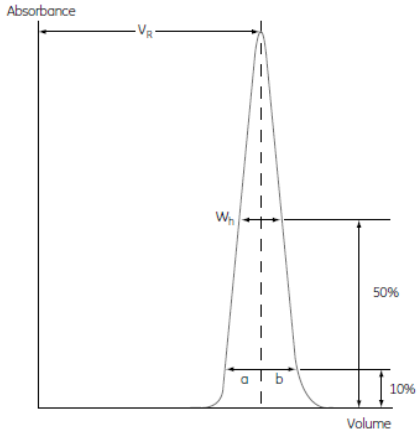

Figure 1. An example UV chromatogram of a 1 – 3 % acetone sample during a column efficiency test.

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.

Ordering Information

To place your order, simply contact us via email, or telephone using the information at the base of this sheet.

If you wish to discuss your purification challenges with a specialist, we have dedicated experts on-hand, across the globe to provide knowledgeable, same day technical assistance.

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