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Technical note

J.T.Baker® BAKERBOND® PROchievA™ column packing guidelines

J.T.Baker®

INTRODUCTION

BAKERBOND® PROchievA™ affinity resin is designed for affinity chromatographic purification of mAbs and Fc-fusion protein manufacturing. It is based on spherical agarose particles, with average particle size of 70-80 µm, coupled with optimized ligand for selectivity and alkaline stability. The spherical particles with average particle size of 70 to 80 µm allow manual or automated packing and operation of process chromatography column with wide pressure and flow range of up to 500 cm/hour under 3 bar. Please see product technical data sheets for additional product attributes, typical properties and application information

GENERAL GUIDELINES BEFORE PACKING

1. Calculate the resin volume needed to pack the desired final column volume. The resin is shipped as approximately 50% (v/v) slurry in 200 mM sodium acetate at pH 5.5 containing 2% benzyl alcohol. Depending on the column type and buffer, the compression factor ranges from 1.1 to 1.2.

   Desired packed column volume (Vc) is calculated from the equation:

   $$Vc = Ac \times L$$

   where Ac is the surface area of the column
   L is the desired packed bed height

   Required volume of gravity settled media (Vgs) for packing is:

   $$Vgs = Vc \times CF$$

   where CF is the compression factor for the media

   Since CF is dependent on the buffer, it is suggested to record Vgs and final Vc from the first packaging. This CF can be used in the future in the same buffer system.

2. Re-suspend the settled resin by mixing the container end over end. A magnetic stir bar should never be used, as it will physically damage the media. Transfer the required amount to pack the column to a clean container that is compatible with the shipping buffer.
3. The media can be packed as supplied and does not require removal of the shipping solution provided that all column materials are compatible. Once the material is suspended, the column should be packed as soon as practical to avoid settling of any residual fines on top.
4. Various solutions such as water, 1X PBS, 50-100 mM sodium acetate, or other suitable buffers can be used for suspending the media or packing the column.

COLUMN PACKING INSTRUCTION (PRESSURE PACKING)

1. Prepare the column as per the column manufacturer's guidelines.
2. Slurry (40 – 50% v/v) the media in a desired column packing buffer.
3. Remove any bubbles trapped in the column after washing, using the column manufacturer's recommendation, and close the column outlet. Add the slurry to the column and make sure there is enough space to put the adapter back without immersing the adapter into the slurry.
4. Connect the top flow adapter, remove the air bubbles from the column inlet, open the outlet of the column, and slowly pump the packing buffer though the column while monitoring the column pressure. Make sure the column pressure is below the column hardware limit and below 3 bar on the resin as this can cause permanent damage to the resin.

5. Increase the flow rate to about 50-100 cm/hour above the intended highest operating linear velocity and maintain the flow rate for 30-45 minutes.
6. Switch off the pump, close the column outlet, and disconnect the column inlet tubing from the equipment. Lower the top adapter to approximately 1 cm above the resin bed, following the column manufacturer's instructions. At this point, a clear liquid should come out of the tube attached to the top adapter.
7. Reconnect the column inlet tubing and open the column outlet. Continue to pack at the desired flow rate and note the settled media height while column is being packed. Continue the packing process for another 15 minutes or until the media bed height is constant.
8. Switch off the pump, close the column outlet, and disconnect the column inlet tubing from the equipment. Lower the top adapter following the column manufacturer's instructions. At this point, the clear liquid should come out of the tube attached to adapter. Lower the adapter until it is in contact with the media, trying to get as close to the mark as possible without putting too much stress on the media.
9. Reconnect the column inlet tubing and equilibrate the column with a suitable solution. The column is now packed and ready for use.
10. Test the column for column integrity if needed.

COLUMN PACKING METHODS BY OTHER METHODS

The above procedure is intended to provide detailed packing guidance using the pressure packing method. BAKERBOND® PROchievA™ can be packed using other packing methods depending on the type of column being packed; however, be sure to follow the above general procedure before transferring the slurry into the column and follow the column manufacturer's recommended conditions to put the adapter in place.

COLUMN INTEGRITY TEST

For column integrity testing, 2.0 M NaCl and/or 1% acetone in water can be injected. Injection volume could be from 0.5 to 2% of bed volume and monitored by conductivity for NaCl peak and absorbance at 280 nm for acetone peak. Asymmetry of PROchievA™ for conductivity peak is 0.9 to 1.8.

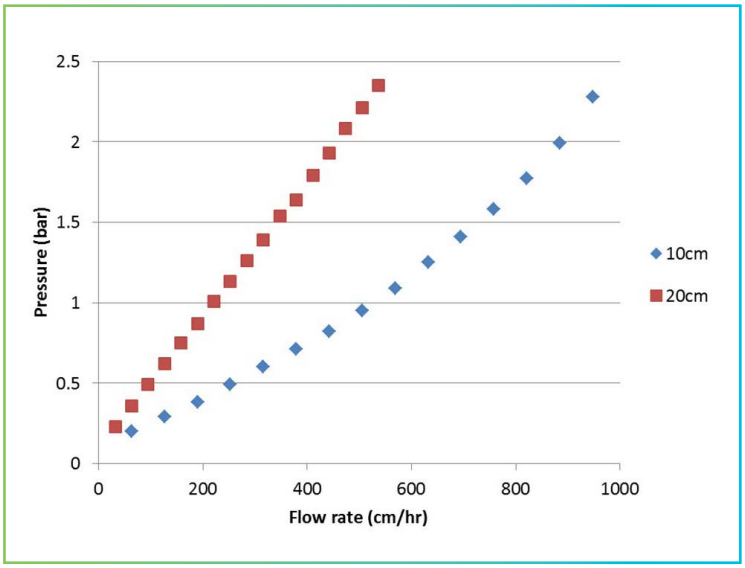

FIGURE 1: Column pressure at different flow rates. Data is generated with 10cm and 20cm bed height columns with 1.1cm diameter.

COLUMN PRESSURE/FLOW

Using the recommended column packing procedure described above yields high efficiencies as well as good asymmetries, and is reproducible in 10 cm and 20 cm bed height, indicating easy process scalability.

STORAGE CONDITIONS

Store the packed BAKERBOND® PROchievA™ column in 200 mM sodium acetate at pH 5.5 containing 2% benzyl alcohol in room temperature for up to 3 months or in 2-8°C for long-term storage. If needed, the column can also be stored in 20% ethanol.

CLEANING CONDITIONS

Clean the column with 0.1N NaOH with 15 to 60 minutes of contact time to sanitize the column. In some cases, 0.5N sodium hydroxide can be used at 2-8°C. For improved long-term stability at ambient conditions, 1M sucrose can be added to NaOH solutions. Other additives such as ethylene glycol or propylene glycol can also be used. Please see PROchievA™ stability technical note for detail.

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