



Large-scale chromatography

Packing Capto™ ImpRes resins with verified packing methods

Capto ImpRes chromatography resins are designed for high-throughput intermediate purification and polishing steps of a wide range of biomolecules. The resins are based on high-flow agarose and display excellent resolution. The ability to run at higher flow velocities and higher bed heights increases productivity and flexibility in process design. However, to achieve effective purification, it is important that the resins are efficiently packed. Poorly packed columns can lead to costly disruptions and loss of valuable product. Robust and verified packing and testing methods can eliminate such concerns and risks. This application note describes packing methods resulting in columns with verified bed stability for Capto SP ImpRes, Capto Q ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes resins in large-scale chromatography columns.

Capto ImpRes resin characteristics

Capto ImpRes resins are based on highly rigid agarose that allows high flow, which is an important factor for high productivity in large-scale operations. The small average bead size of 40 µm gives high resolution. Capto ImpRes resins can be designed with a wide range of bed heights and flow velocities. The pressure-flow specification for these resins in large columns is 220 cm/h at less than 3 bar and at a 20 cm bed height (tested with water at 20°C in 1 m diameter column).

Large-scale columns for packing with Capto ImpRes resins

AxiChrom™ columns

AxiChrom columns are low-pressure, axial mechanical compression chromatography columns designed for process development and biopharmaceutical manufacturing. Mechanical axial compression enables accurate and reproducible control of the packing, even with large diameter columns.

AxiChrom columns are available in many different configurations and materials (see data file 28929041 for more details).

AxiChrom columns are designed to be scalable and will give predictable results over the entire range of scales by enabling a uniform plug flow through the bed, irrespective of size.

The columns feature Intelligent Packing with preprogrammed methods that support all column sizes. Intelligent Packing enables straightforward operation and high packing success rates. The packing methods described here apply to bed heights up to 40 cm in AxiChrom columns, depending on column diameter.

BPG columns

BPG columns are glass columns for process development and manufacturing. The single-screw adapter allows easy and efficient packing and running. The columns have diameters from 100 to 450 mm. The packing methods described here apply to all BPG columns, except for BPG 450, which is not pressure rated for use with Capto ImpRes resins.

Chromaflo™ columns

Chromaflo columns are acrylic or steel, pack-in-place columns for GMP manufacturing. The columns have diameters ranging from 400 to 2000 mm. The packing method described here applies to Chromaflo columns up to 600 mm having a standard configuration.

Packing

Definitions

The bed height of a gravity-settled bed differs from the bed height of a bed settled under low flow (consolidated). Therefore, the compression factor (*CF*) has to be separated from the packing factor (*PF*). For example in water, when consolidated at 30 to 60 cm/h, the *PF* is 1.15 and the *CF* is 1.12 for Capto ImpRes resins.

Equations to calculate *CF*, *PF*, and column volume (V_c) are shown below:

$$\text{Compression factor, } CF = \frac{L_{\text{settled}}}{L_{\text{packed}}}$$

$$\text{Packing factor, } PF = \frac{L_{\text{cons}}}{L_{\text{packed}}}$$

where

L_{settled} = Bed height measured after settling by gravity (cm)

L_{cons} = Consolidated bed height, that is bed height measured after settling the resin at a given flow velocity (cm)

L_{packed} = Packed bed height (cm)

Column volume, $V_c = L_{\text{packed}} \times A_c$

where

A_c = Cross-sectional area of the column (cm²)

When packing BPG and AxiChrom columns, *PF* is used in the packing procedure to calculate the packed bed height after the consolidation step. *CF* is used in the chromatography resin preparation step to calculate the resin volume needed to pack a desired bed height. Because Chromaflow columns are pack-in-place columns, they have no registered consolidated bed heights and the *CF* is used throughout the packing process.

Properties of Capto ImpRes resins in various packing solutions

Capto ImpRes resins settle quickly in both water and in 20% ethanol and, when using these solutions, it should be remembered that tubing and nozzles must be rinsed directly after packing to prevent clogging of the flow path. Adding salt to packing solutions slows the settling of the resin beads and also allows them to settle less tightly. As a consequence, it is very difficult to measure slurry concentration by gravity with salt-based solutions. However, the slurry concentration method described below allows quick and accurate determination of slurry concentration.

When Capto ImpRes resins are settled at 30 to 60 cm/h, the consolidated bed height will be 3% to 7% higher in salt solution compared to in water or 20% ethanol. The effect is almost the same for 10 mM NaCl as for 0.4 M NaCl and can be compensated for by using different *PF*. Table 1 shows *PF* for water and a variety of other packing solutions. However, for optimal performance of the packed bed, specific packing solutions are recommended for different column types and sizes.

Table 1. *PF* required for a 20 cm bed of Capto ImpRes resins in different solutions for optimal bed performance where the bed is consolidated at 30 to 60 cm/h

Solution	Capto Q ImpRes and Capto adhere ImpRes	Capto SP ImpRes and Capto MMC ImpRes
Water	1.15	1.15
20% ethanol	1.15	1.15
10 mM NaCl	1.20	1.20
0.4 M NaCl	1.20	1.20
20% ethanol with 0.2 M sodium acetate	N/A	1.20

Slurry preparation

When preparing the slurry, start by calculating the chromatography resin volume (*V*) needed to pack the desired bed height. The slurry concentration can be determined in a number of ways, but to get an accurate slurry concentration determination of Capto ImpRes resins in salt-containing solutions, use the method described below.

When calculating the slurry volume (*V*), use the compression factor in water/20% ethanol (*CF* = 1.12).

Note: The slurry concentration determined by the method below corresponds to the gravity-settled concentration in water.

$$V = \frac{A_c \times L_{\text{packed}} \times CF}{C_{\text{slurry}}}$$

where

C_{slurry} = Concentration of the slurry

Preparing the resin to form a slurry can be performed manually or mechanically, for example by using Media Wand™ slurry mixing and transfer tool. Shaking gives good results, but is often not practical for larger volumes. When stirring, it is preferable to use soft stirrers without sharp edges. Media Wand suspends the resin directly in the container and transfers the slurry to the slurry tank in one operation, which makes it suitable for large-scale packing.

Capto SP ImpRes and Capto MMC ImpRes are supplied in 20% ethanol with 0.2 M sodium acetate. Capto Q ImpRes and Capto adhere ImpRes are supplied in 20% ethanol. Before packing, transfer the resin to the packing solution as described in the packing instructions for the relevant column.

Measuring slurry concentration

To achieve the correct amount of chromatography resin for packing to target bed height or compression, it is important to measure the slurry concentration correctly. Measuring slurry concentration can be performed with a Tricorn™ 10/100 column. GE Healthcare offers a Slurry Concentration Kit (see Ordering information) with all of the materials required for determination of slurry concentration.

Packing Capto ImpRes resins in AxiChrom columns

When packing AxiChrom 50 to 200 columns for use with ÄKTATM systems, Intelligent Packing control is managed by UNICORN™ control software. For AxiChrom 300 to 1000 columns, Intelligent Packing is performed by AxiChrom Master, a separate unit that comprises a touch-screen operated user interface, or from UNICORN control software on ÄKTAProcess™ system.

Intelligent packing in AxiChrom columns – general considerations

Packing methods are created by entering values for the packing variables, for example, resin, slurry concentration, and target bed height, in the Intelligent Packing wizard.

When packing AxiChrom 50 to 200 columns, the slurry is introduced into the column by hand and adapter movement that is driven by internal hydraulics. After the wizard method has been created and the resin has been equilibrated to the packing solution, the column is primed and filled with slurry. The method controls the flow of hydraulic fluid to drive the adapter and pack the bed (Fig 1).

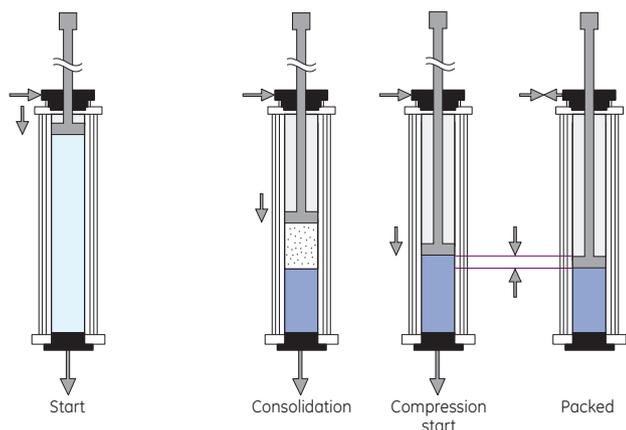


Fig 1. Intelligent Packing in AxiChrom 50 to 200 columns. The adapter is mounted to the column tube and the wizard is started (**Start**). The adapter moves down, forcing packing liquid out through the bottom bed support. The resin forms a consolidated bed (**Consolidation**). When the adapter comes in contact with the consolidated bed surface, the operator initiates bed compression in the UNICORN wizard (**Compression start**). Compression occurs according to a predetermined *PF*. Finally, the target bed height is attained (**Packed**).

In AxiChrom 300 to 1600 columns, slurry is introduced via a resin valve in the center of the bottom bed support and the adapter is driven by an electric servo-motor. The two-position resin valve enables filling, packing, and unpacking without adjusting the assembled column.

After the column is primed, the adapter rises from its lowest position and the column fills with slurry via the resin valve. The slurry volume is calculated automatically from the target bed height, slurry concentration, and *PF*. Also the volume of the tubing connection between the column and slurry tank is taken into consideration. As an electric servo-motor controls the movement of the adapter, its position is monitored with millimeter accuracy.

When the correct slurry volume has been drawn into the column, the adapter starts to lower and packing buffer is forced out through the bottom bed support and bed consolidation starts. The time to complete consolidation (i.e., when the adapter reaches the bed), is also automatically calculated (as for the AxiChrom 50 to 200 columns), allowing the operator to carry out other tasks in the meantime. As the adapter hits the consolidated bed, a distinct dip is seen on the pressure curve. When this occurs, the operator confirms that the adapter has hit the bed.

The compression of the resin starts and a graphical interface shows on the control screen of UNICORN or AxiChrom Master. This graphical interface assists the operator in finishing the packing, giving a perfectly packed bed. When the adapter symbol is within the range of approved packing factors and bed height limits, the operator can end the packing.

Specific considerations for packing Capto ImpRes resins in AxiChrom columns

A general, bed height interval for Capto ImpRes resins in AxiChrom columns is 10 to 40 cm. When packing Capto ImpRes in AxiChrom, the Intelligent Packing wizard custom resin option is used. When applying this option, manual input is needed regarding additional packing variables as *PF* and adapter velocity. Flow conditioning with desired flow velocity and time can be applied. Packing variables and recommended packing buffers are given in Table 2. The actual packing procedure using the custom resin option works the same way as for resins included in the Intelligent Packing wizard.

To achieve optimal bed performance, flow conditioning is recommended for AxiChrom 50 to 200 columns but is optional for AxiChrom 300. The flow velocities shown during flow conditioning are recommended for liquids with the same viscosity as water at 20°C.

Note: The maximum recommended bed height is 30 cm for AxiChrom 600 to 800 columns and 20 cm for AxiChrom 1000 columns using the recommended packing factor. Bed heights above 20 cm for AxiChrom 1000 column is possible at lower levels of compression and reduced flow performance. For more information, contact your GE Healthcare representative.

If selected in the UNICORN wizard, Intelligent Packing will automatically run a packed bed evaluation test after the packing. For large-scale columns, automated methods for priming and unpacking can also be created with the Intelligent Packing wizard.

Note: When unpacking AxiChrom 300 to 1600 columns, use the same Intelligent Unpacking method that is used for the corresponding Capto resins (i.e., Capto S, Capto Q, Capto DEAE, Capto adhere, or Capto MMC).

Table 2. Packing variables for packing Capto ImpRes resins in AxiChrom columns

Packing variable	AxiChrom 50 to 200		AxiChrom 300 to 1600
	Capto SP ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes	Capto Q ImpRes	Capto ImpRes resins
Packing solution	10 mM NaCl	0.4 M NaCl	Water
Packing speed/velocity	30 cm/h	30 cm/h	60 cm/h
Packing factor	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	1.13 to 1.15/1.16 to 1.18*
Flow conditioning	230 cm/h for 30 min	230 cm/h for 30 min	Optional for AxiChrom 300: 230 cm/h at 20 cm bed height, linearly decreasing to 150 cm/h at 40 cm bed height for 30 min

* It is important to stay within the *PF* interval of 1.13 to 1.15 when packing AxiChrom 300 to 1000 columns, and within 1.16 to 1.18 for AxiChrom 1200 to 1600 columns. Note this range is smaller than the range for verified packing methods of other resins in AxiChrom process columns. Lower *PF* might result in instable bed with differences in bed efficiency and selectivity over time, especially for lower beds. Higher *PF* will result in high packing forces, especially for higher beds.

Table 3. Packing variables for packing Capto ImpRes resins in BPG columns

Packing variable	BPG 100		BPG 300
	Capto SP ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes	Capto Q ImpRes	Capto ImpRes resins
Packing solution	10 mM NaCl	0.4 M NaCl	Water
Packing speed/velocity	60 cm/h	60 cm/h	60 cm/h
Packing factor	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	Bed heights < 30 cm: 1.20 Bed heights ≥ 30 cm: 1.16	Bed heights ≤ 20 cm: 1.13 Bed heights > 30 cm: 1.10 Bed heights 20–30 cm, decreasing linearly from 1.13 to 1.10
Flow conditioning	3 bar for 5 CV	3 bar for 5 CV	3 bar for 5 CV

Packing Capto ImpRes resins in BPG columns

Capto ImpRes resins can be packed in BPG 100 to 300 columns. BPG 450 is, however, not recommended for Capto ImpRes resins because of its low pressure limits. The bed height interval recommended for BPG 100 to 300 columns is 10 to 40 cm.

In BPG columns with small diameters (BPG 100), the increased influence of the wall support means that salt solution is required for packing. Capto SP ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes resins are therefore packed with 10 mM NaCl and Capto Q ImpRes resin with 0.4 M NaCl in BPG 100. In BPG 300, all Capto ImpRes resins are packed with water.

Chromatography resin preparation

Equilibration of the resin to the packing solution can be performed by using the BPG column as a “filter”.

Pour the resin into the column (for calculation of amount see “Slurry preparation”), mount the adapter, tighten the adapter O-ring, move the adapter down and compress the bed slightly, connect the pump, and wash the resin with at least three column volumes (CV) of the packing solution. Unpack and resuspend the slurry and pack according to the method below.

Note: Equilibration is critical for Capto SP ImpRes and Capto MMC ImpRes in BPG 300, as the delivery solution contains salt, and the recommended packing solution is water. Measure the conductivity of the flow through solution during equilibration and continue to equilibrate until the conductivity is zero.

Column and system preparation

A detailed description of column preparation is available in the BPG column operating instruction 29193223. The packing pump should be as pulsation-free as possible. Screw and rotary lobe pumps are the most suitable for this task but multi-headed diaphragm pumps are also satisfactory.

Caution: Ensure that the column has no visible scratches in the glass tube and that the adapter moves smoothly in both upward and downward directions before packing. In addition, there should be no difficulty in tightening the adapter O-ring to the column inner wall.

1. Place a new 10 µm net on both the adapter and the bottom end pieces.
2. Level the column with the aid of a spirit level.
3. A pressure relief valve should be used for safety reasons, especially against pressure spikes. Position this valve on the pump outlet and add a pressure gauge to the adapter.
4. Mount one 4-port, 2-way valve on the bottom inlet and one on top of the pressure gauge, 10 mm i.d. for BPG 300 and 6 mm i.d. for BPG 100.

Packing

1. Set the pressure alarm or pressure relief valve according to the pressure rating of the column. Purge the system and tubing of air.
2. Purge the end piece net of trapped air by draining some packing solution through the column outlet. Leave about 2 cm of solution in the column and close the bottom valve. If air is still trapped under the end piece net, add more packing solution and connect tubing to the suction side of a pump. Start the pump and place the tubing on the bottom net and extract any remaining air.
3. Add the slurry to the column and, if needed, additional packing solution to about 40 cm. Mix the resin and the packing solution to a homogeneous slurry.

Note: The available height to allow the adapter to be inserted into a 50 cm column tube (for filling slurry) is only 40 cm. Use a longer column tube when packing beds higher than 20 cm: 75 cm and 95 cm tubes are available.

4. Rinse the wall from particles and let the resin settle until there is about 1 cm clear liquid on top of the slurry. This reduces the risk of particles sticking between the O-ring and the column wall, which can cause column leakage.
5. Insert the top adapter and secure it to the column top flange. Lower the adapter to the surface of the slurry and allow some clear liquid to pass the O-ring. Tighten the adapter O-ring.
6. Make sure that the column top valve is open. Slowly move the adapter down until no air bubbles can be seen leaving the top valve.
7. Start the pump and adjust the settling velocity to 60 cm/h (4.7 L/h for BPG 100 and 42.4 L/h for BPG 300). Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste.
8. Run the settling flow until the bed is completely consolidated. Note the consolidated bed height and calculate the packed bed height. For BPG 100, $PF = 1.20$ in 10 mM or 0.4 M NaCl for bed heights up to 30 cm; for higher beds $PF = 1.16$. For BPG 300, $PF = 1.13$ in water for bed heights up to 20 cm, decreasing linearly to 1.10 for bed heights of 30 cm and higher. The packed bed height is the ratio between the consolidated bed height and the PF . Use a marker pen to indicate on the column the packed bed height.
9. Stop the flow and close the bottom valve. Loosen the O-ring and lower the adapter down to 1 cm above the settled bed and seal the adapter O-ring. Shift the top valve to waste and use the adapter to mechanically compress the bed to the mark on the column (Step 8). Excessive packing solution is removed through the adapter tube.

Note: Compressing resins in BPG columns, especially the larger BPG 300 column, is physically demanding. Do not use extension rods on the adapter height adjuster to compress the resin.

To increase the performance and stability of the bed, flow condition the column downwards with 5 CV of water or packing solution at 3 bar.

10. Connect the pump to the top of the column. Purge the system and tubing by running the mobile phase to waste by bypassing the column inlet with the 4-port valve. Start at a low flow velocity.
11. Shift the top valve to direct the flow into the column and immediately open the bottom mobile phase to waste or connect it to the buffer tank for recirculation.
12. Increase the flow until a pressure of 3 bar is reached, or to the maximum flow/pressure if 3 bar cannot be reached. Run the column at this pressure for 5 CV.
13. Slowly decrease the flow for 2 min to avoid disturbance of the conditioned bed.
14. Test the packing at the optimal test velocity (30 cm/h).

Packing Capto ImpRes resins in Chromaflow columns

The largest recommended Chromaflow column for Capto ImpRes resins is Chromaflow 600, with a recommended bed height interval of 20 to 40 cm when using the packing method described below. As the pump is stopped 5 mm from the adapter, as seen in the packing method, lower beds will not be compressed sufficiently. Moreover, the extreme velocities needed to efficiently pack a shorter bed cannot easily be achieved using standard equipment.

To pack Capto ImpRes resins in Chromaflow 600, the slurry is introduced from the top nozzle by the use of Chromaflow Packing Station Pack 100.

Note: Operational flow velocity is reduced in Chromaflow columns compared with AxiChrom and BPG columns, from at least 220 cm/h to at least 150 cm/h at 20 cm bed height.

Note: The flow capacity of Chromaflow Packing Station Pack 100 is required for packing Capto ImpRes resins in Chromaflow 600 for operational flow velocities of at least 150 cm/h.

Chromatography resin preparation

The recommendation is to use 10 mM NaCl as packing solution for Chromaflow columns and that this packing solution is used throughout the whole procedure.

Note: Water can potentially be used as packing solution but compression and flow velocities have not been verified by GE Healthcare.

To avoid introducing air into the column when packing, additional slurry is required for the extra volumes in the tank and tubing. Add the slurry to the slurry tank and stir the resin. Dilute the suspension to a slurry concentration of about 50%.

Column and system preparation

For more detailed description about the column and packing station preparation, see Chromaflow Columns Operating instruction 28962232 and Chromaflow Packing stations Operating instruction 29046228. In this application note, standard Chromaflow equipment is used for the connections on the column and the packing station.

Note: It is important that the supply air flow rate follows the specification of Chromaflow Packing Station Pack 100 (1000 L/min) and that the supply air pressure into the packing station is 6 to 7 bar.

1. Set up the column according to the Chromaflow Columns Operating instructions 28962232.
2. A pressure relief valve should be used for safety reasons. Position this on the slurry inlet top (SIT). Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount a 3-port, 2-way valve on top of the pressure gauge and the mobile phase bottom (MPB). The top valve should lead in two directions: one side into the system and one to the waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5" to 2" tubing leads to the waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to eliminate air entering through the MPB.
3. Connect appropriate tubing (1" or 1.5" i.d.) and tanks to the column and packing station. If a flow meter is used, place it between the SIT and the packing station.
4. Level the adapter to the desired bed height. Remember to loosen the nuts on the adapter rods to allow the adapter to be raised or lowered. Flush the adapter rods with 20% ethanol as lubrication.
5. Prime the column, packing station, and tubing with water according to the Chromaflow Operating instruction.

Packing

Note: Packing Chromaflow columns is a rapid procedure compared with other packing procedures and it is therefore important to thoroughly read the packing instructions and go through the packing steps in advance of the packing.

1. Set both nozzles in run position to prime the tubing with slurry. Lead the slurry outlet top (SOT) tubing back to the slurry tank and secure it. Stir the slurry to keep it homogeneous, select slurry and SIT on the packing station, open the slurry tank and start the packing pump.
2. The initial flow velocity should be at least 1700 cm/h, corresponding to an air pressure on the packing station of at least 3.5 bar for packed bed heights of 20 and 30 cm.
3. When the tubing is primed and the flow velocity set, set the SIT/slurry inlet bottom (SIB) to the position between SIT and SIB. This blocks the flow during Step 4 while maintaining the correct flow rate for the next step.
4. Move the top nozzle down into the packing position.
5. Two operators should simultaneously open the bottom mobile phase valve to waste and turn the SIT/SIB valve to SIT on the packing station. The column starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.

Note: Column pressure must not exceed the operating pressure limit of the column (i.e., 3 bar). If this pressure is reached, gently decrease the packing flow so that the pressure remains just below 3 bar. Typically, the final pressure in the column is 2 to 3 bar depending on the viscosity of the packing solution, column diameter, bed height, and so forth.

6. Stop the pump when the building bed is 5 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in 3. Once flow is stopped, the bed will expand to meet the adaptor.

Note: If a nontransparent column tube is used, stop the packing flow when the calculated volume of slurry has been introduced into the column. Check the volume in the slurry tank or use a volume totalizer.

7. Immediately move the top nozzle back to the run position.
8. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar.
9. Use packing solution to rinse residual resin from the tubing and the top nozzle. Pump the packing solution through the top nozzle back into the slurry tank.
10. Close the slurry tank and empty the tubing between the tank and packing station.
11. Pump liquid upwards through the column until the air is expelled.

Figure 2 shows the increase in pressure and decrease in flow velocity when packing a 20 cm bed height in Chromaflow 600.

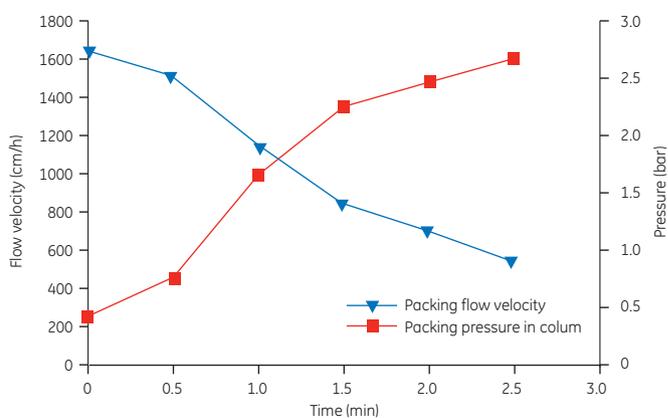


Fig 2. Column pressure and flow velocity during packing of a 20 cm bed height in Chromaflow 600 by the use of Chromaflow Packing Station Pack 100.

Testing the performance of the packed column

Process-scale packed columns must perform with a high degree of efficiency over many processing cycles (i.e., display very high stability). The efficiency of a packed column can be expressed in terms of height equivalent to a theoretical plate (HETP) and asymmetry factor (A_s). This test should be repeated regularly to monitor the state of the packed bed throughout the working life of the column. If the test results are to be comparable over time, conditions such as, flow velocity (cm/h), liquid pathway, sample composition, and elution buffer should be kept constant. The requirements for the test have to be set in accordance with the test conditions and the goal of the purification. This is further described in application note 28937207.

To compare the performance of columns packed with chromatography resins of different particle diameters, the reduced plate height ($h = \text{HETP}/\text{average bead diameter } [dp]$) is typically used. As a guideline, a value of $h < 3$ is very good at optimal test conditions.

Test conditions used in this study

In this study, optimal test conditions have been chosen. This also includes the use of recommended tubing for each column setup.

Sample:	2% v/v acetone
	0.8 M NaCl for AxiChrom 50, 100, and 200 columns
Sample volume:	1% of the column volume (V_c)
Test velocity:	30 cm/h
Eluent:	Water
	0.4 M NaCl for AxiChrom 50, 100, and 200 columns

Examples of results

The columns packed with the methods outlined above were tested for plate number, asymmetry, pressure-flow velocity, and stability.

AxiChrom columns

Examples of efficiency and stability results for Capto ImpRes resins packed in AxiChrom 50, 100, and 200 columns can be seen in Table 4. Results for Capto ImpRes in AxiChrom 300 and 300 to 1600 columns are shown in Table 5.

Capto SP ImpRes resin showed good and similar (in some cases identical) results from the different column sizes of AxiChrom 50, 300, and 600 (Tables 4 and 5) and for different bed heights in AxiChrom 300 and 600 columns (Table 5). These results are also similar to the results for Capto Q ImpRes, Capto adhere ImpRes, and Capto MMC ImpRes. Thus, the method developed, gave similar results for all four resins independent of column size and bed height.

The stability test showed that the packed beds are stable when running in water at the velocities given in Tables 4 and 5, and also that the packed beds stabilize with time as seen in the continued running of stability tests.

Pressure-flow curves provide a simple, effective illustration of column performance in terms of the maximum operating velocity at which the purification process can be run. They also show the magnitude of the backpressure in the system at a certain flow velocity. All AxiChrom columns can be utilized at the maximum flow velocity of Capto ImpRes (Fig 3 and 4).

Table 4. Column efficiency data for AxiChrom 50, 100, and 200 (pilot-scale) columns packed with Capto ImpRes resins

Resin	Column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h) [†]	Change after stability test (%) [†]	
							h	A_s
Capto SP ImpRes	AxiChrom 50	20	15 800	1.4 to 1.5	0.9 to 1.0	300	-4; -2 [‡]	14; 2 [‡]
Capto SP ImpRes	AxiChrom 100	10	16 500	1.4	1.1 to 1.2	500	2	3
Capto Q ImpRes	AxiChrom 50	40	15 400	1.5	1.0	150	0	4
Capto Q ImpRes	AxiChrom 200	30	13 900	1.6 to 1.7	1.2 to 1.4	200	3	-9
Capto MMC ImpRes	AxiChrom 50	40	14 000	1.6 to 1.8	0.8 to 1.0	150	3; -1	16; 4
Capto MMC ImpRes	AxiChrom 100	10	15 700	1.4 to 1.5	1.1 to 1.2	500	-1	5
Capto adhere ImpRes	AxiChrom 50	40	13 700	1.6 to 1.8	1.0 to 1.1	150	1	9

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at least three packings, except for Capto Q ImpRes in AxiChrom 200 where only two packings were performed.

[†] Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

[‡] Data from two consecutive stability tests.

Table 5. Column efficiency data for AxiChrom 300, 600, 1000, and 1600 (process-scale) columns packed with Capto ImpRes resins

Resin	Column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h) [†]	Change after stability test (%) [†]	
							h	A_s
Capto SP ImpRes	AxiChrom 300	20	16 300	1.5 to 1.6	1.1 to 1.3	300	-7	7
Capto SP ImpRes	AxiChrom 300	40	14 000	1.5 to 1.9	1.0 to 1.2	150	-14; 4 [‡]	13; 3 [‡]
Capto SP ImpRes	AxiChrom 600	10	15 700	1.4 to 1.6	1.2 to 1.4	500	-8	1
Capto SP ImpRes	AxiChrom 600	30	15 900	1.4 to 1.5	1.0 to 1.3	170	-10	5
Capto SP ImpRes	AxiChrom 1000	20	15 800	1.6 to 1.6	1.2 to 1.3	240	6	4
Capto SP ImpRes	AxiChrom 1600	15	13 500	1.6 to 2.2	1.0 to 1.3	310	-8	6
Capto Q ImpRes	AxiChrom 300	20	15 100	1.6 to 1.7	1.0 to 1.2	300	-13; 2 [‡]	17; 0 [‡]
Capto adhere ImpRes	AxiChrom 300	20	14 200	1.6 to 1.8	1.2 to 1.4	300	-5	2

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at least three packings.

[†] Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

[‡] Data from two consecutive stability tests.

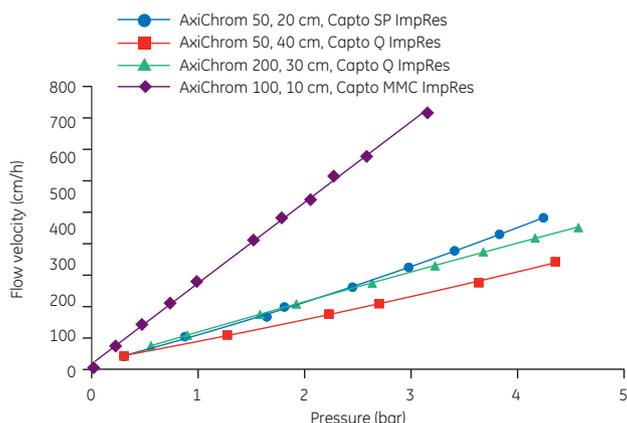


Fig 3. Pressure-flow curves for AxiChrom pilot-scale columns packed to different bed heights with Capto ImpRes resins. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded. Different chromatography resin batches were used to cover a larger operational space.

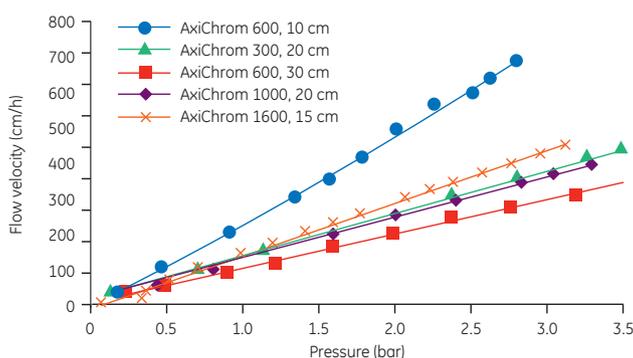


Fig 4. Pressure-flow curves for AxiChrom process-scale columns packed to different bed heights with Capto SP ImpRes resin. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded. Different chromatography resin batches were used to cover a larger operational space.

BPG columns

Examples of efficiency and stability results for Capto ImpRes packed in BPG 100 and BPG 300 columns are shown in Table 6. The results were very similar both for different column sizes and for bed heights. In general, the packing method works well for these resins in BPG columns of different sizes and at different bed heights. The stability test showed that the packed bed was stable at the flow velocities given in the table.

The pressure-flow curves for BPG 100 and 300 columns, packed with Capto SP ImpRes resin to a bed height of 20 cm, are shown in Figure 5. For both column sizes, these curves show linear behavior. The pressure-flow profile for BPG 300 column shows that the resin can be run at 220 cm/h with less than 3 bar backpressure from the bed.

Chromaflow columns

Examples of efficiency results for Capto SP ImpRes packed in Chromaflow 600 column are shown in Table 7. Both 20 and 30 cm bed heights give good results.

The pressure-flow curve for Capto SP ImpRes in Chromaflow 600 column is shown in Figure 6. As the optimal CF is difficult to achieve in standard pack-in-place columns, the maximum flow velocity that can be run through the packed bed is limited. The highest operating velocity recommended for this type of column is 150 cm/h at a 20 cm bed height. Note that bed efficiency and bed stability are good, provided that the 150 cm/h guideline is met.

Table 6. Column efficiency data for BPG 100 and 300 columns packed with Capto ImpRes resins

Resin	BPG column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto SP ImpRes	100	20	14 800	1.5 to 1.6	1.0 to 1.1	300	4	1
Capto SP ImpRes	300	20	14 900	1.4 to 1.7	1.2	300	3	4
Capto SP ImpRes	300	30	13 900	1.5 to 2.0	1.2 to 1.3	190	7	7
Capto Q ImpRes	300	20	15 700	1.5 to 1.7	1.1 to 1.2	300	-2	-1

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at least three packings, except for Capto SP ImpRes resin in BPG 100 column where only two packings were performed.

† Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

Table 7. Column efficiency data for Chromaflow 600 column packed with Capto SP ImpRes resin

Resin	Chromaflow column	Bed height (cm)	Average plates/m*	Reduced plate height (h) range*	Asymmetry factor (A_s) range*	Flow velocity for stability test (cm/h)†	Change after stability test (%)†	
							h	A_s
Capto SP ImpRes	600	20	10 900	1.9 to 2.4	1.1 to 1.3	150	16	13
Capto SP ImpRes	600	30	11 400	1.9 to 2.3	1.2 to 1.5	100	10	2

* Test performed at optimal test conditions. Average and ranges of upflow and downflow tests for at three packings.

† Stability tests were run once for each bed height/resin/column combination in water for 16 h at given velocity.

Pressure-flow curves for AxiChrom (Fig 4) and Chromaflow columns (Fig 6) showed higher backpressure in AxiChrom columns than in the Chromaflow 600 column at the same bed height and flow velocity. This results from the higher compression when packing AxiChrom columns compared with when packing Chromaflow columns. This higher compression is needed to utilize the full flow velocity of Capto ImpRes resins. Still, the backpressure over AxiChrom columns at maximum flow velocity is far from the maximum operating range of the columns (4 bar).

Efficiency tests at different flow velocities

Efficiency tests were run at different velocities. Figure 7 shows that the curves follow the van Deemter theory. The A_s is stable at the different flow velocities. The reduced plate height increases with the flow velocity and the optimal result is achieved at 20 to 40 cm/h. When running at higher flow velocities, the A_s and reduced plate height continue to behave linearly. This indicates that the efficiency test can be run at any flow velocity, but the expectation of the result has to be changed and compared to the optimal result.

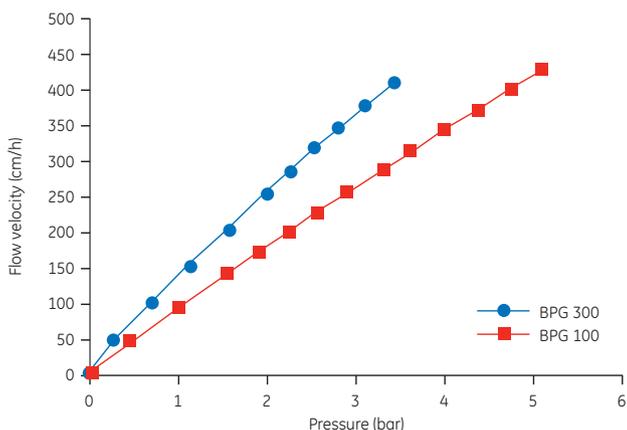


Fig 5. Pressure-flow curves for BPG columns packed to 20 cm bed height with Capto SP ImpRes resin. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded.

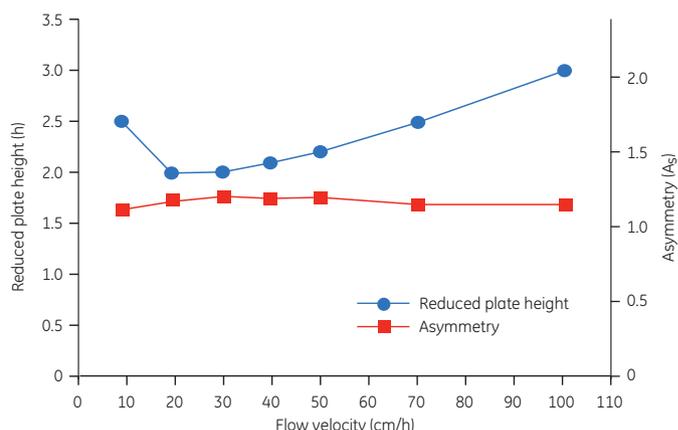


Fig 7. Reduced plate height and A_s values at different flow velocities run on a 20 cm bed of Capto SP ImpRes in a Chromaflow 600 column. This is an example but similar behavior can be expected for packed beds in other columns such as AxiChrom and BPG.

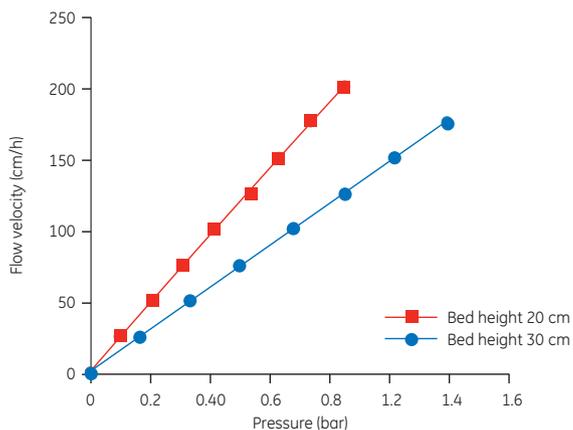


Fig 6. Pressure-flow curves Chromaflow 600 column packed to different bed heights with Capto SP ImpRes resin. Measurements were made with water at 20°C. The contribution of hardware pressure is excluded.

Conclusions

This application note describes packing of Capto SP ImpRes, Capto Q ImpRes, Capto MMC ImpRes, and Capto adhere ImpRes resins in AxiChrom columns using the custom mode feature of Intelligent Packing, as well as packing of Capto ImpRes resins in BPG and Chromaflow columns.

Capto ImpRes resins can generally be packed in AxiChrom, BPG, and Chromaflow columns to bed heights between 10 and 40 cm (see specific column type for details). The flexibility of column diameters and bed heights enables full utilization of the flow capacity of the Capto ImpRes resins, allowing for processes with increased bed heights if floor space is limited or at lower bed heights and larger diameters to decrease process time. Each packing method described is related to a specific packing solution. Deviation from use of the packing solutions described can have significant impact on the *PF* and subsequently on the packing result.

To utilize the full flow potential of the Capto ImpRes resins, AxiChrom columns are recommended.

Ordering information

Product	Quantity	Product code
Capto SP ImpRes	25 mL	17546810
	100 mL	17546802
	1 L	17546803
	5 L	17546804
	10 L	17546805
Capto Q ImpRes	25 mL	17547010
	100 mL	17547002
	1 L	17547003
	5 L	17547004
	10 L	17547005
Capto MMC ImpRes	25 mL	17371601
	100 mL	17371602
	1 L	17371603
	5 L	17371604
	10 L	17371605
Capto adhere ImpRes	25 mL	17371501
	100 mL	17371502
	1 L	17371503
	5 L	17371504
	10 L	17371505
Media Wand 50	1	28922764
Media Handling unit	1	28922769
Slurry Concentration Kit	1	29096100

For more information about AxiChrom, BPG, and Chromaflow columns as well as Chromaflow Packing Stations, visit gelifesciences.com

Related literature

Products	Product code
Data files	
Capto SP ImpRes and Capto Q ImpRes	28983763
Capto MMC ImpRes	29035674
Capto adhere Impres	29034497
AxiChrom columns	28929041
BPG columns	18111523
Chromaflow columns	18113892
Media Wand	28923101
Application notes	
Column efficiency testing	28937207
Instructions	
Capto SP ImpRes and Capto Q ImpRes	28977655
Capto MMC ImpRes	29027184
Capto adhere ImpRes	29027182
AxiChrom 50, 70, and 100 columns	28933108
AxiChrom 140 and 200 columns	28943123
AxiChrom 300–1600 columns	29065430
BPG Columns	29193223
Chromaflow columns	28962232
Chromaflow Packing Station 50, 100, 200, and 400	29046228

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