HiScale™ columns

HiScale is a family of pressure-stable, empty columns based on the well-established XK column line and designed for process development and preparative chromatography. The user-friendly design allows for an optimized process, through simplified and reproducible operation. The range of functional advantages makes HiScale columns well-suited for work that requires ease-of-use, robustness, and process control.

Features and benefits of HiScale columns include:

- Pressure stability up to 20 bar, providing compatibility with modern BioProcess™ media
- Axial compression of the gel bed and a plunger mechanism enabling a wide range of packing protocols
- Column measurement scale and ergonomical design of the end cap, enabling precise packing
- Adapter QuickLock mechanism, facilitating column handling and cleaning
- Dual adapters, offering high flexibility and dynamic protocols
- Compatibility with all methods currently used with GE Healthcare's XK columns

HiScale columns were developed to provide a range of functional improvements with the goal of simplifying and accelerating process development work. Improvements include features that enable precise column packing, easy handling, and increased method reproducibility. The column tubing material is PEEK, which allows for the increased pressure specifications. The QuickLock mechanism of the adapter shaft facilitates rapid and easy movement of the adapter, simplifying adjustments as well as disassembly and cleaning. The ability to rinse the adapter shaft after packing promotes excellent column hygiene even after prolonged use.

Fig 1. HiScale 16/40 column for preparative chromatography, scale-up, and process development.

Column characteristics

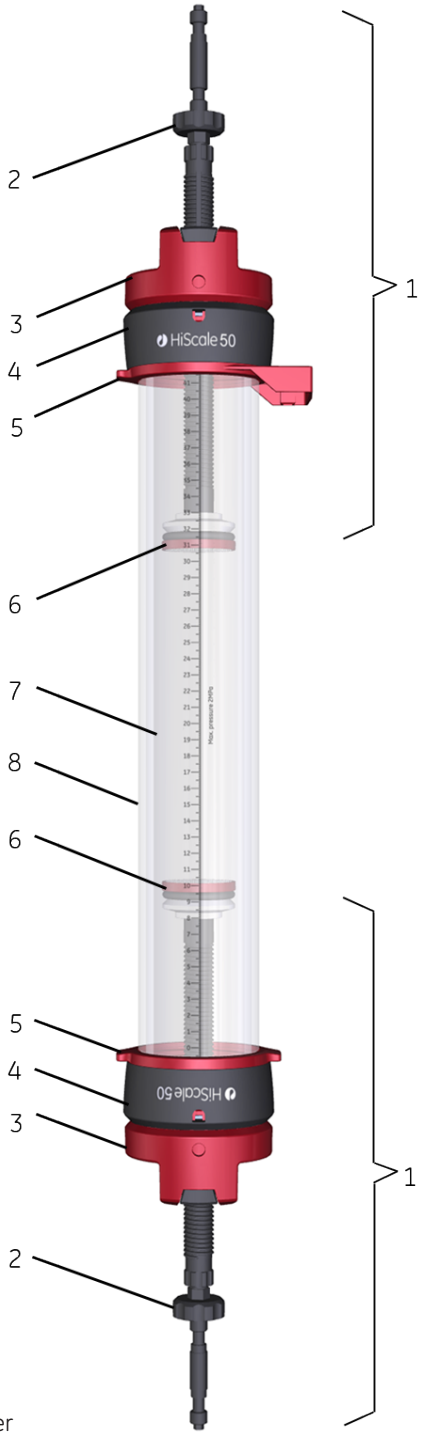
HiScale columns are available in a number of sizes (Table 1). Columns are comprised of an inner glass tube and an outer protective polycarbonate tube. The default filter size is 20 µm, but other sizes are available (for details see the accessories).

HiScale columns are equipped with a measurement scale, making it easer to predict and reach a predefined packing height, thereby increasing process control as well as reproducibility. The end cap as well as the end knob have been ergonomically designed to facilitate adapter adjustment and movement during axial compression of the bed.

Table 1. Maximum bed heights and volumes for HiScale columns

Column	Max bed height (cm)	Max volume (ml)
HiScale 16/20	20	40
HiScale 16/40	40	80
HiScale 26/20	20	106
HiScale 26/40	40	212
HiScale 50/20	20	393
HiScale 50/40	40	785

HiScale columns are equipped with two adapters, allowing for a large range of bed heights (Fig 2, Table 1). The columns can be operated at temperatures from 4°C to 40°C and at pressures up to 20 bar (2.0 MPa).



1. Adapter
2. End knob
3. End cap
4. End housing
5. Tube holder
6. Plunger
7. Glass tube (borosilicate)
8. Protective tube (polycarbonate)

Fig 2. Main components of HiScale columns.

Column functionality

HiScale columns have a range of mechanical features designed to simplify and improve column functionality. When packing high beds, a packing tube can easily be connected. HiScale columns are supplied with 1/16" (1 mm i.d.; HiScale 16 and 26) or 1/8" (2 mm i.d.; HiScale 50) PEEK tubing and Valco fittings (Fig 3) that are compatible with ÄKTA™ and other standard chromatographic equipment and allow safe and easy exchange of parts.

The end caps of HiScale columns are ergonomically shaped and easily adjusted, providing a simple means of achieving axial compression of the gel bed (Fig 4), a feature that is particularly suitable during packing of rigid high flow media.

Fig 3. Valco fittings allow for easy exchange of tubing.

Fig 4. Adustment of the end cap on HiScale columns enables controlled axial compression of the gel bed.

When packing rigid gels, a spanner (wrench) can be used to assist bed compression (Fig 5).

The QuickLock mechanism of the end cap allows rapid and accurate movement of the adapter, facilitating column handling and cleaning (Fig 6).

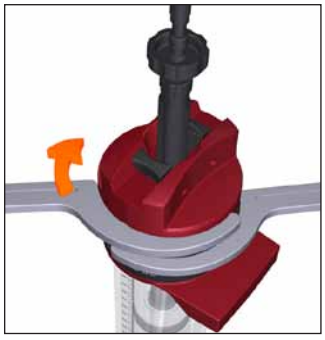


Fig 5. Turning the end cap with spanners.

Fig 6. QuickLock mechanism on the column end caps enables simplified adapter movement.

Wetted materials

Under normal operating conditions, the only materials in contact with the liquid phase are: Polypropylene, borosilicate glass, polyamide, fluoro-rubber, PEEK capillary tubing, and Tefzel™ ferrules.

Chemical resistance

The columns can be used with nearly all organic solvents commonly used in liquid chromatography of macromolecules, with the following few exceptions: Chlorinated hydrocarbons, acetone and other ketones, aliphatic esters, and phenol. Solutions containing more than 2 M NaOH, 1 M HCl, other strong mineral acids, or 75% acetic acid should not be used.

Applications

Scale-up experiments

One of the primary aims of process development is to obtain a robust, scalable process with the highest possible throughput at the lowest possible cost. As a result, method development of a purification is normally initiated at small scale for convenience and to save time. HiScale columns were developed to provide a column for scale-up, enabling packing of high-flow agarose media, which require higher pressure during packing.

Two typical scale-up applications were performed: The first experiment was a capture purification of IgG performed on MabSelect SuRe™ media, where the process was scaled up from HiScreen to HiScale 16/40 columns. The second application was an ion exchange chromatography (IEX) purification of green fluorescent protein (GFP) on Capto™ Q media using the same column formats.

The results were compared between HiScreen and HiScale columns (Figs 7 and 8). In both experiments, the curve overlays of the relative retention volumes demonstrate the excellent reproducibility of HiScale columns (as well as the utilized BioProcess media and ÄKTA systems). HiScale columns provide nearly identical levels (> 96%) of high yield relative to HiScreen columns (Table 2), demonstrating that HiScale columns are an excellent choice for scale up work.

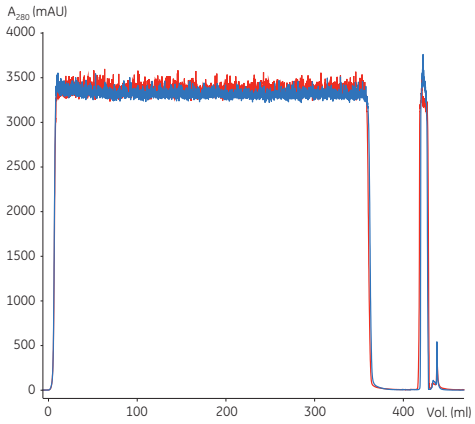


Fig 7. Purification and scale-up of IgG on MabSelect SuRe chromatography medium. The figure is an overlay of UV absorbance (280 nm) for HiScreen MabSelect SuRe (blue) and HiScale 16/40 packed with MabSelect SuRe (red). The curves are normalized against the volume for HiScreen. Running conditions are described in Table 2.

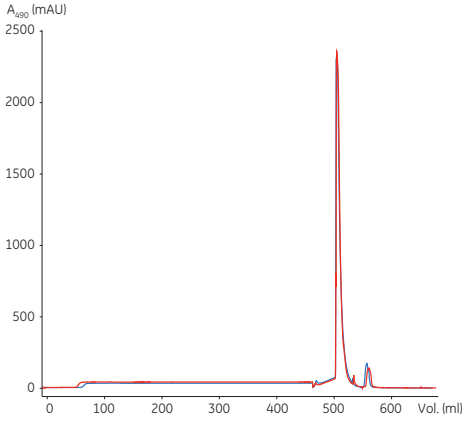


Fig 8. Purification and scale-up of GFP on Capto Q chromatography medium. The Figure is an overlay of UV absorbance (490 nm) for HiScreen Capto Q (blue) and HiScale 16/40 packed with Capto Q (red). The curves are normalized against the volume for HiScreen. Running conditions are described in Table 2.

Table 2. Summary of running conditions and results from scale-up experiments using HiScreen and HiScale columns

Medium	Column	CV (ml)	Sample (sample conc.)	Sample load (mg)	Residence time (min)	Flow rate (ml/min)	System	Yield (%)
MabSelect SuRe	HiScreen	9.3	CHO supernatant (0.8 mg/ml IgG)	291	4	2.4	ÄKTAexplorer™ 100	≥ 96
	HiScale	42.6	CHO supernatant (0.8 mg/ml IgG)	1318	4	10.7	ÄKTAexplorer 100	≥ 96
Capto Q	HiScreen	9.3	*E. coli* supernatant (4 mg/ml GFP)	150	4	2.4	ÄKTA avant 25	≥ 97
	HiScale	40.8	*E. coli* supernatant (4 mg/ml GFP)	753	4	10.7	ÄKTA avant 25	≥ 97

Ordering information

Column*	Quantity	Code no.
HiScale 16/20	1	28-9644-41
HiScale 16/40	1	28-9644-24
HiScale 26/20	1	28-9645-14
HiScale 26/40	1	28-9645-13
HiScale 50/20	1	28-9644-45
HiScale 50/40	1	28-9644-44

* Each HiScale column is delivered with two adapters

Accessories

	HiScale 16	HiScale 26	HiScale 50	No. per pack
Spanner wrench	28-9647-76	28-9647-77	28-9647-78	2
Long column holder	18-1126-32	18-1126-32		1
Short column holder	18-1113-17	18-1113-17		1
Column holder ÄKTA avant	28-9562-82	28-9562-82		1
Column clamp ÄKTA avant	28-9563-19	28-9563-19		1
Column holder, steel			28-9644-99	1
Superloop, 1/16" fittings 10 ml	18-1113-81	18-1113-81	18-1113-81	1
Superloop, 1/16" fittings 50 ml	18-1113-82	18-1113-82	18-1113-82	1
Superloop, M6 fittings 150 ml	18-1023-85	18-1023-85	18-1023-85	1
Union 5/16" female - 1/16" male			18-1142-08	8
Fingertight union 1/16" male/M6 female	18-1112-58	18-1112-58	18-1112-58	8
Connector 1/16" male/Luer female	18-1112-51	18-1112-51		2
Net ring 10 µm	18-8761-01	18-8760-01	18-8759-01	5
Net ring 80 µm			18-1000-69	5
Tubing 1 mm i.d.	18-1115-83	18-1115-83		2 m
Tubing 2 mm i.d.			28-9663-76	1 m
Tubing cutter	18-1112-46	18-1112-46		1
Packing connector	28-9645-03			1
O-ring, packing connector	28-9666-53			2
Column tube 20	28-9666-46	28-9666-48	28-9666-49	1
Column tube 40	28-9666-52	28-9666-51	28-9666-50	1
Packing tube 20		28-9803-83	28-9802-51	1
Packing tube 40		28-9645-05	28-9645-06	1
O-ring, packing tube		28-9666-54	28-9666-55	2
Accessory kit*	28-9663-67	28-9663-74	28-9663-75	1

* The accessory kit includes 1 net ring, 1 support screen, 1 O-ring, 2 ferrules and 2 fingertight stop plugs

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