

HiScale columns

EMPTY CHROMATOGRAPHY COLUMNS

HiScale™ is a family of pressure-stable empty columns designed for preparative chromatography, process development, and process validation such as scale-down studies. 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:

- Easy to pack – with axial compression capabilities, a nonrotating plunger mechanism, and column measurement scale
- Compatible with modern BioProcess™ resins allowing operation up to 20 bar (2 MPa, 290 psi) pressure
- Adapter QuickLock mechanism for easy column handling and cleaning
- Equipped with two adapters allowing for a large range of bed heights
- The narrow diameter and flexible bed height of HiScale 10/40 makes the column an excellent choice for scale-down studies

Column characteristics

HiScale columns offer versatile features to simplify and accelerate process development work. HiScale columns allow robust column packing, easy handling, and increased method reproducibility. The column tubing material is PEEK, which allows for the high pressure specifications.

Precise and robust packing of the column

HiScale columns are equipped with a measurement scale, making it easier to predict and reach a predefined bed 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.

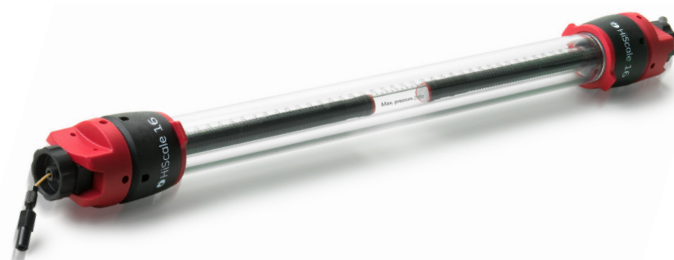


Fig 1. HiScale 16/40 column for preparative chromatography, process validation such as scale-up and scale-down studies, and process development.

QuickLock mechanism for easy column handling

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.

Range of column sizes for greater flexibility

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 *Accessories*). HiScale columns are equipped with two adapters, allowing for a large range of bed heights (Table 1 and Fig 2).

Table 1. Maximum bed heights and volumes for HiScale columns

Column	Max. bed height (cm)	Max. volume (mL)
HiScale 10/40	40	31
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

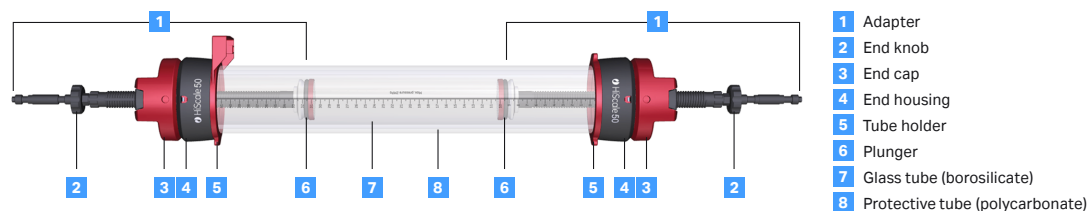


Fig 2. Main components of HiScale columns.

Column operation

HiScale columns have a range of mechanical features designed to simplify and improve column operation. When packing high beds, a packing tube can easily be connected. HiScale columns are supplied with PEEK tubing and connectors (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 resins.

When packing rigid resins, 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).

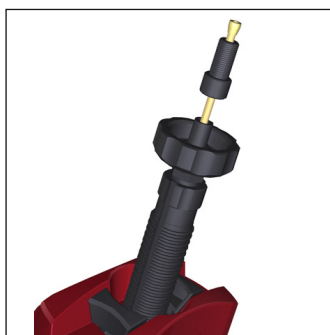


Fig 3. Connectors allow for easy exchange of tubing.



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

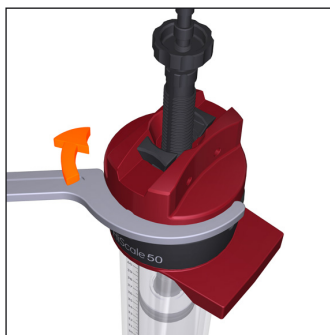


Fig 5. Turning the end cap with spanners.

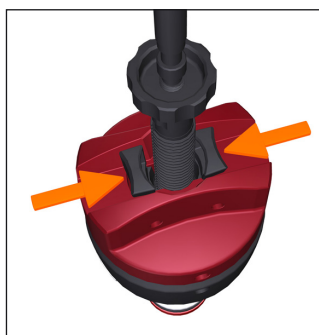


Fig 6. QuickLock mechanism on the column end caps enables simplified adapter movement.

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 a wide range of 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 resins, which require high pressure during packing.

Two typical scale-up applications were performed: the first experiment was a capture purification of IgG performed on MabSelect SuRe™ resin, 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 chromatography resin using the same column formats.

The results were compared between HiScreen and HiScale columns (Fig 7 and Fig 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 resins 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.

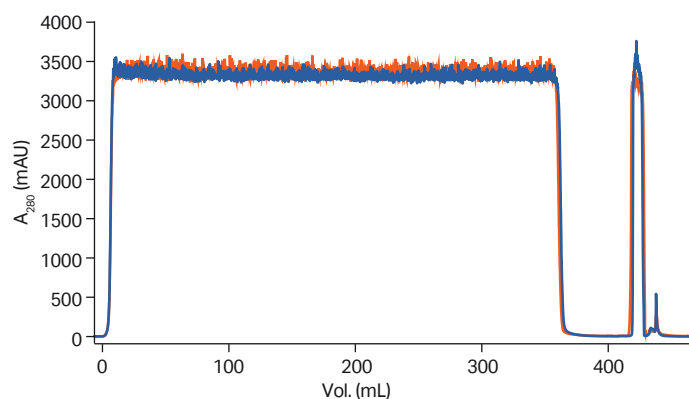


Fig 7. Purification and scale-up of IgG on MabSelect SuRe chromatography resin. The figure is an overlay of UV absorbance (280 nm) for HiScreen MabSelect SuRe (blue) and HiScale 16/40 packed with MabSelect SuRe (orange). The curves are normalized against the volume for HiScreen. Running conditions are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

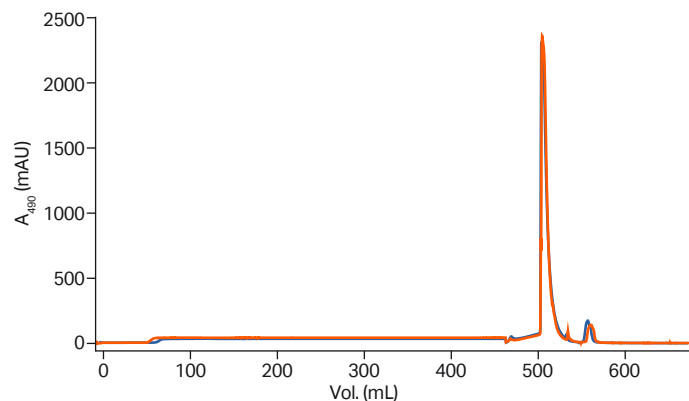


Fig 8. Purification and scale-up of GFP on Capto Q chromatography resin. The Figure is an overlay of UV absorbance (490 nm) for HiScreen Capto Q (blue) and HiScale 16/40 packed with Capto Q (orange). The curves are normalized against the volume for HiScreen. Running conditions are described in Table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

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

Resin	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	<i>E. coli</i> supernatant (4 mg/mL GFP)	150	4	2.4	ÄKTA avant 25	≥ 97
	HiScale	40.8	<i>E. coli</i> supernatant (4 mg/mL GFP)	753	4	10.7	ÄKTA avant 25	≥ 97

Table 3. HiScale 10/40 column packed with MabSelect PrismA: virus clearance (\log_{10}) of xMuLV and MVM in relation to load density and residence time

Experiment	Load density	Residence time (process/load)	xMuLV reduction factor	MVM reduction factor
Run 1 (standard)	65.5 g/L	6.0/12.0 min	2.85 \log_{10}	3.09 \log_{10}
Run 2 (standard)	64.3 g/L	5.9/11.9 min	2.98 \log_{10}	2.87 \log_{10}
Run 3 (low load)	20.4 g/L	5.9/11.8 min	3.64 \log_{10}	2.47 \log_{10}
Run 4 (short residence time)	65.5 g/L	3.9/4.0 min	3.47 \log_{10}	2.60 \log_{10}

Scale-down studies with HiScale 10/40 columns

Viral clearance is essential in process development of bioengineered monoclonal antibodies and recombinant proteins. To evaluate the efficiency of virus removal, virus clearance studies are usually performed with a scaled down model of the full-scale process. The scale down is performed by maintaining the same column bed height and flow velocity (cm/h) while decreasing the column diameter (Fig 9).

A robust column with flexible adapters to mimic large-scale bed heights is a preferred feature of the scale-down column. In addition, the column should be easy to pack and provide reproducible results since several columns are used in these types of studies. HiScale 10/40 column is an excellent column format for scale-down studies and provides all the above features.

HiScale 10/40 allows determination of virus log reduction by use of small resin volumes (from 8 mL) and low amounts of sample. In this application, a virus clearance study was performed using HiScale 10/40 column packed with MabSelect™ Prisma (bed height 20 cm). Two different viruses were chosen: xenotropic murine leukemia virus (xMuLV), which is an enveloped RNA virus; and minute virus of mice (MVM), which is a nonenveloped small DNA virus.

The experimental setup involved four columns where the sample load and residence time were varied to challenge method robustness.

A reduction factor in the range of $\sim 2.5 \log_{10}$ is regarded as a good outcome as shown in Table 3, this was achieved irrespectively of load and residence time for both virus types.

The viral clearance study with HiScale 10/40 verified a good viral log reduction with MabSelect Prisma resin, and shows the suitability of the column format for scale-down applications.

Chromatography scale-down
Use a column with same bed height as in manufacturing and smaller inner diameter

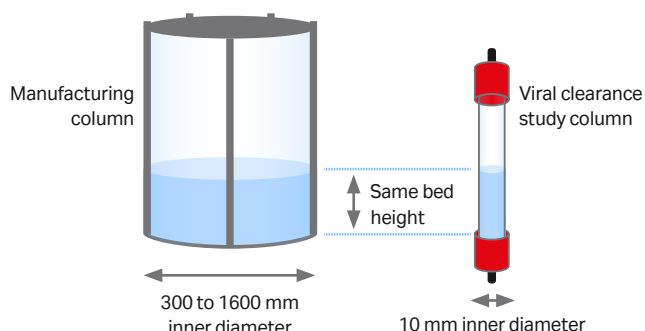


Fig 9. Scale-down studies for viral clearance evaluation are typically performed using a chromatography column with the same bed height as in the large-scale column, but with a smaller inner diameter. This reduces the amount of resin and sample used.

Ordering information

Column ¹	Quantity	Product code
HiScale 10/40	1	29360550
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513
HiScale 50/20	1	28964445
HiScale 50/40	1	28964444

¹ Each HiScale column is delivered with two adapters

Accessories	HiScale 10	HiScale 16	HiScale 26	HiScale 50	No. per pack
Spanner wrench	28964776	28964776	28964777	28964778	2
Long column holder, for classic ÄKTA systems	18112632	18112632	18112632	–	1
Short column holder, for classic ÄKTA systems	–	18111317	18111317	–	1
Column holder, ÄKTA avant and ÄKTA pure	28956282	28956282	28956282	–	1
Column holder, steel	–	–	–	28964499	1
Superloop™, 1/16" fittings 10 mL	18111381	18111381	18111381	18111381	1
Superloop, 1/16" fittings 50 mL	18111382	18111382	18111382	18111382	1
Superloop, M6 fittings 150 mL	18102385	18102385	18102385	18102385	1
Union 5/16" female - 1/16" male	–	–	–	18114208	8
Fingertight union 1/16" male/M6 female	18111258	18111258	18111258	18111258	8
Connector 1/16" male/Luer female	18111251	18111251	18111251	–	2
Net ring 10 µm	29360547	18876101	18876001	18875901	5
Net ring 80 µm	–	–	–	18100069	5
Tubing 0.75 mm i.d., 1.16" o.d.	18111253	–	–	–	2 m
Tubing 1 mm i.d., 1.16" o.d.	–	18111583	18111583	–	2 m
Tubing 2 mm i.d., 1/8" o.d.	–	–	–	28966376	1 m
Tubing cutter	18111246	18111246	18111246	–	1
Column tube 20, complete	–	28966646	28966648	28966649	1
Column tube 40, complete	29372270	28966652	28966651	28966650	1
Packing tube 20	29360551	28986816	28980383	28980251	1
Packing tube 40	–	28986815	28964505	28964506	1
O-ring, packing tube	18103579	28966653	28966654	28966655	2
Accessory kit ²	29360581	28966367	28966374	28966375	1

² The accessory kit includes 1 net ring 20 µm, 1 support screen (not included in HiScale 10), 1 O-ring, 2 ferrules and 2 fingertight stop plugs.

Spare parts	HiScale 10	HiScale 16	HiScale 26	HiScale 50	Material	No. per pack
Adapter	29372404	28966383	28966384	28966385	–	1
Fingertight stop plug, 1/16" male	–	11000355	11000355	–	F	5
Stop plug 5/16" male	–	–	–	18111250	F	5
Union 1/16" female - 1/16" female	–	11000339	11000339	–	F	5
Union 5/16" female - 5/16" female	–	–	–	18117351	F	2
Ferrules for 1/16" tubing	–	18112706	18112706	–	G	10
Ferrules for 1/8" tubing	–	–	–	18112118	G	10
Connector for 1/16" tubing	–	29018382	29018382	–	F	2
Connector for 1/8" tubing	–	–	–	18112117	F	10
Tubing 2 mm i.d. cut	–	–	–	28966656	F	0.295 m
O-ring	29372271	19016301	28978227	28978228	D, H	5
Support screen	–	19065101	18937701	19066401	I	5
Net ring 20 µm	29372273	28966379	28966380	28966381	I, J	5
Wrench, HiScale 10	29372274	–	–	–	J	2

A = Borosilicate glass
B = Polycarbonate

C = Polyamide (reinforced glass fibre)
D = Ethylene propylene diene polymer (EPDM)

E = Polyoxymethylene (POM)
F = Polyether ether ketone (PEEK)

G = Ethylene tetrafluoroethylene (ETFE)
H = Fluoro-rubber (Viton™)

I = Polypropylene
J = Polyamide (nylon)

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