HiPrep 26/10 Desalting

Instructions for Use

HiPrep™ 26/10 Desalting is a prepacked, ready to use column for group separation of high (M_r > 5 000) from low molecular weight substances (M_r < 1 000).

Column data

Matrix	Sephadex™ G-25 Fine, cross-linked dextran
Mean particle size	90 µm
Bed volume	53 ml
Bed height	100 mm
i.d.	26 mm
Column composition	Polypropylene
Void volume	15 ml
Sample dilution	1.2–3 fold
Exclusion limit, proteins, peptides, M_r	5000
Recommended flow rate[1]	9–31 ml/min, (100–350 cm/h)
Maximum flow rate[1]	40 ml/min, (450 cm/h)
Maximum pressure over the packed bed during operation, Δp[2]	0.15 MPa, 1.5 bar, 22 psi
HiPrep column hardware pressure limit[2]	0.5 MPa, 5 bar, 73 psi
pH stability	
Long term	pH 2–13
Short term	pH 2–13
Storage	+4 to +30 °C in 20% ethanol

[1] Water at room temperature. Flow rate is determined by $v \cdot \eta \leq 10$ ml/min where v=flow rate and η=viscosity.

[2] Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the pre-column pressure, the pressure drop over the gel bed, and the post column pressure. It is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the gel is overpressured.

If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

To avoid breaking the column, the post-column pressure must never exceed 3.5 bar.

Step	Action
1	Connect a piece of tubing in place of the column.
2	Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the back pressure as total pressure.
3	Disconnect the tubing and run at the same flow rate used in step 2. Note this back pressure as pre-column pressure.
4	Calculate the post-column pressure as total pressure minus pre-column pressure. If the post column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors) and perform steps 1-4 again until the post column pressure is below 3.5 bar. When the post column pressure is satisfactory, add the post column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

First time use

Ensure an appropriate pressure limit has been set.

Equilibrate the column for first time use or after long-term storage by running:

At least 265 ml (5 CV[1]) buffer at 15 ml/min.

HiPrep 26/10 Desalting can be used directly on ÄKTAdesign systems without the need for any extra connectors.

Try these conditions first

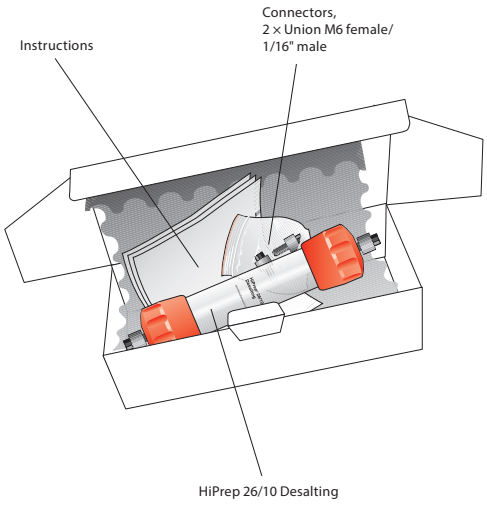
Buffer:	50 mM phosphate buffer, 0.15 M NaCl, pH 7.0
Flow rate:	20 ml/min at room temperature
Sample volume:	15 ml
Separations:	Elute the column with 106 ml (2 CV) of buffer

Equilibration before a new run

Equilibrate with at least 265 ml (5 CV) buffer at 20 ml/min. Extended equilibration may be needed with buffers containing detergents.

Equilibration is not necessary between runs with the same buffer

Please read the back of this instruction for information on optimizing a separation.



Buffers and solvent resistance

To increase column life time, filter all solutions through a 0.45 µm filter.

Daily use

All commonly used aqueous solutions, pH 2-13

Guanidine hydrochloride, up to 6 M

Urea, up to 8 M

Ethanol, up to 25%

Methanol, up to 25%

Propanol, up to 25%

Ionic and non-ionic detergents

Cleaning

Sodium hydroxide, up to 0.2 M

Hydrochloric acid, up to 0.1 M

Acetic acid, up to 1.0 M

Avoid

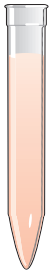
Solutions < pH 2

Solutions > pH 13

Oxidizing agents

Sample recommendations

Sample concentration	< 70 mg/ml, proteins
	< 5 mg/ml, dextrans
Sample volume	< 15 ml*
Preparation	0.45 µm filter or 10 000 x g for 10 min



Note:

Larger sample volumes can be applied but will have a negative influence on resolution/ desalting.

To increase sample volumes connect several HiPrep 26/10 Desalting columns in series.

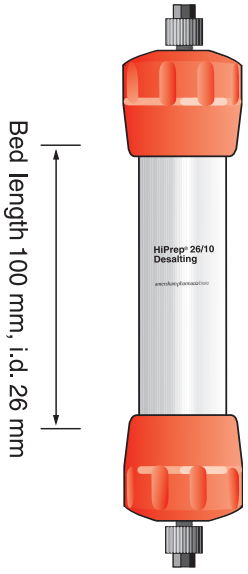
2 x HiPrep 26/10 Desalting columns give a sample volume of 30 ml.

Up to 4 x HiPrep 26/10 Desalting columns in series have been tested using 60 ml sample volume, resulting in very good results.

Delivery/storage

The column is delivered equilibrated with 20 % ethanol. If the column is to be stored for more than two days after use, clean the column according to the procedure described under *Cleaning-in-Place (CIP), on page 3*. Then equilibrate with at least 265 ml (5 CV) of 20% ethanol at a flow rate of 15 ml/min.

DO NOT OPEN THE COLUMN!



1 1 CV = 1 column volume ≈ 53 ml

Choice of buffer

The buffer should be selected to ensure that the sample is fully soluble. Also, it should ideally be chosen to simplify a later stage, e.g. lyophilization or another purification step. For substances carrying charged groups an buffer containing a salt is recommended. A salt concentration of at least 0.15 M is recommended to prevent possible ionic interactions with the matrix. Sodium chloride is often used for this purpose. Suggested volatile buffers are listed in *Table 1, on page 3*

Table 1. Volatile buffer systems.

pH	Substance
3–5	Trimethylamine/formic acid
4–6	Trimethylamine/acetic acid
6.8–8.8	Trimethylamine/HCl
8–9.5	Ammonium carbonate/ammonia
8.5–10	Ethanolamine/HCl

Optimization

Perform your first run according to *Try these conditions first, on page 1*. If the obtained results are unsatisfactory, consider the following:

Action	Effect
Decrease sample volume	Improved resolution, but greater dilution of recovered material
Decrease flow rate	Improved resolution
Decrease sample concentration	Improved resolution if close to 70 mg/ml
Connect two or more columns in series	Maintains resolution with a larger sample volume

For more information, please refer to the handbook *Gel filtration. Principles and Methods* from Cytiva.

Cleaning-in-Place (CIP)

Regular cleaning

The frequency of cleaning will depend on the nature of the sample material and should be worked out on a case-by-case basis. A general cleaning procedure is the following:

Step	Action
1	Reverse flow direction and wash the column with 106 ml (2 CV) 0.2 M sodium hydroxide or a solution of a non ionic detergent at a flow rate of 10 ml/min. Ensure that the pressure drop does not exceed 0.15 MPa (1.5 bar, 22 psi).
2	Wash the column with 265 ml (5 CV) of distilled water at a flow rate of 15 ml/min.
3	Before the next run, equilibrate the column with at least 265 ml (5 CV) buffer until the UV base line and pH are stable.

If you suspect that the column is still contaminated, refer to the *More rigorous cleaning* section below.

More rigorous cleaning

To remove precipitated proteins and peptides, fill the column with 1 mg pepsin/ml in 0.1 M acetic acid, 0.5 M NaCl and leave it at room temperature overnight or 1 hour at +37 °C. After enzymatic treatment, repeat steps 1–3 in the Section *Regular cleaning* above.

DO NOT OPEN THE COLUMN!

Troubleshooting

Symptom	Remedy
Increased back pressure over column and/or loss of resolution	Follow the procedure described in the section *Cleaning-in-Place (CIP)*. If back pressure is not decreased reverse the flow direction again and follow the Section *More rigorous cleaning*.
Air in the column	Reverse the flow direction and pump 200 ml well de-gassed buffer at a flow rate of 15 ml/min through the column. The quality of the packed bed will not normally be affected.

Column performance control

We recommend checking the column performance at regular intervals. The performance of the column can be checked as described in Figure 1 below.

Sample:	2 mg Bovine Serum Albumin (BSA)/ml, 0.07 mg N-Hydroxysuccinimide (NHS)/ml in 50 mM sodium phosphate, 0.15 M NaCl, pH 7.0
Sample volume:	10 ml
Eluent:	50 mM sodium phosphate, 0.15 M NaCl, pH 7.0
Flow rate:	26.5 ml/min (300 cm/h)
Instrumentation:	ÄKTAexplorer 100 with 1 mm i.d. tubing installed

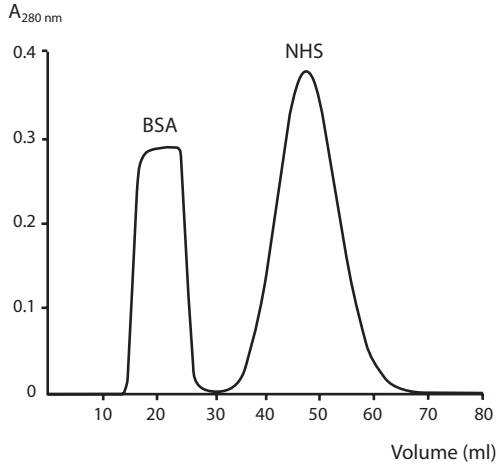


Fig 1. Typical chromatogram from a function test of HiPrep 26/10 Desalting.

Ordering information

Product	No. per pack	Product code
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02

Companion products

HiTrap™ Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml[1]	11-0003-29

[1] Special pack size delivered on specific customer order.

Accessories

To connect columns with 1/16" connectors to FPLC System:		
Union M6 female/1/16" male[1]	5	18-3858-01
HiTrap/HiPrep 1/16" male connector to ÄKTAdesign	8	28-4010-81

[1] 2 or 8 units (red polypropylene) are included in HiPrep package.

Literature

Handbook, Gel Filtration, Principles and Methods	1	18-1022-18
Gelfiltration, Chromatography Media and Column Guide	1	18-1124-19

Further information

Check *cytiva.com/protein-purification* for more information.

cytiva.com/protein-purification

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