Sephadex G-25 resins and prepacked formats

DESALTING/BUFFER EXCHANGE AND SIZE EXCLUSION CHROMATOGRAPHY

Size exclusion chromatography (SEC) or as it is also commonly named, gel filtration (GF), has been successfully employed for size-based separations of macromolecules since the introduction of the first commercially available Sephadex™ chromatography resin in 1959. SEC may be performed mainly in two principally different modes, depending on the size differences of the solutes to be separated: group separation and fractionation.

The main use for the Sephadex product family is group separation, such as desalting, buffer exchange, and sample cleanup. Sephadex resins are members of the BioProcess™ resin family and carry comprehensive technical and regulatory support documentation.

Sephadex G-25 resins and prepacked columns offer:

- Quick desalting, removal of contaminants, and buffer exchange in a single step
- Excellent recovery and minimum sample dilution
- Well-established resins for industrial applications

Separation principle

"Group separation" separates molecules according to their relative sizes: small molecules such as salts, free labels, and other impurities are efficiently separated from high-molecular weight molecules of interest. In Sephadex, the degree of cross-linking of the dextran determines the extent to which macromolecules can permeate the beads. Large molecules are excluded while smaller sized molecules enter the beads to varying extents according to their different sizes. Large molecules thus leave the column first, followed by smaller molecules.

Fig 1. Sephadex G-25 is available for packing in columns and batch separations. Prepacked columns are available in HiTrap™, HiPrep™, PD-10 Desalting, PD MiniTrap™ G-25, PD MidiTrap™ G-25, and PD SpinTrap™ G-25 columns. Prepacked PD MultiTrap™ G-25 96-well filter plates are also available. The wide range of prepacked formats gives greater choice to enable fast and convenient group separations between high- and low-molecular weight substances.

Sephadex G-25 has a fractionation range for globular proteins of molecular weights (M_r) 1000 to 5000, with an exclusion limit of approximately M_r 5000. Proteins and peptides larger than M_r 5000 are therefore easily separated from molecules with molecular weights of less than 1000. Separations are conveniently performed using different prepacked column and 96-well plate formats, either manually with gravity flow, centrifugation, vaccum, a peristaltic pump, or a chromatography system such as ÄKTA™, depending on the format selected.

The principles of SEC and the structures of Sephadex and other SEC resins are explained in more detail in the handbook *Size Exclusion Chromatography, Principles and Methods*, 18102218 (cytiva.com/handbooks).

Chromatography resin characteristics

Sephadex resins are prepared by cross-linking dextran with epichlorohydrin. The resins are supplied dry, except when prepacked in columns and plate format. The resins swell in aqueous solutions (Table 1). Sephadex G-25 is one of six different Sephadex G-types. The different types of Sephadex resins differ in their degree of cross-linking and hence in their degree of swelling and molecular fractionation range.

The effect of particle size

Sephadex G-25 is available in four different particle size grades (Coarse, Medium, Fine, and Superfine). These four grades form the basis of a range of group separation products. Coarse and Medium grades are preferred for group separations at process scale where high flow rates and low operating pressures are required. The Fine and Superfine grades are for routine laboratory work including preparative separations. The smaller particle sizes of the Fine and Superfine grades give shorter diffusion distances and allow for highly efficient separations at high flow rates. Examples of different uses are shown under *Applications* and illustrate several of these properties.

The particle sizes of Sephadex resins are usually reported as dry diameter in micrometer (µm). However, as the resins are swollen before use, it is the wet bead diameter that is of practical importance when choosing the correct type of equipment to use. The dry particle diameters as well as the hydrated-to-dry diameter ratios for Sephadex G-25 resins are shown in Table 1. Note that the degree of swelling in organic solvents is not the same as in aqueous solutions and must be determined experimentally for each solvent to be used. For routine use in organic solvents, lipophilic Sephadex LH-20 is recommended.

Table 1. Particle sizes for Sephadex G-25 resins

Sephadex G-25	Dry particle diameter (µm)	Diameter ratio (hydrated:dry)
Superfine	20 to 50	~ 1.7
Fine	20 to 80	~ 1.7
Medium	50 to 150	~ 1.7
Coarse	100 to 300	~ 1.7

Stability

The mechanical strength and pH stability of Sephadex SEC resins depend on the degree of cross-linking.

Sephadex G-25 is one of the more rigid members of the family and has an operational pH range of 2 to 13. In trials, Sephadex G-25 has been exposed to 0.1 M HCl for 1 to 2 h and to 0.02 M HCl for 6 mo without impact on its chromatographic properties. Leakage studies have shown that Sephadex G-25 also withstands long-term exposure to NaOH, and can be stored in 0.01 M NaOH[1] without alterations in its performance. For storage, 20% ethanol can also be used. Cleaning and sanitization by 60 to 90 min exposure to 0.2 M NaOH, followed by flushing with water or buffer, is recommended and can be repeated for hundreds of cleaning cycles.

Sephadex resins can be autoclaved in their wet form (pH 7.0) for 30 min at 121°C.

Batch-to-batch reproducibility

Consistent quality and performance from batch to batch is important for all separation resins, but is most significant when the resins are routinely used in industrial processes. Quality control data gathered for more than 50 yr show outstanding batch-to-batch consistency of the Sephadex resins.

Working with Sephadex G-25

Column dimensions

In SEC, column length is important for resolution and column diameter determines loading capacity. For group separations at high sample loadings, short (5 to 30 cm) and wide columns give excellent results at sample loadings of about 30% of the column volume.

Column packing

As Sephadex is supplied as a dry powder, it must be swollen in buffer before packing in the column. Laboratory columns can be packed by pouring the swollen G-25 slurry into the column and using a pump to pack the resin bed. For packing flow rates, see the instructions for detailed information.

Packing process-scale columns requires modified techniques but gives excellent results and consistent performance.

Parameters affecting desalting

The most critical parameter affecting resolution in desalting applications is the sample-to-resin volume ratio. To minimize dilution and still retain good separation, sample volumes of up to approximately 30% of the total bed volume are recommended. Figure 2 illustrates how the resolution of a group separation run on HiPrep 26/10 Desalting is affected by sample volume. For baseline separation, resolution between peaks (R_s) should be at least 1.5. As flow rate has a minor impact on resolution, desalting can be performed at high flow rates (Fig 3).

[1] In most cases, no long term stability data has been generated by Cytiva in 0.01 M NaOH. In some cases, accelerated studies at elevated temperature indicate that storage in 0.01 M NaOH can be a viable option but no guarantees can be made regarding retained function of the product.

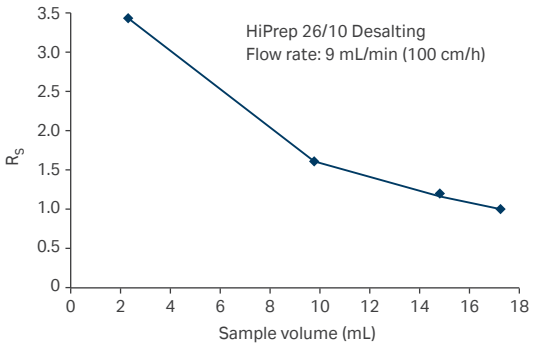


Fig 2. Influence of sample volume on the resolution (R_s).

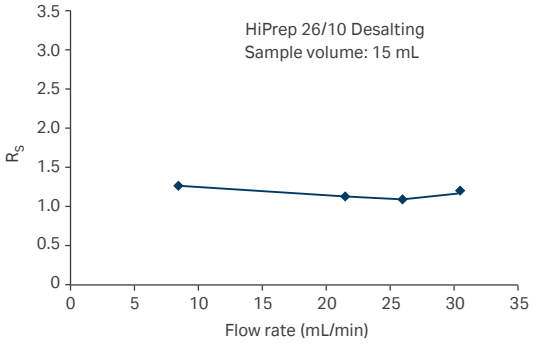


Fig 3. Influence of flow rate on the resolution (R_s).

Cleaning packed columns

For columns that have been in use for some time, it can be necessary to remove precipitated proteins or other contaminants. Columns packed with Sephadex G-25 may be cleaned with 2 column volumes of 0.2 M NaOH or a nonionic detergent solution (60 to 90 min exposure). The frequency of cleaning will depend on the nature of the sample material and should be worked out on a case-by-case basis.

Scale-up

Scaling up a separation based on Sephadex G-25 from small-scale to large-scale routine production is a straightforward procedure. Well known examples of successful commercial applications include buffer exchange in processes for removing endotoxins from albumin (see Applications below) and as a preparative step during the production of vaccines.

Applications

As noted earlier, group separations such as buffer exchange and desalting are the most widely used applications for Sephadex G-25. Buffer exchange is often necessary when the buffer composition of the sample needs to be changed between chromatography steps. Desalting of a sample is often performed before ion exchange (IEX) and multimodal chromatography (MMC), as well as after hydrophobic interaction (HIC) and affinity chromatography (AC), and prior to analysis. Cleanup of small contaminants is, for example, needed after labeling to remove free labeling molecules prior to analysis. At small scales, SEC with Sephadex G-25 has replaced much slower and more cumbersome dialysis procedures.

Figure 4 shows process data from a buffer exchange step using Sephadex G-25 Coarse in a process for removal of endotoxins and/or ethanol from albumin. The throughput of Sephadex G-25 Coarse grade resulted in a rapid and efficient separation, despite the high concentration of protein in the feedstock.

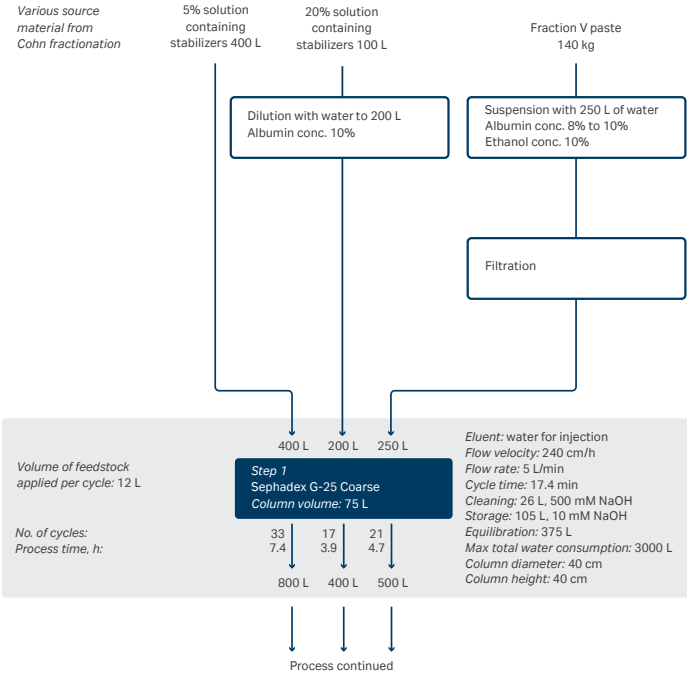


Fig 4. Process data from a buffer exchange step using Sephadex G-25 Coarse in a production-scale process to remove endotoxins and/or ethanol from albumin.

Other desalting applications at larger scale can be exemplified by intermediate group separation of allergen extract from low-molecular weight impurities using a BPG 450 column packed with Sephadex G-25 Superfine. The bed height was 15 cm, giving a bed volume of 25 L. The sample volume was up to 6 L (24% load) and the flow velocity of 1.1 cm/min, giving a cycle time of 17 min. The dilution factor was 1.4.

A classic example of desalting is given by the de-ethanolization of human serum albumin (1). In this example, 12 L of a 9% protein solution was purified at a cycle time of 17.4 min using a 60 × 40 cm i.d. column packed with Sephadex G-25 Coarse, corresponding to a productivity of 50 g/h/L.

Large-scale desalting of samples can be performed effectively with Sephadex G-25 Coarse. To demonstrate this, a crude enzyme preparation was desalted in 2500 L of Sephadex G-25 Coarse, which was packed in a stainless-steel column (i.d. 100 × 180 mm). The sample volume was 875 L (i.e., 35% of the column volume) and the flow rate was 62.5 L/min, giving a cycle time of 1 h. Further, the productivity of Sephadex G-25 Coarse was compared to that of Sephadex G-25 Medium (Table 2). The results show that Sephadex G-25 Coarse provides the highest productivity of these two resins.

Table 2. Comparison between the productivity of Sephadex G-25 Coarse with G-25 Medium for desalting of albumin from ammonium sulfate

Parameter	Sephadex G-25 Coarse	Sephadex G-25 Medium
Sample volume (L)	31	37
Relative sample volume (%)	25	30
Sample capacity (L/h)	70	18
Salt concentration in product (%)	0.4	0.04
Salt removed (%)	98.0	99.9
Albumin concentration in product (%)	4.0	5.0
Dilution factor of albumin	1.5	1.2
Albumin processed (g/h)	4125	1170
Productivity (g/h/L)	33	9
Cost per kg of albumin (USD)	0.64	2.20

Note: Albumin concentration in feed is 6%, salt concentration in feed is 24%. Column: 100 × 40 cm i.d., giving a bed volume of 125 L.

Size exclusion chromatography can offer a very robust purification step as in the use of Sephadex G-25 Coarse for initial buffer exchange of raw plasma in large-scale fractionation of albumin. The column, containing 75 L of resin, was used for more than 6 yr and processed 70 000 L of material.

Prepacked columns with Sephadex G-25

Prepacked column and 96-well plate formats can be used when removal of low-molecular weight components is needed.

Examples of applications are:

- Preparation of samples prior to or after IEX, MMC, AC, and HIC
- Removal of free low-molecular weight labels or contaminants from proteins/peptides
- Removal of substrates, inhibitors, or cofactors from enzymes
- Preparation of samples for concentration, freeze-drying, or storage
- Termination of a reaction between a macromolecule and a low-molecular weight reagent

HiTrap Desalting

HiTrap Desalting column is packed with Sephadex G-25 Superfine. The column is made of biocompatible polypropylene with polyethylene frits. The column is delivered with a stopper on the inlet and a snap-off end on the outlet. The column cannot be opened or refilled. Operation is easy, using a syringe, a peristaltic pump, or a liquid chromatography system. Larger sample volumes or, if required, improved resolution can easily be achieved by connecting up to five columns in series.

Table 3 shows the main characteristics of HiTrap Desalting.

Desalting in a fraction of a minute

Because of the rigidity of the Sephadex G-25 matrix and an optimized column packing method, HiTrap Desalting column has a very low back pressure (0.25 bar [0.025 MPa, 3.7 psi] at 10 mL/min). The low back pressure makes it possible to run separations in a fraction of a minute using a syringe or a pump. The separation of bovine serum albumin (BSA) and NaCl is shown in Figure 5. The whole separation took only 45 s and the protein was eluted in less than 30 s.

Column: HiTrap Desalting
Sample: 1.4 mL of BSA (2 mg/mL) in 50 mM sodium phosphate, 500 mM sodium chloride, pH 7.0
Buffer: 50 mM sodium phosphate, 150 mM sodium chloride, pH 7.0
Flow rate: 10 mL/min

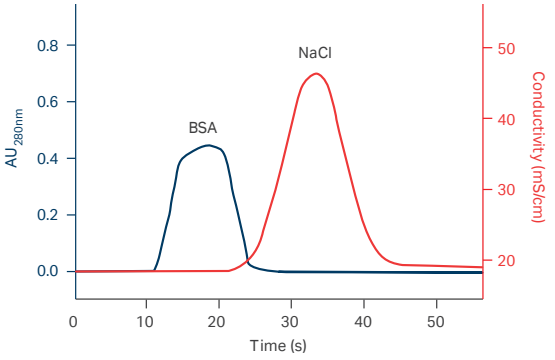


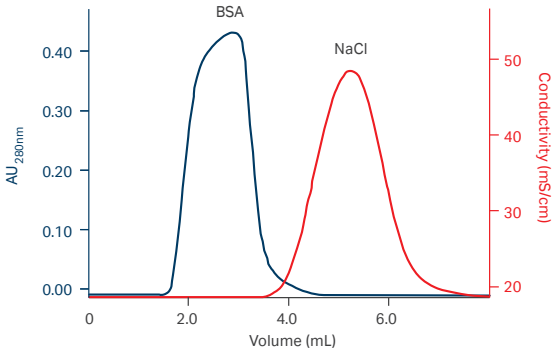
Fig 5. Highly efficient desalting in in under 45 s using HiTrap Desalting.

Scaling up by connecting HiTrap Desalting columns in series

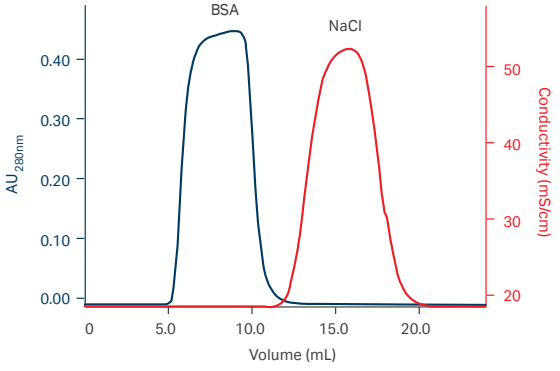
Larger sample volumes or, if required, improved resolution can easily be achieved by connecting up to five columns in series. Figures 6 A to C show the results obtained when one, three, and five HiTrap Desalting columns were connected in series. The sample volumes were 1.4, 4.3, and 7.1 mL, respectively.

Columns:	HiTrap Desalting, 1 × 5 mL, 3 × 5 mL, 5 × 5 mL
Sample:	2 mg/mL BSA in 50 mM sodium phosphate, 500 mM sodium chloride, pH 7.0
Sample volume:	28% of column volume (1.4, 4.3, and 7.1 mL, respectively)
Buffer:	50 mM sodium phosphate, 150 mM sodium chloride, pH 7.0
Flow rate:	5 mL/min

(A) HiTrap Desalting 1 × 5 mL



(B) HiTrap Desalting 3 × 5 mL in series



(C) HiTrap Desalting 5 × 5 mL in series

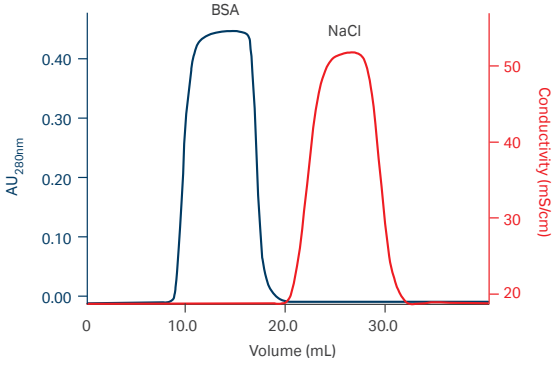


Fig 6. Separation of BSA from NaCl on HiTrap columns connected in series.

Online buffer exchange

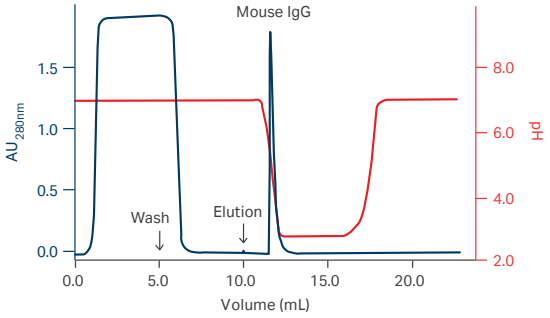
HiTrap Desalting columns can easily be attached to other columns for online buffer exchange before or after a chromatographic step. An example is shown in Figure 7, where a HiTrap Desalting column was connected serially after a HiTrap Protein G HP column in order to adjust the pH of the eluted material. The HiTrap Desalting column was equilibrated with 20 mM sodium phosphate, pH 7.0.

A mouse monoclonal antibody (IgG1) from a serum-free cell culture supernatant was purified using a HiTrap Protein G HP 1 mL column. After loading of a 5 mL sample and washing with 5 mL of binding buffer (20 mM sodium phosphate, pH 7.0), bound material was eluted with 5 mL of 100 mM glycine, pH 2.7. The first 1.3 mL eluted from the HiTrap Protein G HP column was discarded before the column was connected to the inlet of the HiTrap Desalting column. The eluate from the columns was monitored for UV absorbance and pH was measured in the collected 0.5 mL fractions.

Figure 7A shows the original separation without the extra desalting step. As shown in Figure 7B, the obtained yield was 18% higher when the HiTrap Desalting column was used for online desalting.

Columns:	(A) HiTrap Protein G HP, 1 mL (B) HiTrap Desalting, 5 mL
Sample:	Eluted mouse monoclonal IgG from HiTrap Protein G HP
Binding buffer:	20 mM sodium phosphate, pH 7.0
Elution buffer:	100 mM glycine, pH 2.7
Flow rate:	2 mL/min

(A) Original separation without the desalting step



(B) Separation of mouse IgG including a desalting step

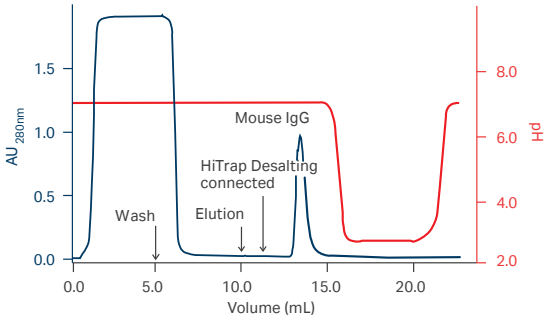


Fig 7. Online buffer exchange with HiTrap Desalting improves yield at a mouse IgG purification. (A) Original separation without the desalting step. (B) Separation including a desalting step.

HiPrep 26/10 Desalting column

HiPrep 26/10 Desalting is packed with Sephadex G-25 Fine. The column has an internal diameter of 2.6 cm and a bed height of 10 cm. The bed volume is approximately 53 mL.

The HiPrep column is made of biocompatible polypropylene with nylon frits. The supplied set of connectors makes it easy to connect the column to different chromatography systems. The column is not designed to be opened or repacked. The characteristics of HiPrep 26/10 Desalting column are summarized in Table 3.

Table 3. Characteristics of HiTrap Desalting and HiPrep 26/10 Desalting

	HiTrap Desalting	HiPrep 26/10 Desalting
Resin	Sephadex G-25 Superfine	Sephadex G-25 Fine
Matrix	Cross-linked dextran	Cross-linked dextran
Bed volume	5 mL	53 mL
Bed dimension	1.6 × 2.5 cm	2.6 × 10 cm
Void volume	1.5 mL	15 mL
Recommended sample volume	0.1 to 1.5 mL	≤ 15 mL
Sample dilution, syringe operation	1.3 to 4 fold	1.2 to 3 fold
Exclusion limit [M_r] Globular proteins	M_r ~ 5000	M_r ~ 5000
Particle size distribution, dry (µm)	20 to 50*	20 to 80*
Maximum operational flow rate†	15 mL/min	40 mL/min
Recommended operational flow rate†	1 to 10 mL/min	9 to 31 mL/min
Column hardware pressure limit	5 bar (0.5 MPa, 70 psi)	5 bar (0.5 MPa, 70 psi)
Chemical stability	Stable to commonly used aqueous buffers	Stable to commonly used aqueous buffers
pH stability, operational‡:	2 to 13	2 to 13
pH stability, CIP¶:	2 to 13	2 to 13
Avoid	Oxidizing agents	Oxidizing agents
Storage	4°C to 30°C, 20% ethanol	4°C to 30°C, 20% ethanol

* ≥ 80% volume share within given range.
† Room temperature, aqueous buffers.
‡ pH range where resin can be operated without significant change in function.
¶ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Buffer exchange of mouse plasma

Buffer was exchanged on mouse plasma within 1.5 min on HiPrep 26/10 Desalting column. A 10 mL sample was applied at a flow rate of 22 mL/min (flow velocity 250 cm/h). Figure 8 shows the chromatogram of the separation. The protein was eluted in a volume of 19 mL.

Column:	HiPrep 26/10 Desalting
Sample:	Mouse plasma, centrifuged at 10 000 × g for 10 min
Sample volume:	10 mL
Buffer:	25 mM sodium acetate, pH 7.0
Flow rate (flow velocity):	22 mL/min (250 cm/h)
System:	ÄKTA system (1 mm i.d. tubing installed)

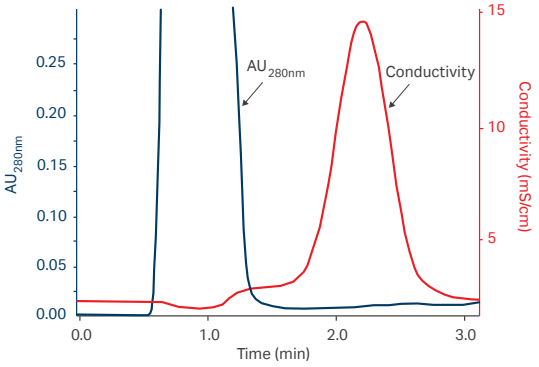


Fig 8. Buffer exchange of mouse plasma on HiPrep 26/10 Desalting.

Reproducible removal of N-Hydroxysuccinimide from BSA

Figure 9 shows the removal of N-Hydroxysuccinimide (M_r 115) from BSA (M_r 67 000) in three parallel runs. Highly efficient and reproducible desalting was achieved in all three runs.

Column:	HiPrep 26/10 Desalting
Sample:	2 mg/mL BSA, 0.07 mg/mL of N-Hydroxysuccinimide (NHS) in 50 mM sodium phosphate, 150 mM NaCl, pH 7.0. Filtered through a 0.45 µm filter
Sample volume:	13 mL
Buffer:	50 mM sodium phosphate, 150 mM NaCl, pH 7.0
Flow rate (flow velocity):	31 mL/min (350 cm/h)
Instrumentation:	ÄKTA system (1 mm i.d. tubing installed)

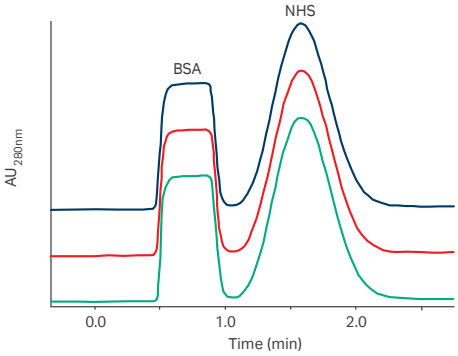


Fig 9. Reproducible removal of N-Hydroxysuccinimide from BSA.

Scaling up sample volumes

Samples of 15 mL (30% of the total bed volume) or less can be applied to a single column, and by coupling of up to four columns in series (Fig 10), a maximum sample volume of 60 mL can be run. Even with four columns in series, high flow rates can be maintained without back-pressure problems, resulting in fast separations. In fact, up to 60 mL of sample can be desalted or buffer exchanged in 20 to 30 min.

Table 4 shows run data and results for HiPrep 26/10 Desalting columns in series. Samples consisted of either 30 or 60 mL of a fungal culture supernatant containing a secreted recombinant protein. Samples were run on two (30 mL sample) or four (60 mL sample) columns connected in series.

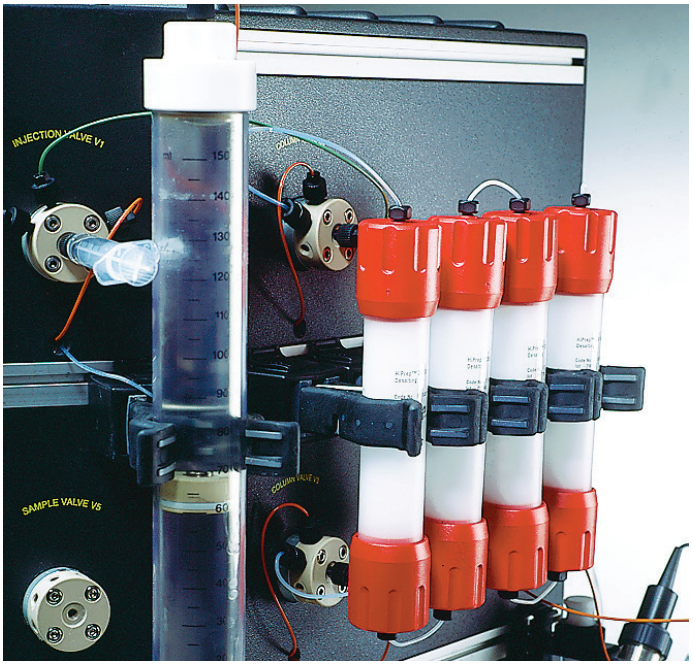


Fig 10. Four HiPrep 26/10 Desalting columns connected in series.

Table 4. Run data for buffer exchange of 30 and 60 mL samples on HiPrep 26/10 Desalting columns connected in series

Columns	2 × HiPrep 26/10 Desalting in series (total volume, V_t = 106 mL) for 30 mL sample 4 × HiPrep 26/10 Desalting in series (V_t = 212 mL) for 60 mL sample
Sample	30 or 60 mL *Pichia pastoris* culture supernatant containing a secreted recombinant protein
Sample preparation	Filter through 0.45 µm filter
Sample loop	Superloop™, 150 mL
Sample elution	
30 mL loading	35 mL (dilution factor 1.2)
60 mL loading	70 mL (dilution factor 1.2)
Buffer	100 mM Tris, 150 mM NaCl, 0.05% Tween™ 20, pH 7.6
Flow rates (30 mL)	
Sample loading	12 mL/min
Elution	17 mL/min
Flow rates (60 mL)	
Sample loading	8 mL/min
Elution	11 mL/min

Disposable PD-10 Desalting Columns, PD MidiTrap G-25, PD MiniTrap G-25, PD SpinTrap G-25, and PD MultiTrap G-25

Prepacked, single-use column and 96-well filter plate formats packed with Sephadex G-25 Medium or Sephadex G-25 Superfine are available for buffer exchange, desalting, and cleanup of biological samples, for example proteins and carbohydrates (Fig 11). These columns and 96-well filter plates provide convenient small-scale preparation of protein samples prior to downstream analytical techniques such as gel electrophoresis, liquid chromatography, and liquid chromatography-mass spectrometry (LC-MS). Sample volumes of up to 2.5 mL are possible using these formats (Table 5).

The columns and 96-well filter plates are manufactured from biocompatible polypropylene. Special column and plate frits protect the resin from running dry during use. Recommended volumes for sample loading and elution with subsequent dilution factors are listed in Table 6.

Fig 11. Simple desalting and buffer exchange with Disposable PD-10 Desalting Columns, PD MidiTrap G-25 and PD MiniTrap G-25 gravity-flow/spin columns, PD SpinTrap G-25 microspin column, and PD MultiTrap G-25 96-well filter plate.

Table 5. Characteristics of PD products prepacked with Sephadex G-25

	PD MultiTrap G-25	PD SpinTrap G-25	PD MiniTrap G-25	PD MidiTrap G-25	PD-10 Desalting Columns
Volume of prepacked resin	500 µL/well	~ 600 µL/column	2.1 mL	2.1 mL	8.3 mL
Packed bed dimensions	N/A	N/A	0.97 × 2.8 cm	1.3 × 2.6 cm	1.45 × 5.0 cm
Well/column volume	800 µL	1 mL	5 mL	8.5 mL	13. 5 mL
Void volume	~ 150 µL	~ 150 µL	~ 0.5 mL	~ 1.0 mL	2.5 mL
Maximum sample volume	130 µL	180 µL	0.5 mL	1.0 mL	2.5 mL
Volume of eluted sample (gravity)	–	–	1.0 mL	1.5 mL	3.5 mL
Volume of eluted sample (spin)*	130 µL	180 µL	0.5 mL	1.0 mL	2.5 mL
Recovery†	70 to 90%	70 to 90%	70 to 95%	70 to 95%	70 to > 95%
Desalting capacity	> 85%	> 85%	> 90%	> 90%	> 90%
Plate/column material	polypropylene and polyethylene	polypropylene and polyethylene	polypropylene and polyethylene	polypropylene and polyethylene	polypropylene and polyethylene
Storage solution	20% ethanol	0.15% kathon™	0.15% kathon	0.15% kathon	0.15% kathon
Storage temperature	4°C to 30°C	4°C to 30°C	4°C to 30°C	4°C to 30°C	4°C to 30°C

* Applied volume = eluted volume
† Biomolecule dependent

Table 6. Recommended sample and elution volumes on desalting/buffer exchange columns and 96-well filter plate

Column	Loaded volume (mL)	Eluted volume (mL)	Dilution factor	Operation
HiPrep 26/10 Desalting	10	10 to 15	1 to 1.5	pump
	15 (max.)	15 to 20	1 to 1.3	pump
2 × HiPrep 26/10 Desalting	30 (max.)	30 to 40	1 to 1.3	pump
3 × HiPrep 26/10 Desalting	45 (max.)	45 to 55	1 to 1.2	pump
4 × HiPrep 26/10 Desalting	60 (max.)	60 to 70	1 to 1.2	pump
HiTrap Desalting	0.25	1.0	4	syringe/pump
	0.5	1.0	3	syringe/pump
	1.0	2.0	2	syringe/pump
	1.5 (max)	2.0	1.3	syringe/pump
2 × HiTrap Desalting	3.0 (max.)	4.0 to 5.0	1.3 to 1.7	syringe/pump
3 × HiTrap Desalting	4.5 (max.)	6.0 to 7.0	1.3 to 1.7	syringe/pump
PD–10	1.5	3.5	2.3	gravity
	2.0	3.5	1.8	gravity
	2.5 (max)	3.5	1.4	
PD MiniTrap G-25	0.1 to 0.5	1.0	2	gravity
	0.2 to 0.5	Up to 0.5	0	spin
PD MidiTrap G-25	0.5 to 1.0	1.5	2	gravity
	0.75 to 1.0	Up to 1.0	0	centrifugation
PD SpinTrap G-25	0.1 to 0.18	0.14 to 0.18	0 to 1.4	centrifugation
PD MultiTrap G-25	0.07 to 0.13	0.1 to 0.13	0 to 1.4	vacuum/centrifugation

Small-scale cleanup

PD SpinTrap G-25 and PD MultiTrap G-25 are designed for small-scale cleanup and are valuable tools for screening purposes and high-throughput applications. SpinTrap columns only require a standard microcentrifuge (Fig 12A). MultiTrap 96-well filter plates allow cleanup by centrifugation, either manually or automated with robotics (Fig 12B).

Typical desalting capacity is above 85% with recoveries between 70% and 90% (biomolecule dependent). In a run of 96 parallel wells with BSA in 1 M NaCl, the salt removal capacity varied by 1% (Fig 13) and the recovery of total loaded material varied by 3%.

(A) (B)

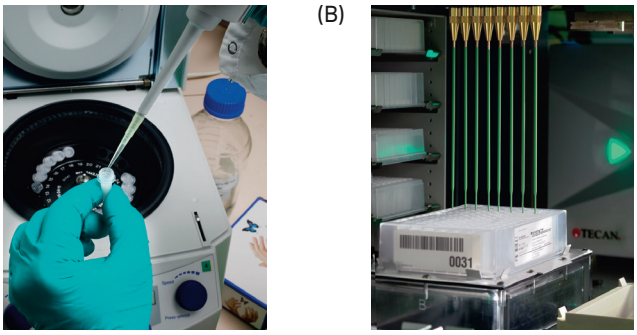


Fig 12. (A) PD SpinTrap G-25 sample preparation. (B) PD MultiTrap G-25 automated sample preparation in a robotic system.

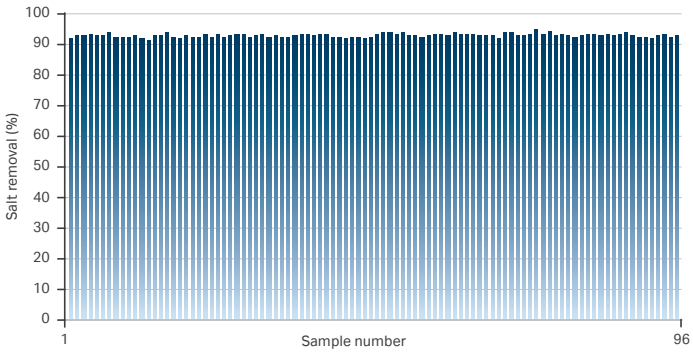


Fig 13. Removal of NaCl from BSA on a PD MultiTrap G-25 96-well filter plate showed highly reproducible results. The average desalting capacity was 93% and the well-to-well variation was 1% (relative standard deviation).

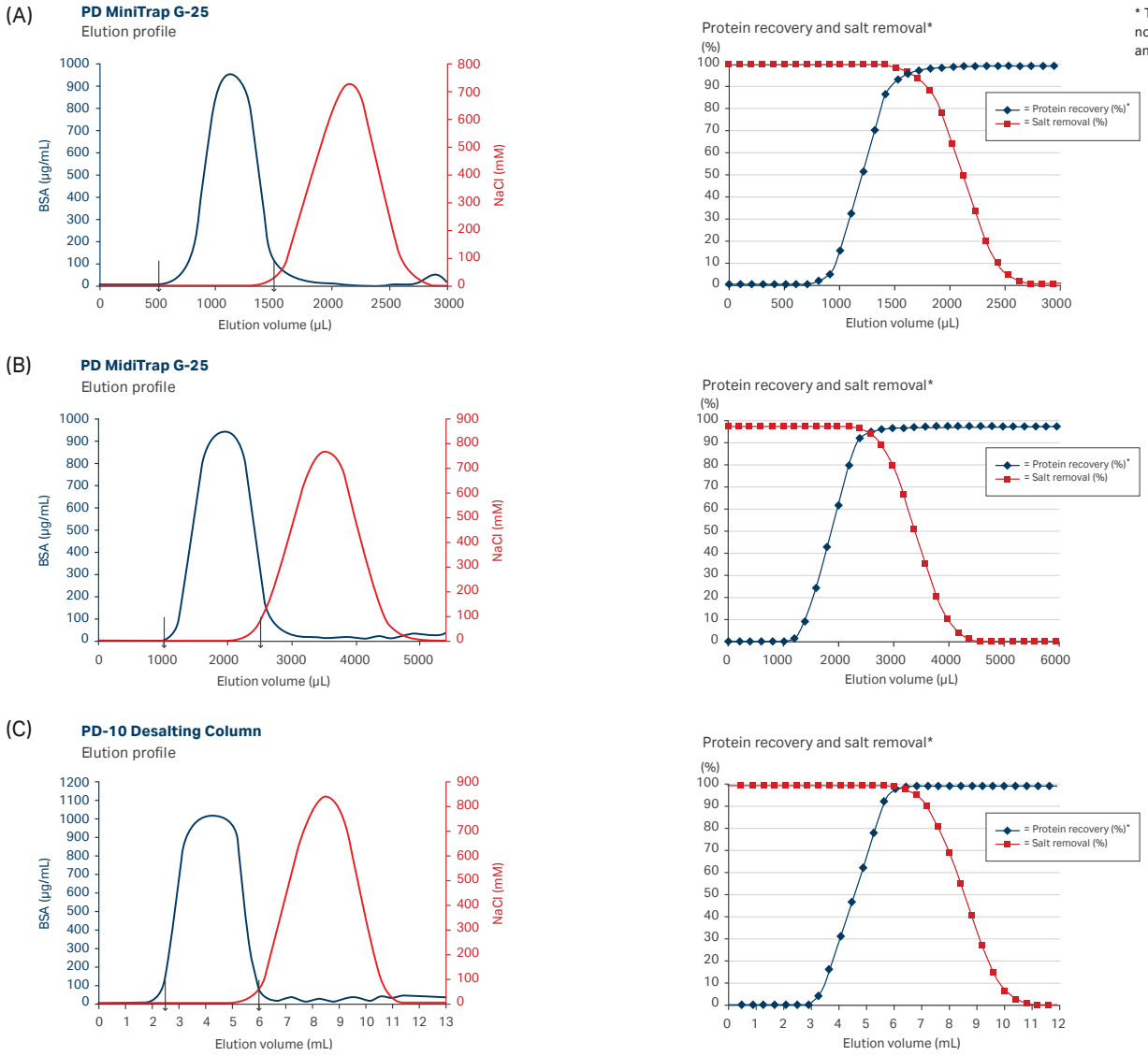
Columns for both gravity and spin protocols

PD-10 Desalting Columns, PD MidiTrap G-25, and PD MiniTrap G-25 provide two possible application protocols, gravity and spin. The typical desalting capacity, for both protocols, is above 90% with recoveries between 70% and 95% (biomolecule dependent).

Gravity protocol

By using the gravity protocol, a simple cleanup of the sample is performed on the lab bench with one or several columns in parallel without the need for a purification system. To simplify the use of PD-10 Desalting Columns with the gravity protocol, the LabMate PD-10 Buffer Reservoir may be used (see *Ordering information*). Using buffer reservoir, wash and equilibration buffers can be applied in one step. Elution profiles for BSA using the gravity protocol are shown in Figure 14.

Columns: (A) PD MiniTrap G-25, (B) PD MidiTrap G-25, and (C) PD-10 Desalting Columns
Sample: 1 mg/mL BSA in 1 M NaCl
Sample volumes: (A) 0.5 mL, (B) 1 mL, and (C) 2.5 mL
Equilibration solution: Milli-Q™ water



* The recovery curves are normalized against the total amount of loaded sample.

Fig 14. Removal of NaCl from BSA using the gravity protocol. The protein recovery (between arrows) was 95% for PD MiniTrap G-25 and PD MidiTrap G-25 (A and B) and > 95% for PD-10 Desalting Columns (C). The desalting capacity was > 98% for PD MidiTrap G-25 (B) and > 99% for PD MiniTrap G-25 and PD-10 Desalting Columns (A and C). The images to the right illustrate the protein recovery and salt removal versus the total elution volume for the used column.

Spin protocol

By using the spin protocol, the samples are run in parallel in a standard centrifuge. The spin protocol gives minimal dilution of the eluted sample. Four adapters are included in each product package to enable easy use of a standard centrifuge (Fig 15). To facilitate increased throughput, a package of 10 adapters can be ordered separately.

Fig 15. Spin adapters are used together with PD-10 Desalting Columns, PD MidiTrap G-25, and PD MiniTrap G-25 to enable use in a standard centrifuge.

Efficient cleanup of carbohydrates before enzymatic cleavage

The PD products prepacked with Sephadex G-25 Medium are excellent for desalting biomolecules other than proteins, for example carbohydrates. To demonstrate this, bovine intestinal ^{3}H-labeled heparan sulfate was eluted from an IEX column using a high salt concentration. The eluate was run on a PD MidiTrap G-25 column before enzymatic cleavage. Due to high peak resolution, the sample was collected with high recovery in a very low concentration of salt. The elution profile is shown in Figure 16.

Column:	PD MidiTrap G-25
Sample:	18 600 cpm bovine intestinal ^{3}H-labeled heparan sulfate ([^{3}H]HS) in 1.5 M NaCl
Sample volume:	0.5 mL
Equilibration solution:	Distilled water

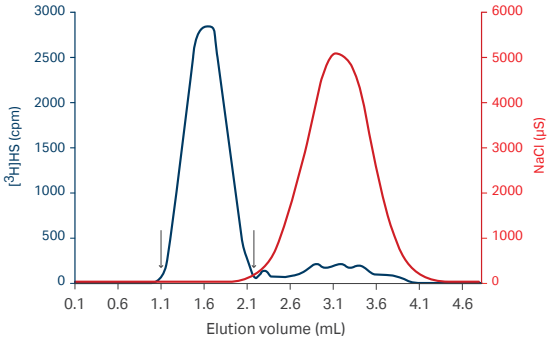


Fig 16. Removal of NaCl from [3H]HS on a PD MidiTrap G-25 column. The fractions were analyzed with regards to conductivity and radioactive content. Fractions 11 through 21 (between arrows) contained 87% of the total recovery of [3H]HS. The recovery volume was 1.1 mL with a NaCl concentration of 20 mM, corresponding to > 98% salt removal.

Conclusions

Sephadex G-25 resins are excellent for desalting of protein, carbohydrate, and DNA preparations. Sephadex G-25 Coarse will generally be the size exclusion chromatography resin of choice for industrial scale. It is especially suitable for processing of large volumes of feed where the requirement for productivity is high. In cases where sample dilution needs to be minimized, the higher resolution of a smaller particle size, for example Sephadex G-25 Medium, might be required.

For laboratory-scale desalting, where less effort needs to be spent on optimization, it is common to use more conservative sample volumes (i.e., a load below 20% of the column volume). For these applications, Sephadex G-25 Medium or Sephadex G-25 Fine can be recommended to minimize the dilution factor. For micropreparative work using very small sample volumes, Sephadex G-25 Superfine will be the resin of choice.

For Sephadex G-25 resins, several different prepacked column and plate formats are available, for which the choice depends on sample volume and existing laboratory equipment.

References

1. Hagel L. and Janson J.-C., Size-exclusion chromatography, in *Chromatography*, 5th edition (E. Heftmann, ed.), Elsevier, Amsterdam, pp. A267–A307 (1992).

Ordering information

Prepacked columns	Quantity	Code number
HiTrap Desalting	1 × 5 mL	29048684
	5 × 5 mL	17140801
	100 × 5 mL	11000329
HiPrep 26/10 Desalting	1 × 53 mL	17508701
	4 × 53 mL	17508702
Bulk resins		
Sephadex G-25 Coarse	100 g	17003401
	500 g	17003402
	5 kg	17003403
	40 kg	17003407
Sephadex G-25 Medium	100 g	17003301
	500 g	17003302
	5 kg	17003303
	40 kg	17003307
Sephadex G-25 Fine	100 g	17003201
	500 g	17003202
	5 kg	17003203
	40 kg	17003207
Sephadex G-25 Superfine	100 g	17003101
	500 g	17003102
	5 kg	17003103
Prepacked disposable products		
PD-10 Desalting Columns	30 columns	17085101
PD SpinTrap G-25	50 columns	28918004
PD MultiTrap G-25	4 × 96-well filter plates	28918006
PD MiniTrap G-25	50 columns	28918007
PD MidiTrap G-25	50 columns	28918008

Accessories	Quantity	Code number
MiniSpin Adapter	10	28923243
MidiSpin Adapter	10	28923244
PD-10 Spin Adapter	10	28923245
Collection plate 500 µl V-bottom (for collection of fractions from MultiTrap)	5 × 96-well filter plates	28403943
LabMate PD-10 Buffer Reservoir	10	18321603
1/16" male/Luer female*	2	18111251
Tubing connector flangeless/M6 female	2	18100368
Tubing connector flangeless/M6 male	2	18101798
Union 1/16" female/M6 male	6	18111257
Union M6 female/1/16" male	5	18385801
Union luerlock female/M6 female	2	18102712
HiTrap/HiPrep, 1/16" male connector for ÄKTA	8	28401081
Stop plug female, 1/16"†	5	11000464
Fingertight stop plug, 1/16"‡	5	11000355

* One connector included in each HiTrap package.
† Two, five, or seven stop plugs female included in HiTrap packages depending on product.
‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature

Handbook: Size Exclusion Chromatography, Principles and Methods	18102218
Selection guide: Size exclusion chromatography columns and resins	18112419
Selection guide: Sample preparation for analysis of proteins, peptides, and carbohydrates	18112862

cytiva.com/protein-purification

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