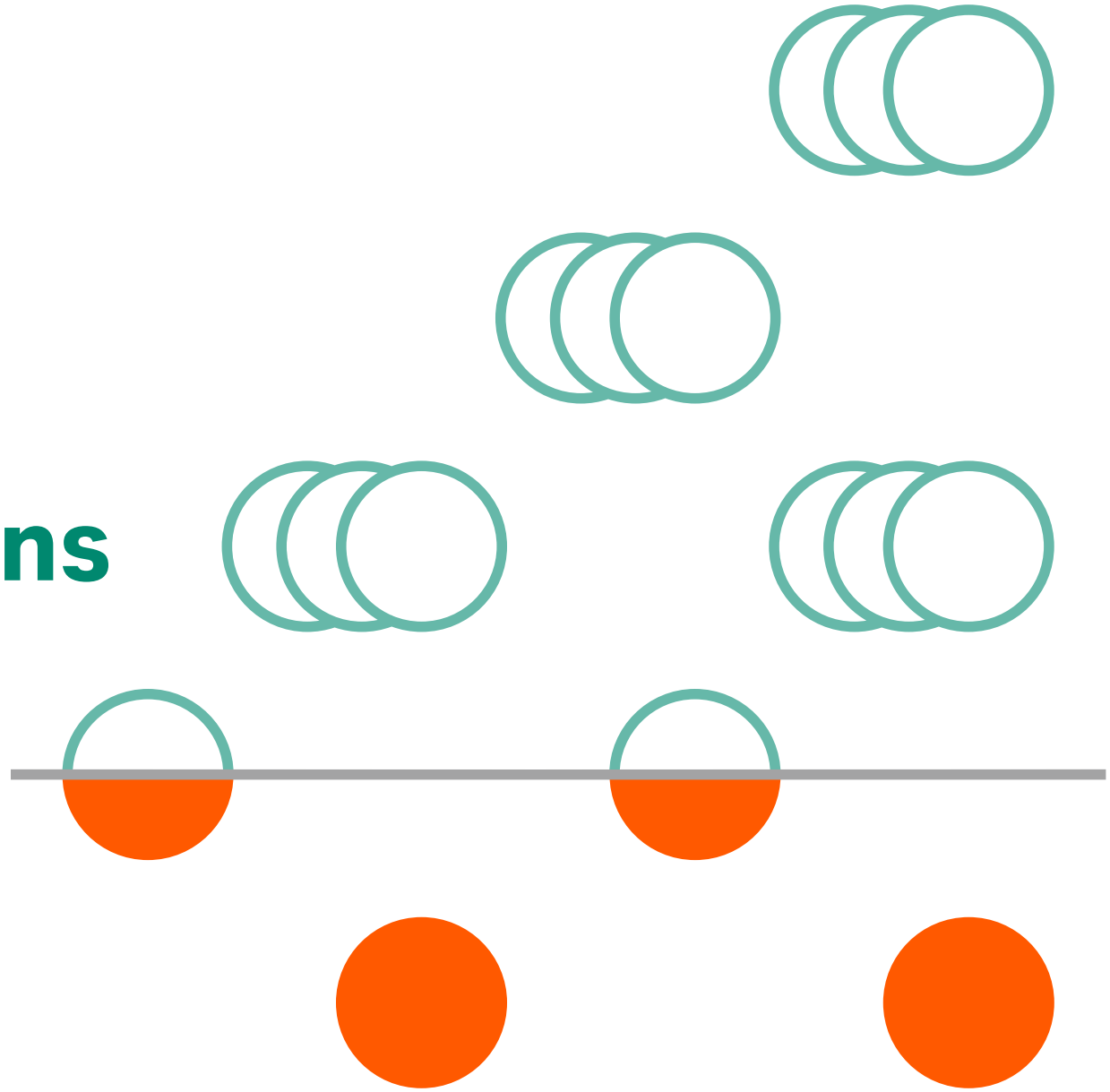
cytiva

Preparative purification of proteins with size exclusion chromatography columns



Content

For direct access, click on your size exclusion chromatography (SEC) topic of interest

When and why should you use SEC

Cytiva SEC columns

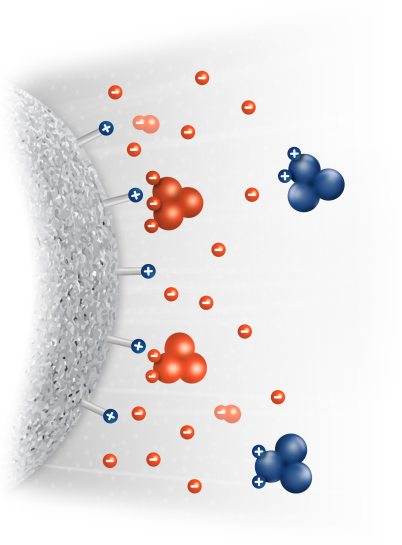
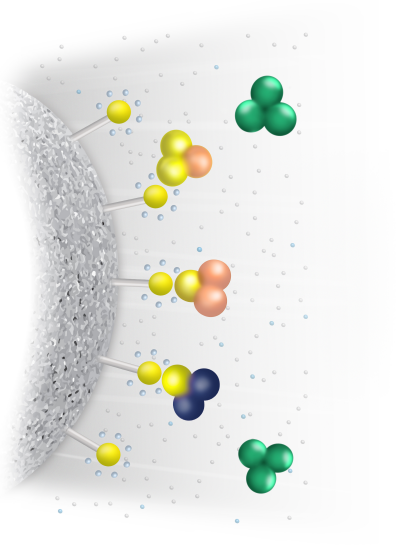
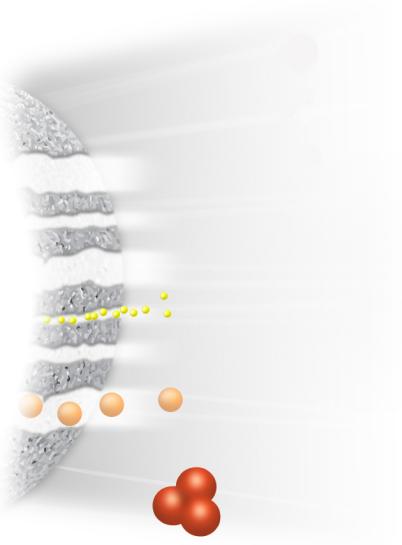
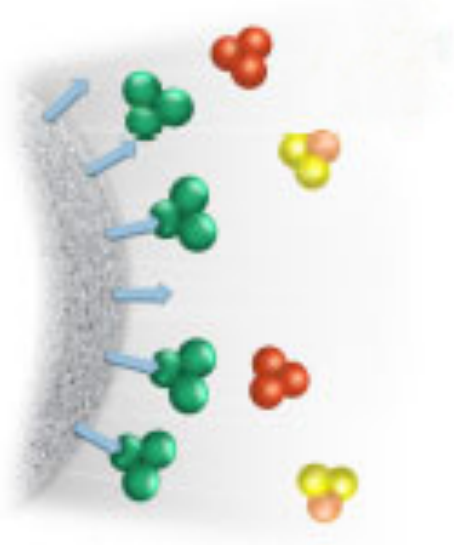
SEC tips, tools, and summary

Appendix

1

SEC fundamentals

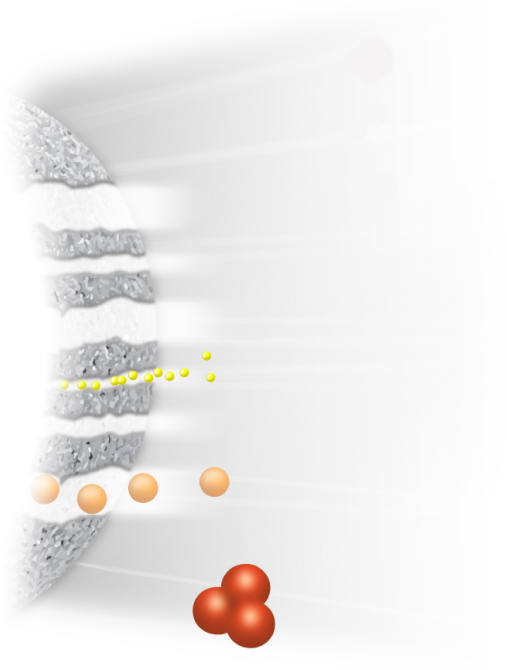
Chromatography techniques commonly used for protein purification

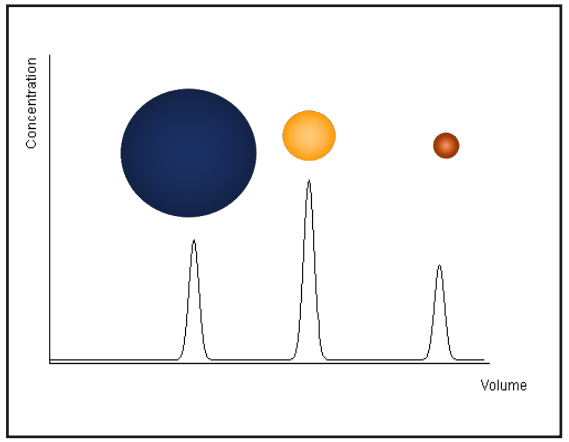
Technique:	Ion exchange Chromatography (IEX)	Hydrophobic interaction Chromatography (HIC)	Size exclusion Chromatography (SEC)	Affinity Chromatography (AC)
				
Separation principle:	**Charge**	**Hydrophobicity**	**Size**	**Biorecognition**

Chromatography techniques enable separation of proteins based on differences in specific properties

Principles of SEC

Separates molecules based on size





Largest molecules elute first

Characteristics

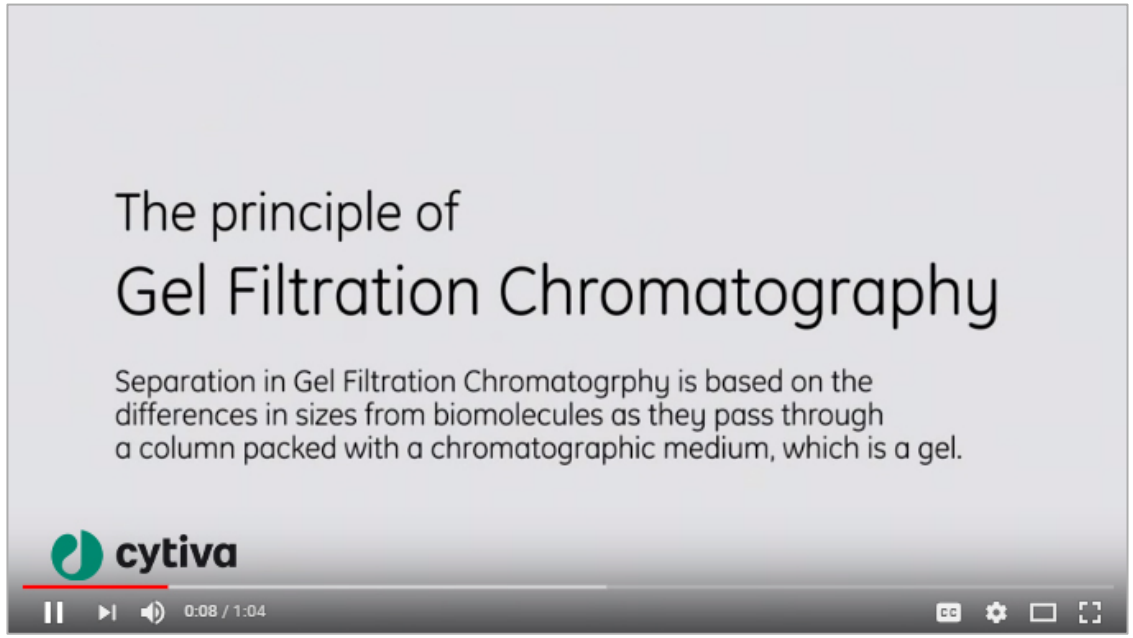
- Nonbinding technique — the separation takes place in only 1 column volume (CV)
- Mild conditions — good for sensitive biomolecules
- Any buffer can be used
- Limited in sample volume
- By nature a slow technique

A well-packed SEC column is critical for high-resolution separations

More on SEC animation and Cytiva handbook

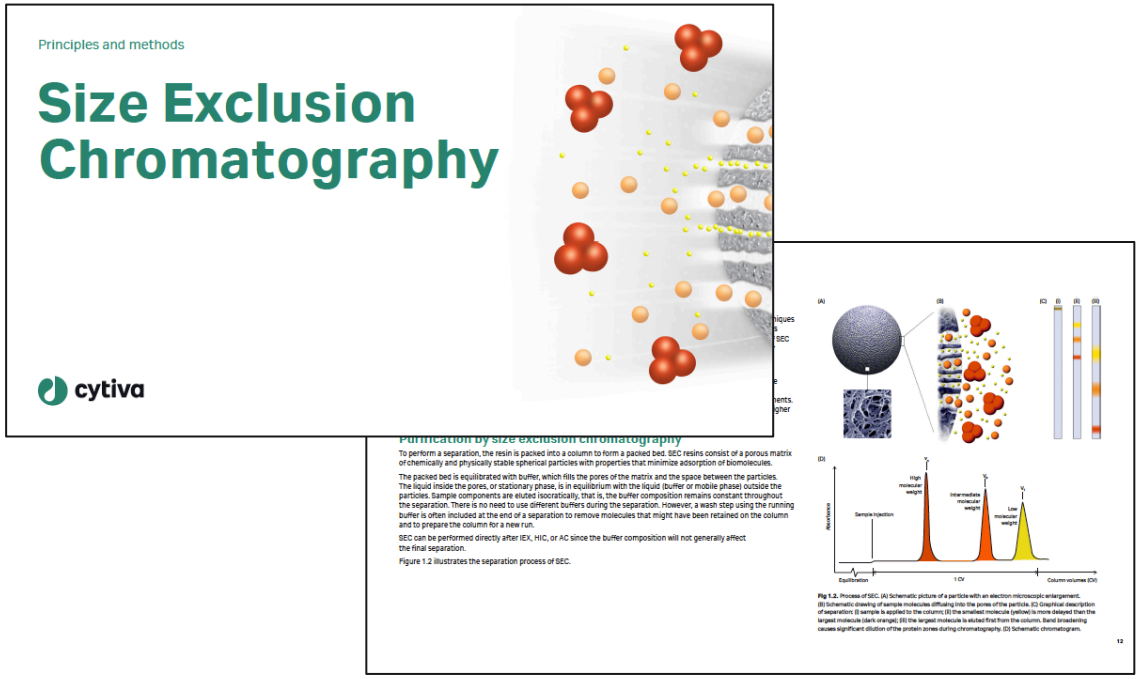
Watch the video on YouTube

The principle of gel filtration (size exclusion chromatography)



Download the handbook

Size Exclusion Chromatography Principles and Methods

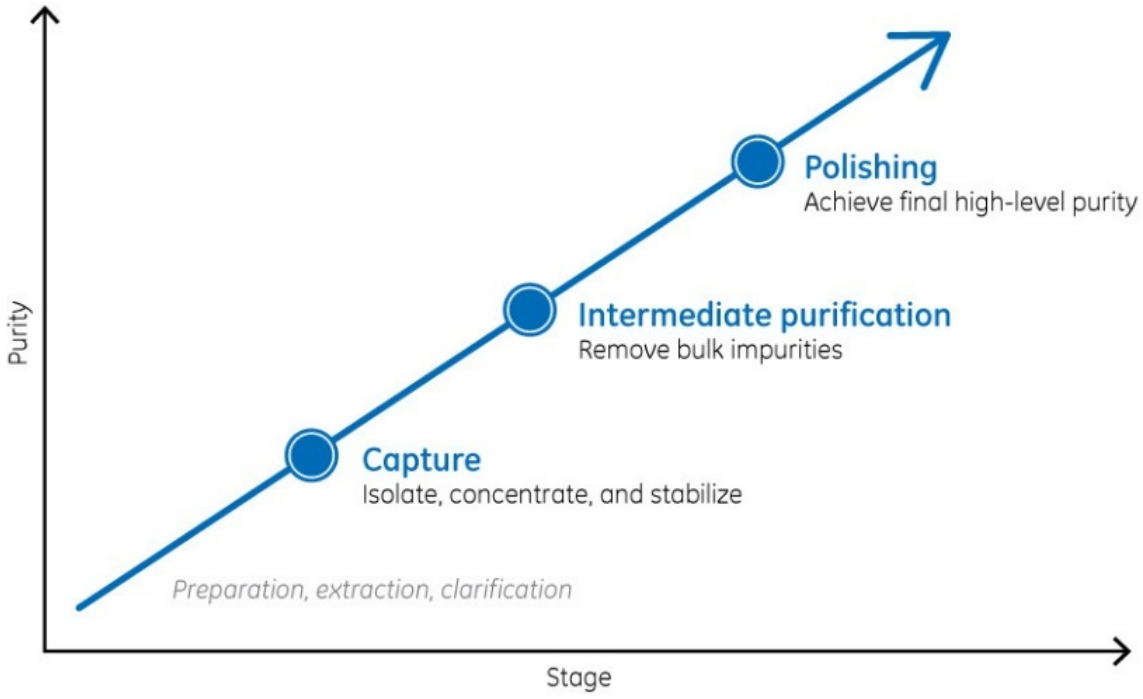


2

Why use SEC in a protein purification protocol?

The CiPP model for simplified protein purification planning

Capture, intermediate Purification, and Polishing (CiPP)



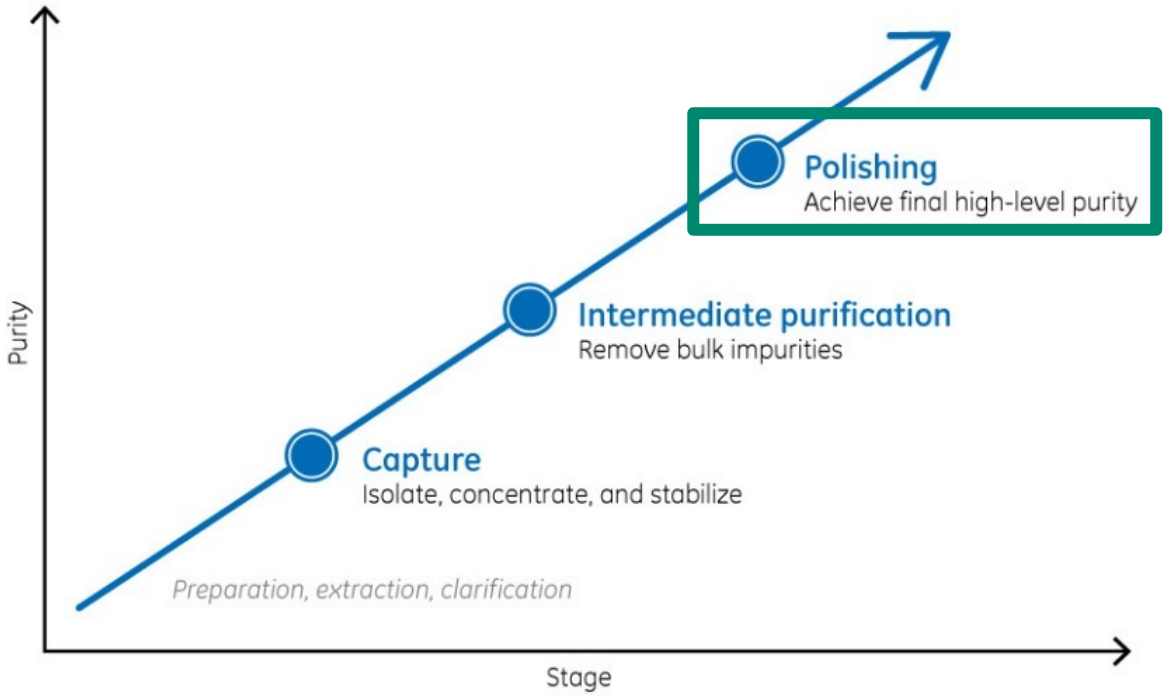
The initial capture stage isolates, concentrates, and stabilizes the target protein.

Intermediate purification removes bulk contaminants.

The final polishing step removes the most difficult impurities, such as aggregates or isoforms of the target protein.

SEC is widely used for polishing

Capture, intermediate Purification, and Polishing (CiPP)

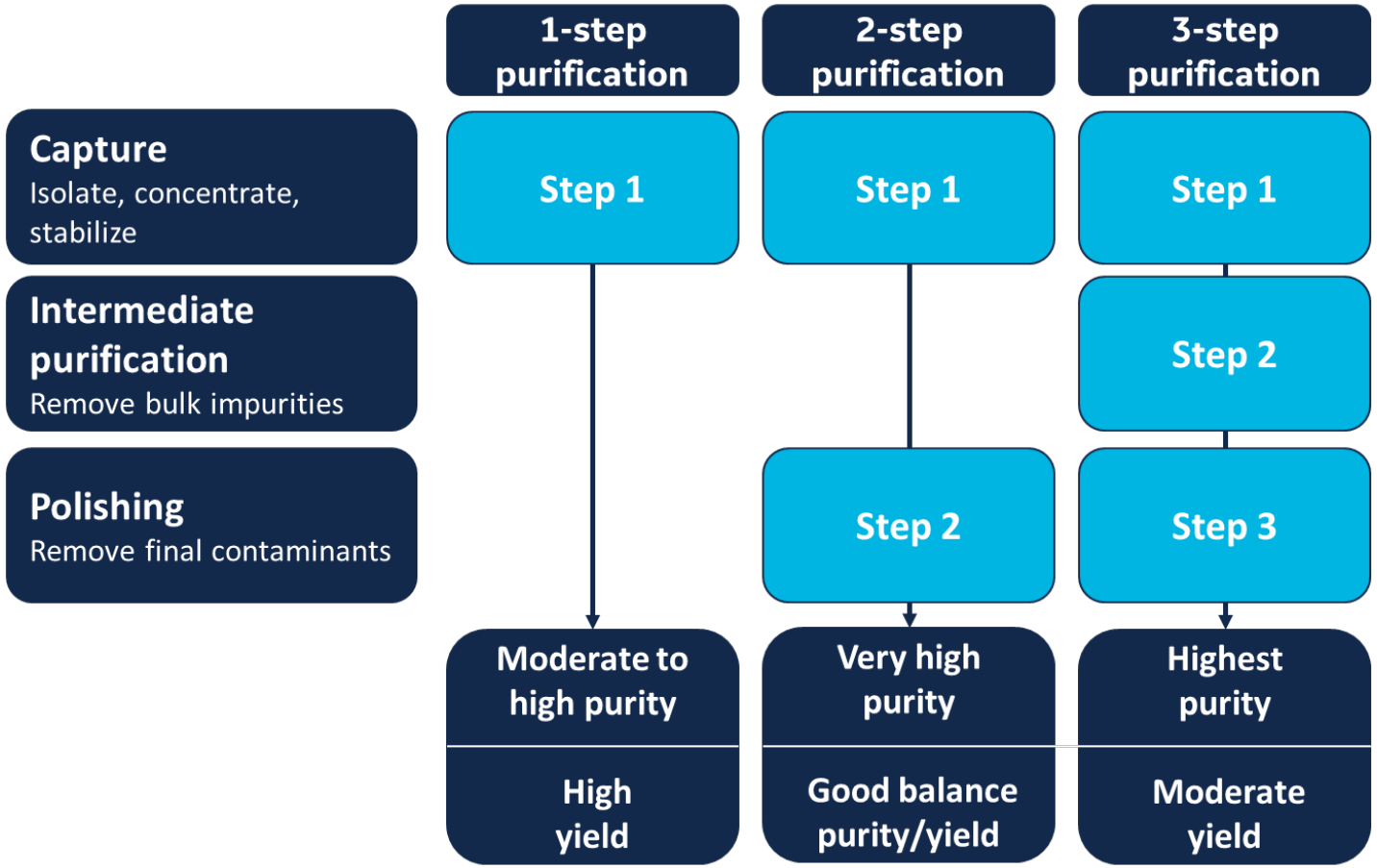


SEC is widely used for polishing:

- It effectively removes dimers and aggregates of the target protein
- The target protein will be size homogeneous

SEC also simultaneously enables the transfer of the target protein to the buffer of choice.

How many chromatography steps should be used in a purification protocol?

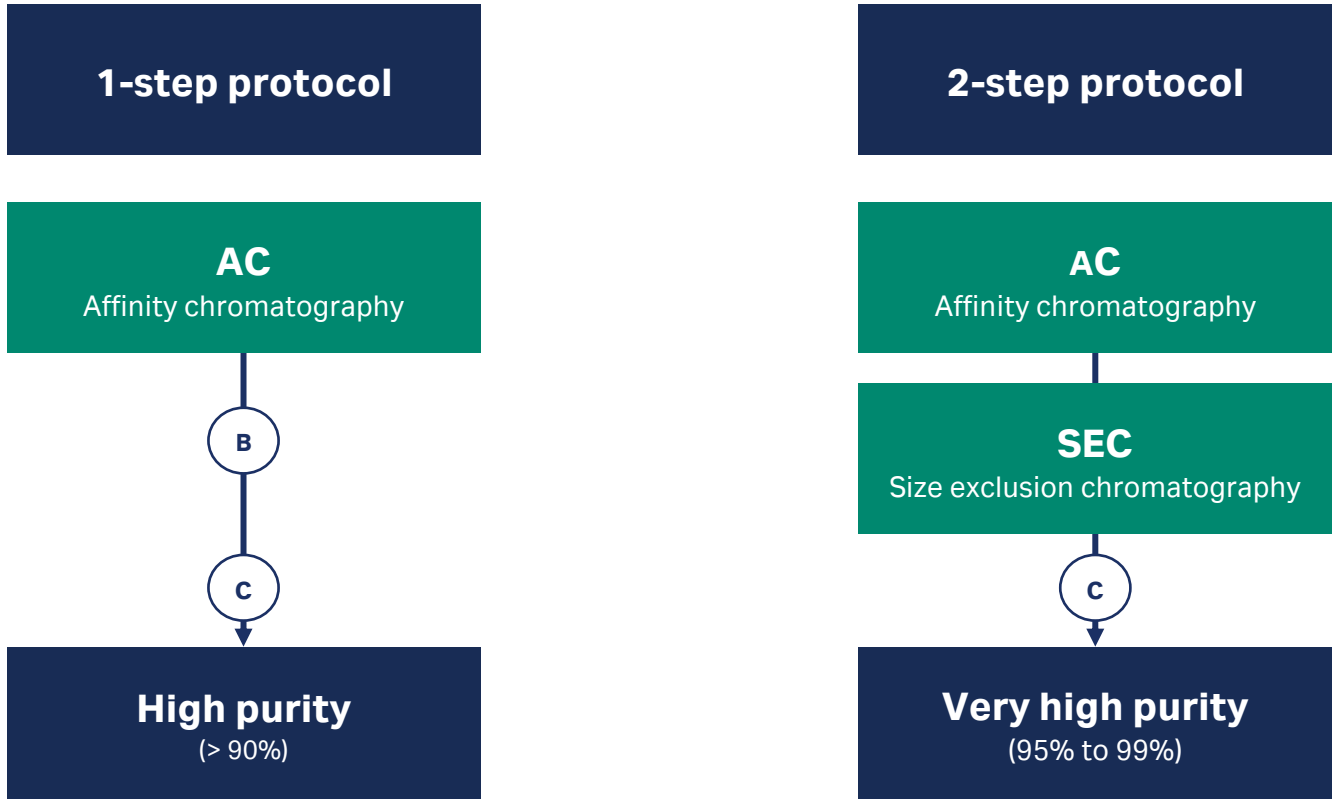


The number of steps to be included will depend on the purity requirements and intended use of the protein.

Addition of chromatography steps will increase purity at the cost of decreased yield of active protein.

Learn more about how to combine chromatography techniques

Use of SEC to improve antibody purity



After the first affinity chromatography step that isolates the antibody from initial sample, a SEC step will remove antibody aggregates and/or fragments to obtain monomeric antibodies.

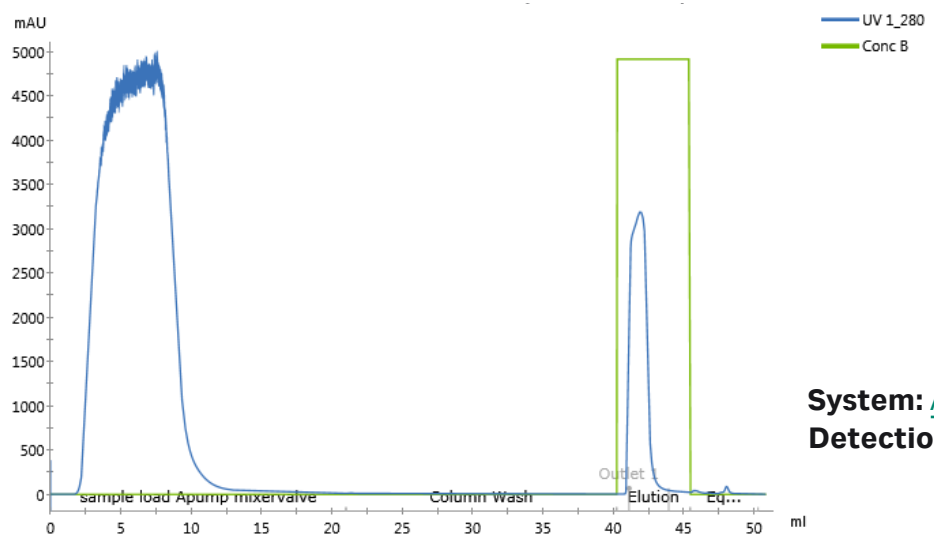
After SEC, the purity is very high: 95% to 99%.

B = buffer exchange to neutralize low pH Ab elution buffer
C = concentration for sample volume reduction. May also be performed before SEC.

Use of SEC to improve antibody purity

The addition of SEC removed antibody aggregates to obtain monomeric antibodies

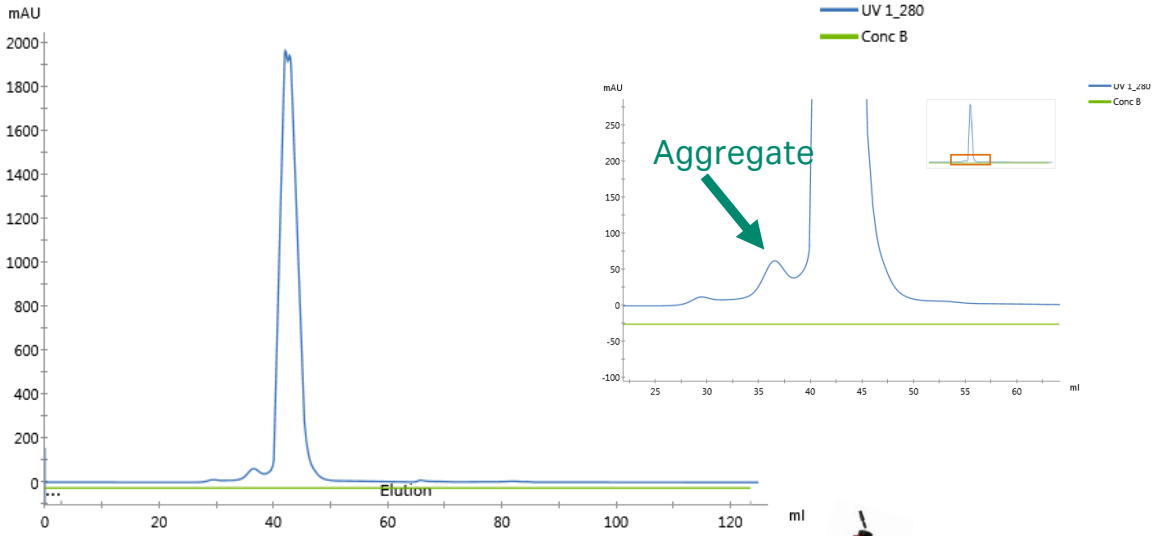
Step 1 = AC



System: ÄKTA™ pure 25
Detection: A_{280}

Column: HiTrap™ MabSelect™ PrismA 1 mL
Binding buffer: 20 mM phosphate, 150 mM NaCl pH 7.4,
Elution buffer: 50 mM sodium acetate pH 3.5
Sample: 6 mL of supernatant containing polyclonal human IgG
Flow rate: 0.5 mL/min

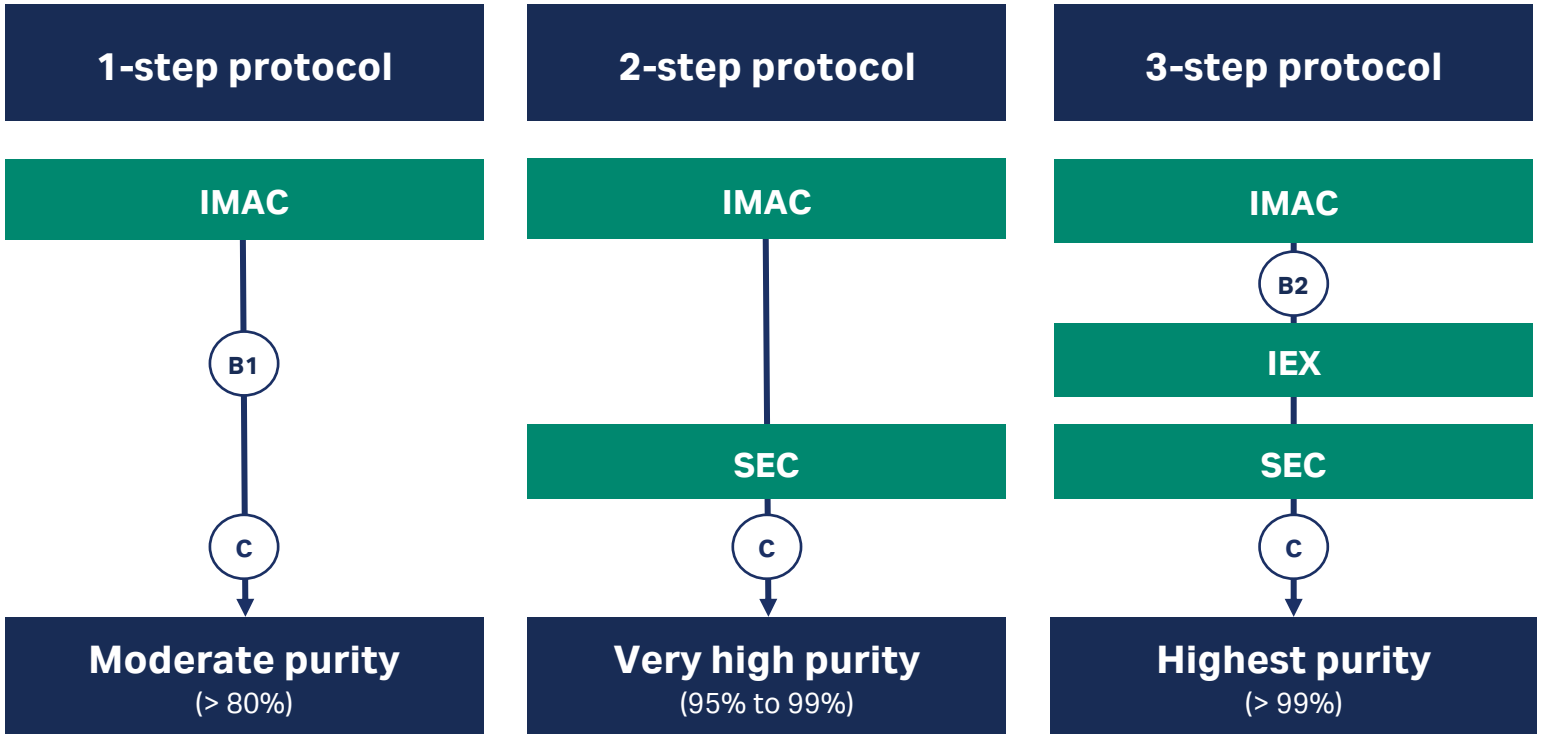
Step 2 = SEC



Column: HiScale™ 16/40 Superdex 200 Increase[1]
Buffer: 20 mM phosphate, 150 mM NaCl pH 7.4
Sample: 3 mL of eluate from HiTrap MabSelect PrismA (supernatant containing polyclonal human IgG)
Flow rate: 1 mL/min

[1] These columns are produced on-demand.

Use of SEC to improve antibody purity



A single IMAC step delivers a moderate protein purity (> 80%).

Whether you choose a 2-step or 3-step protocol, SEC will be used as a last step for removal of remaining impurities.

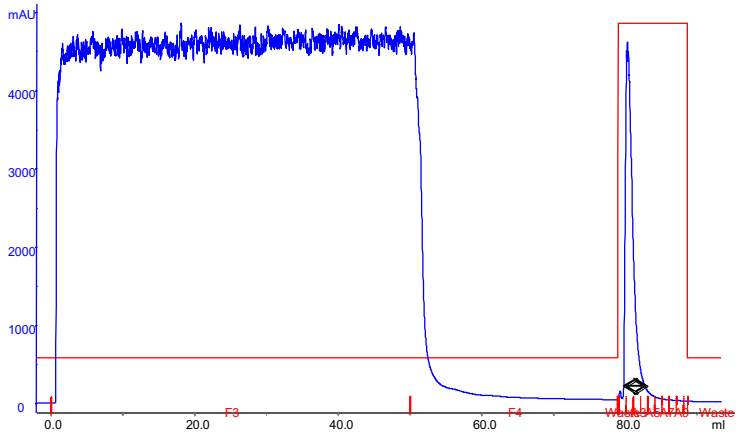
In the 3-step purification protocol, IEX enables removal of impurities such as HCP.

B1 = buffer exchange to remove imidazole or salts
B2 = buffer exchange to prepare for IEX
C = concentration for sample volume reduction. May also be performed before SEC.
HCP = host-cell proteins

Use of SEC to improve purity of his-tagged proteins

The addition of a SEC step removes impurities such as truncated forms and aggregates of your target protein

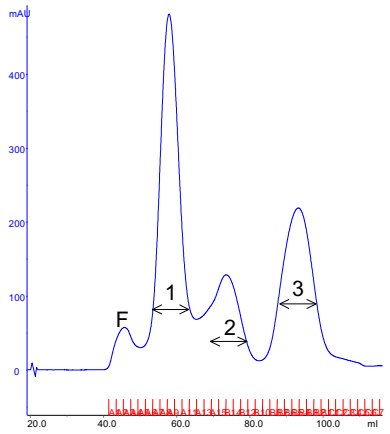
Step 1 = IMAC



Column: HisTrap™ FF 1 mL

Sample: 50 mL of $(his)_{10}$-Trx-P 450 in *E. coli* lysate

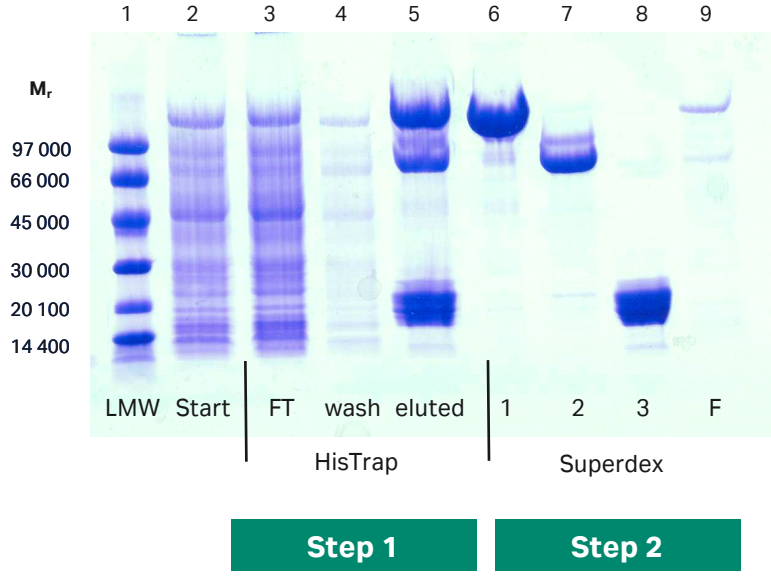
Step 2 = SEC



Column: HiLoad™ 16/60 Superdex™ 200 pg

Sample: 5.2 mL of eluted pool from HisTrap FF

SDS-PAGE analysis

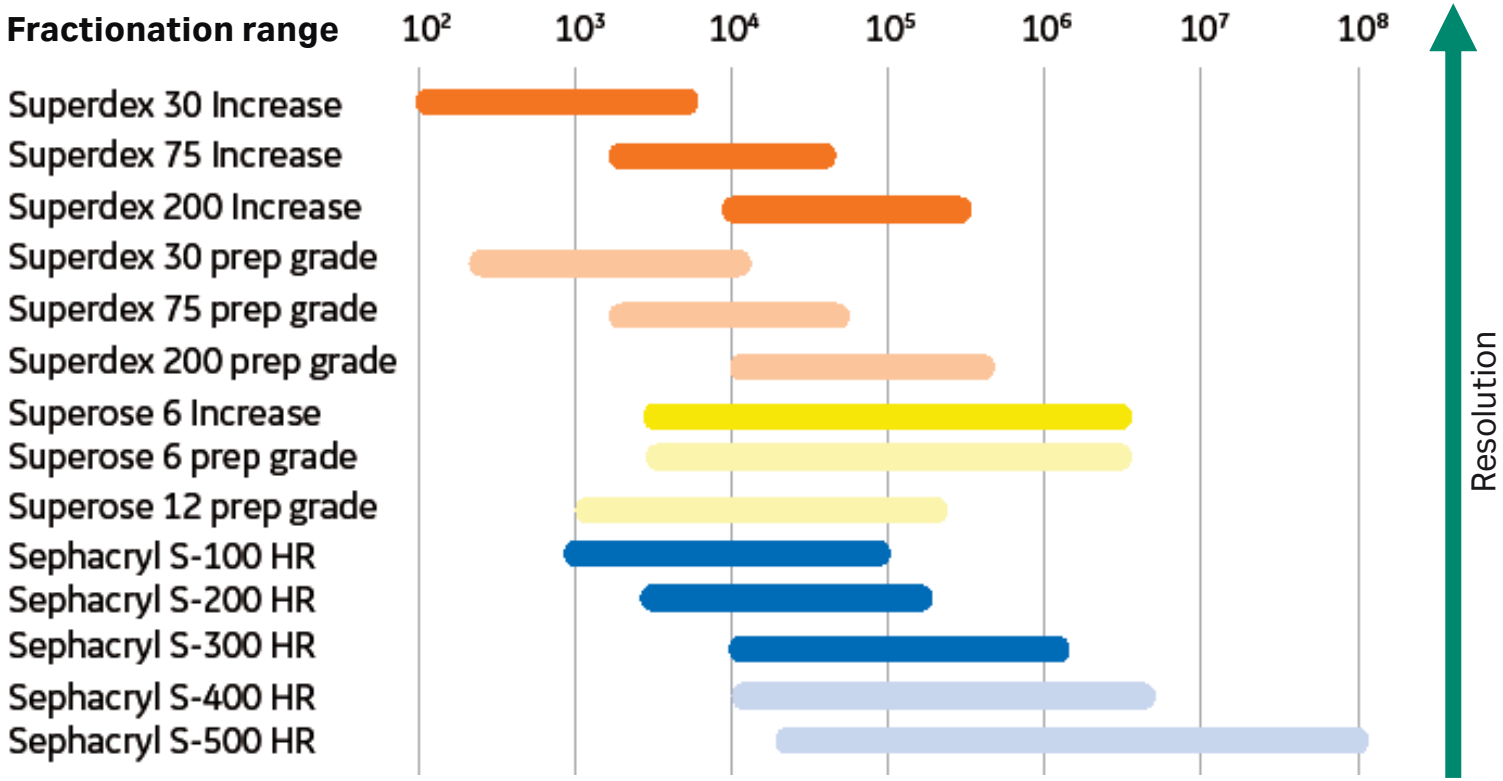


3

How to select the most suitable Cytiva SEC column for your protein purification

Cytiva offers prepacked SEC columns for user convenience and reproducible results

For a broad range of biomolecules and resolutions



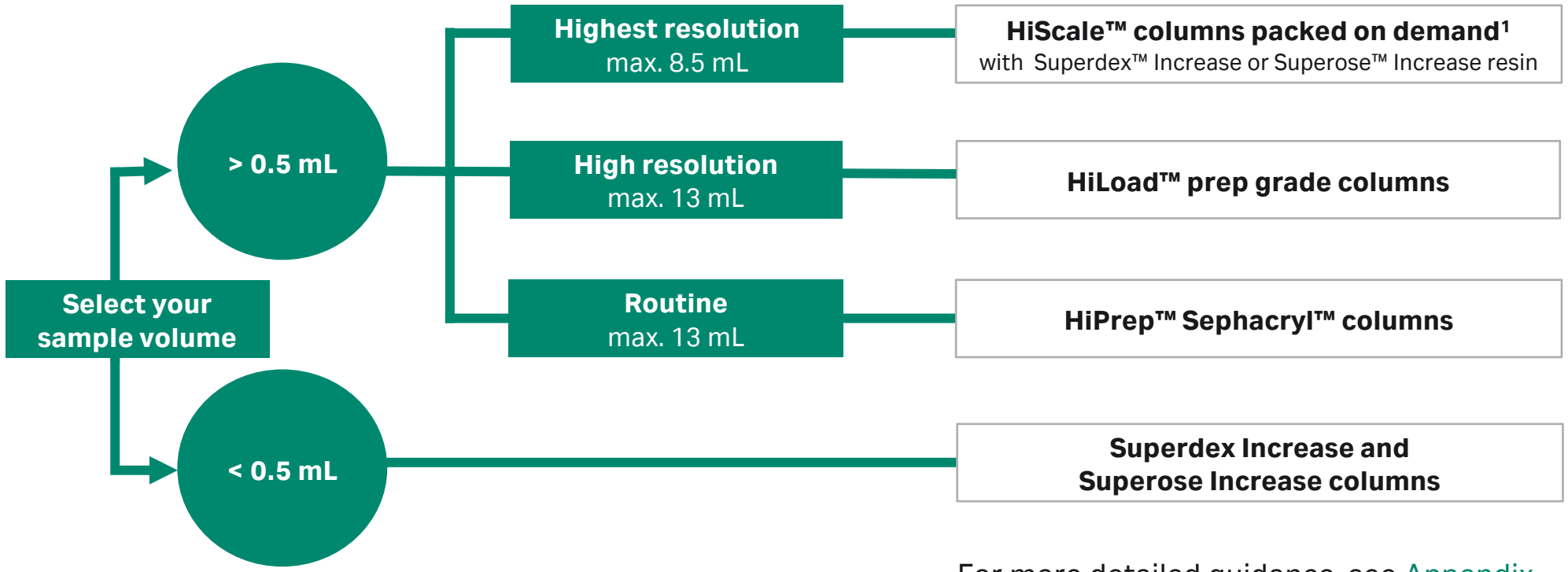
Colored bars indicate the fractionation range for each resin

How to select the best SEC column for a specific application

- Choose the resin that has a fractionation range where the target molecule falls in the middle of the range
- If contaminants are close in size to the target molecule, choose a resin with higher resolution
- Choose column type depending on the sample volume that should be applied

General guidelines for SEC column selection

For more information, click on the column of your interest



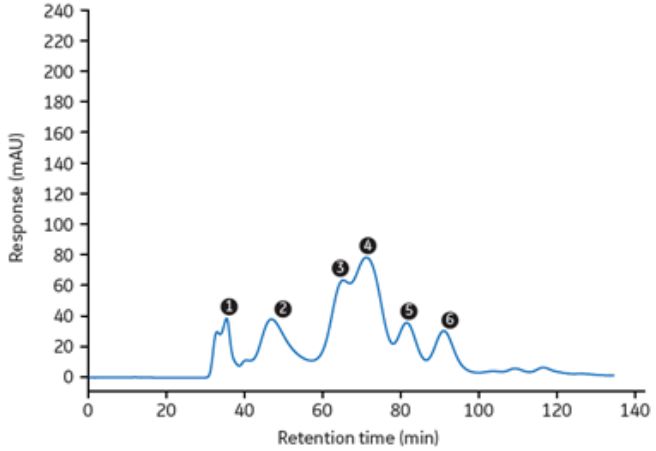
[1]These columns are produced on-demand. Contact your Cytiva representative

For more detailed guidance, see Appendix

For volumes > 0.5 mL, the choice of column depends on the resolution needed

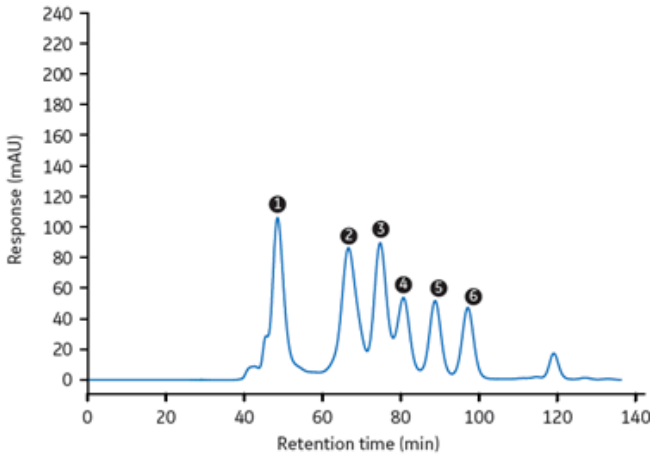
For good resolution: HiPrep™ Sephacryl™

HiPrep 16/60 Sephacryl S-300 HR
M_r range ~ 10 000–1 500 000



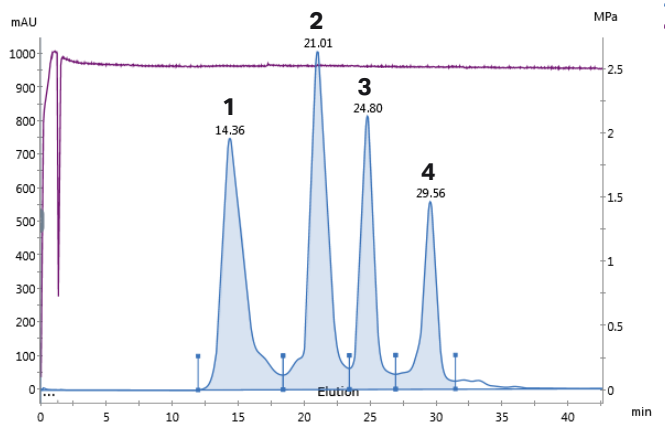
For high resolution: HiLoad™ Superdex™/Superose™ pg

HiLoad 16/600 Superdex 200 pg
M_r range ~ 10 000–600 000



For highest resolution: HiScale™ with "Increase" resin

Superdex 200 Increase HiScale 16/40
M_r range ~ 10 000–600 000



Highest resolution

Protein mix (HiPrep Sephacryl + HiLoad Superdex)

1. Ferritin (M_r 440 000)
2. Aldolase (M_r 158 000)
3. Conalbumin (M_r 75 000)
4. Ovalbumin (M_r 44 000)
5. Carbonic anhydrase (M_r 29 000)
6. Ribonuclease (M_r 13 700)

Running conditions	HiPrep Sephacryl and HiLoad Superdex pg	Superdex Increase HiScale
Sample volume	0.5 mL	1.6 mL
Flow rate	1 mL/min	2 mL/min

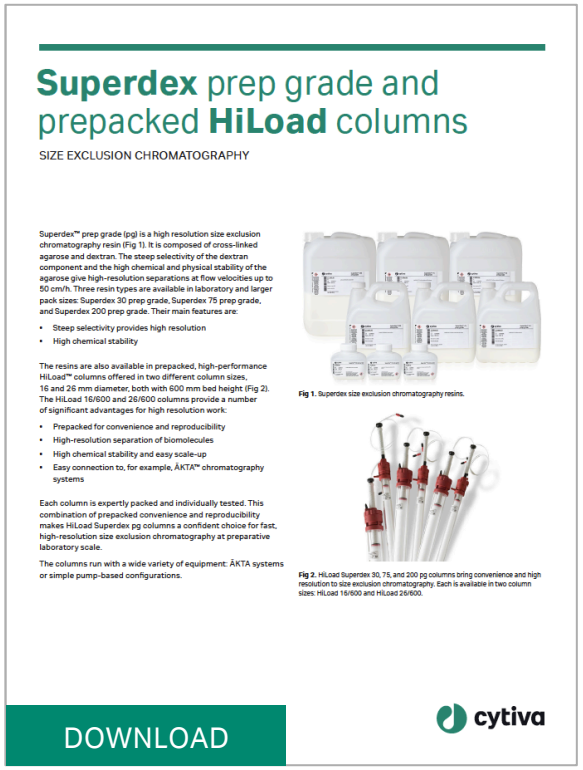
Protein mix (HiScale Superdex Increase)

1. Thyroglobulin (M_r 669 000)
2. Aldolase (M_r 158 000)
3. Ovalbumin (M_r 44 000)
4. Ribonuclease (M_r 13 700)

Data files available for download: sample volume > 0.5 mL

Click on the document of interest

HiLoad™ Superdex™ columns



Superdex prep grade and prepacked **HiLoad** columns

SIZE EXCLUSION CHROMATOGRAPHY

Superdex™ prep grade (pg) is a high resolution size exclusion chromatography resin (Fig 1). It is composed of cross-linked agarose and dextran. The steep selectivity of the dextran component and the high chemical and physical stability of the agarose give high-resolution separations at flow velocities up to 50 cm/h. Three resin types are available in laboratory and larger pack sizes: Superdex 30 prep grade, Superdex 75 prep grade, and Superdex 200 prep grade. Their main features are:

- Steep selectivity provides high resolution
- High chemical stability

The resins are also available in prepacked, high-performance HiLoad™ columns offered in two different column sizes, 16 and 26 mm diameter, both with 600 mm bed height (Fig 2). The HiLoad 16/600 and 26/600 columns provide a number of significant advantages for high resolution work:

- Prepacked for convenience and reproducibility
- High-resolution separation of biomolecules
- High chemical stability and easy scale-up
- Easy connection to, for example, ÄKTA™ chromatography systems

Each column is expertly packed and individually tested. This combination of prepacked convenience and reproducibility makes HiLoad Superdex pg columns a confident choice for fast, high-resolution size exclusion chromatography at preparative laboratory scale.

The columns run with a wide variety of equipment: ÄKTA systems or simple pump-based configurations.

Fig 1. Superdex size exclusion chromatography resins.

Fig 2. HiLoad Superdex 30, 75, and 200 pg columns bring convenience and high resolution to size exclusion chromatography. Each is available in two column sizes: HiLoad 16/600 and HiLoad 26/600.

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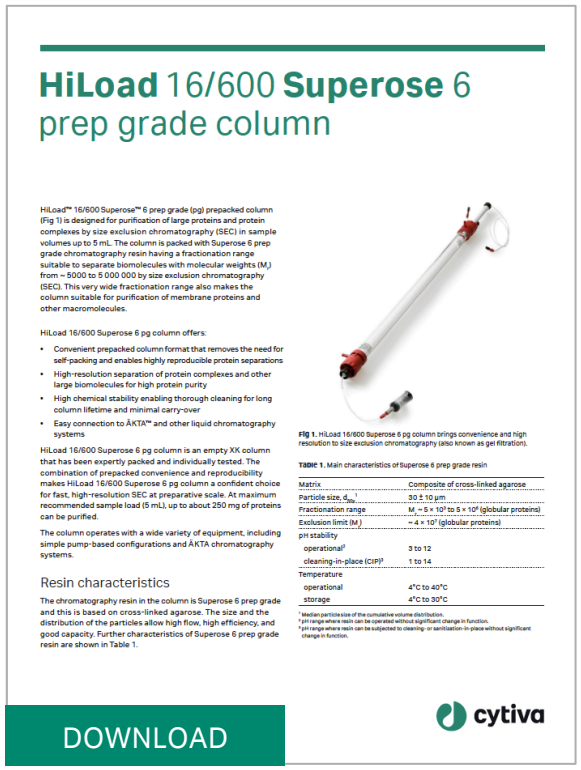
HiLoad 16/600 Superose™ 6 pg



HiLoad 16/600 **Superose** 6 prep grade column

HiLoad™ 16/600 Superose™ 6 prep grade (pg) prepacked column (Fig 1) is designed for purification of large proteins and protein complexes by size exclusion chromatography (SEC) in sample volumes up to 5 mL. The column is packed with Superose 6 prep grade chromatography resin having a fractionation range suitable to separate biomolecules with molecular weights (M_r) from ~ 5000 to 5 000 000 by size exclusion chromatography (SEC). This very wide fractionation range also makes the column suitable for purification of membrane proteins and other macromolecules.

HiLoad 16/600 Superose 6 pg column offers:

- Convenient prepacked column format that removes the need for self-packing and enables highly reproducible protein separations
- High-resolution separation of protein complexes and other large biomolecules for high protein purity
- High chemical stability enabling thorough cleaning for long column lifetime and minimal carry-over
- Easy connection to ÄKTA™ and other liquid chromatography systems

HiLoad 16/600 Superose 6 pg column is an empty XK column that has been expertly packed and individually tested. The combination of prepacked convenience and reproducibility makes HiLoad 16/600 Superose 6 pg column a confident choice for fast, high-resolution SEC at preparative scale. At maximum recommended sample load (5 mL), up to about 250 mg of proteins can be purified.

The column operates with a wide variety of equipment, including simple pump-based configurations and ÄKTA chromatography systems.

Resin characteristics

The chromatography resin in the column is Superose 6 prep grade and this is based on cross-linked agarose. The size and the distribution of the particles allow high flow, high efficiency, and good capacity. Further characteristics of Superose 6 prep grade resin are shown in Table 1.

Fig 1. HiLoad 16/600 Superose 6 pg column brings convenience and high resolution to size exclusion chromatography (also known as gel filtration).

Table 1. Main characteristics of Superose 6 prep grade resin

Matrix	Composite of cross-linked agarose
Particle size, d_{50w} [1]	30 ± 10 µm
Fractionation range	M_r ~5 × 10^3 to 5 × 10^6 (globular proteins)
Exclusion limit (M_r)	~4 × 10^7 (globular proteins)
pH stability	
operational[2]	3 to 12
cleaning-in-place (CIP)[3]	1 to 14
Temperature	
operational	4°C to 40°C
storage	4°C to 30°C

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

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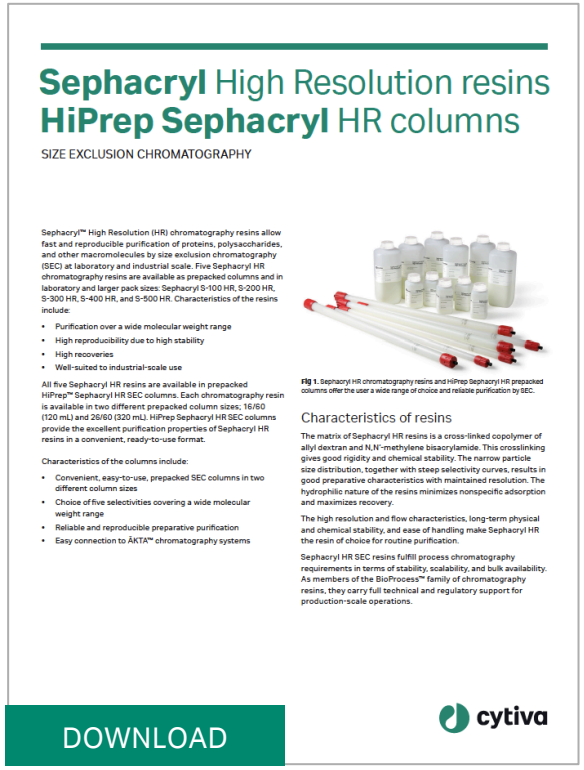
HiPrep™ Sephacryl™ columns



Sephacryl High Resolution resins **HiPrep Sephacryl** HR columns

SIZE EXCLUSION CHROMATOGRAPHY

Sephacryl™ High Resolution (HR) chromatography resins allow fast and reproducible purification of proteins, polysaccharides, and other macromolecules by size exclusion chromatography (SEC) at laboratory and industrial scale. Five Sephacryl HR chromatography resins are available as prepacked columns and in laboratory and larger pack sizes: Sephacryl S-100 HR, S-200 HR, S-300 HR, S-400 HR, and S-500 HR. Characteristics of the resins include:

- Purification over a wide molecular weight range
- High reproducibility due to high stability
- High recoveries
- Well-suited to industrial-scale use

All five Sephacryl HR resins are available in prepacked HiPrep™ Sephacryl HR SEC columns. Each chromatography resin is available in two different prepacked column sizes; 16/60 (120 mL) and 26/60 (320 mL). HiPrep Sephacryl HR SEC columns provide the excellent purification properties of Sephacryl HR resins in a convenient, ready-to-use format.

Characteristics of the columns include:

- Convenient, easy-to-use, prepacked SEC columns in two different column sizes
- Choice of five selectivities covering a wide molecular weight range
- Reliable and reproducible preparative purification
- Easy connection to ÄKTA™ chromatography systems

Fig 1. Sephacryl HR chromatography resins and HiPrep Sephacryl HR prepacked columns offer the user a wide range of choice and reliable purification by SEC.

Characteristics of resins

The matrix of Sephacryl HR resins is a cross-linked copolymer of allyl dextran and N,N'-methylene bisacrylamide. This crosslinking gives good rigidity and chemical stability. The narrow particle size distribution, together with steep selectivity curves, results in good preparative characteristics with maintained resolution. The hydrophilic nature of the resins minimizes nonspecific adsorption and maximizes recovery.

The high resolution and flow characteristics, long-term physical and chemical stability, and ease of handling make Sephacryl HR the resin of choice for routine purification.

Sephacryl HR SEC resins fulfill process chromatography requirements in terms of stability, scalability, and bulk availability. As members of the BioProcess™ family of chromatography resins, they carry full technical and regulatory support for production-scale operations.

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Data files available for download: sample volume < 0.5 mL

Click on the document of interest

Superdex™ 30 Increase >>


Superdex 30 Increase columns
SIZE EXCLUSION CHROMATOGRAPHY
Resin characteristics
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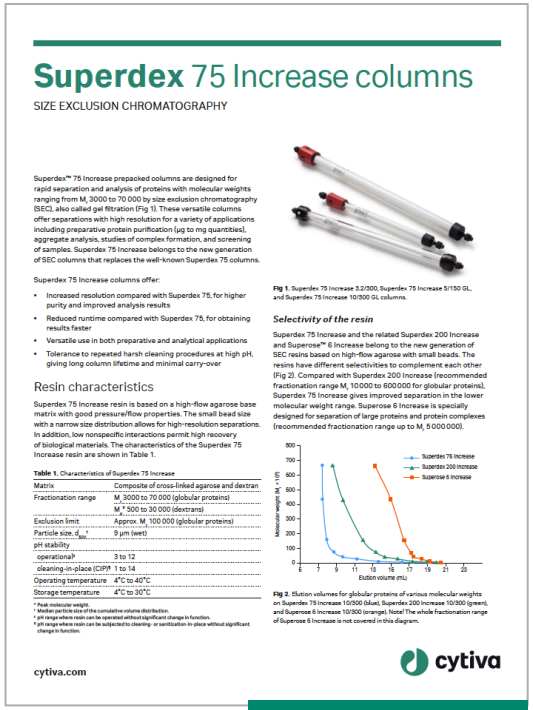
Superdex 75 Increase >>


Superdex 75 Increase columns
SIZE EXCLUSION CHROMATOGRAPHY
Selectivity of the resin
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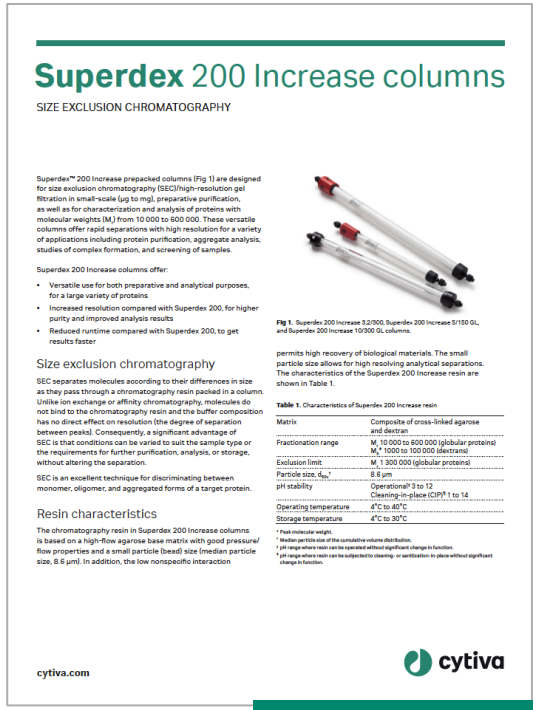
Superdex 200 Increase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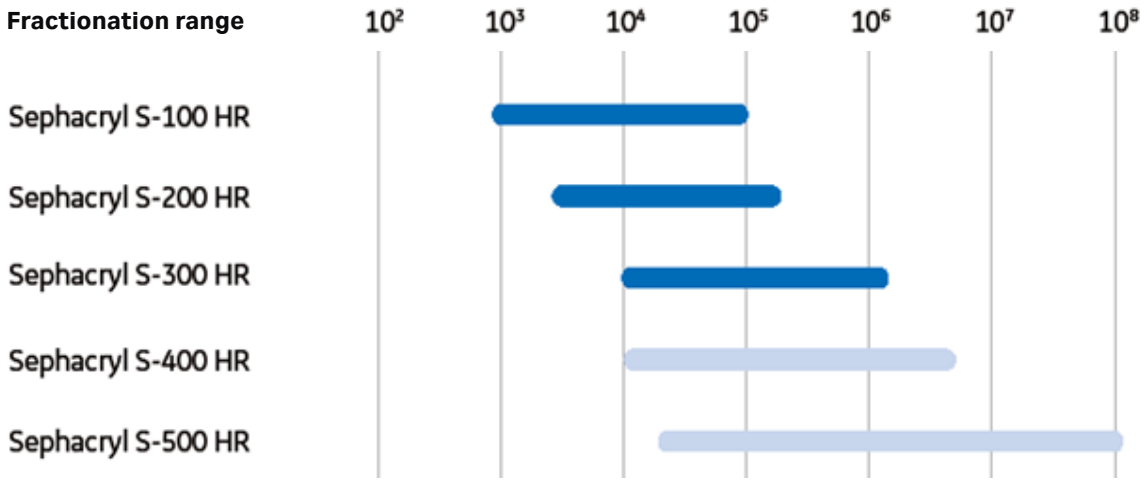
4

HiPrep Sephacryl SEC columns

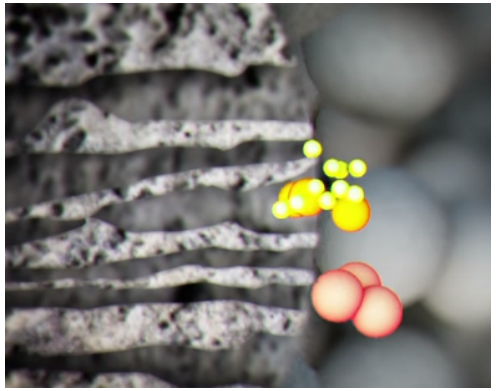
HiPrep Sephacryl columns deliver good resolution over a broad fractionation range for routine SEC

- **Five different fractionation ranges**
- **Two column dimensions**
- **Sample volumes up to 5 mL and 13 mL**



Good price/resolution compromise!

Sephacryl resins technical specifications

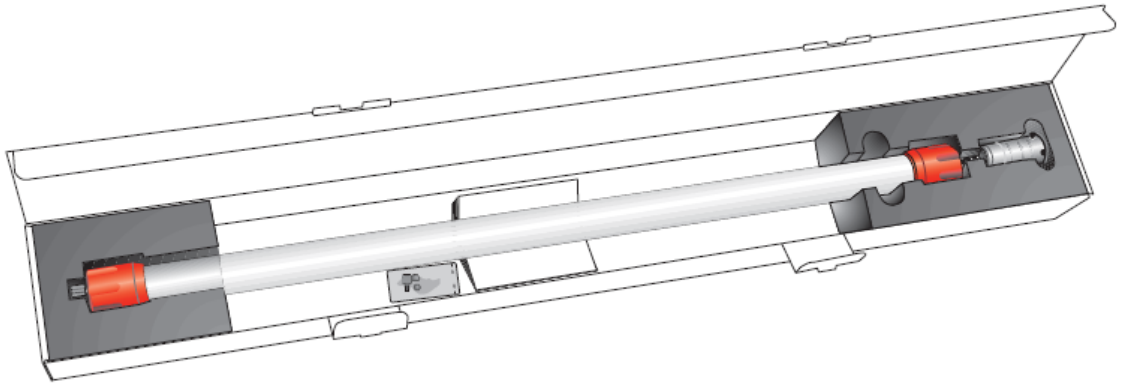


- **Matrix:** Cross-linked copolymer of allyl dextran and N,N-Methylene bisacrylamide
- **Particle size, d_{50V}[1]:** ~ 50 µm
- **pH stability, operational[2]**: 3 to 11
- **pH stability, CIP[3]:** 2 to 13

Fractionation range/Resin	Sephacryl™ S-100 HR	Sephacryl S-200 HR	Sephacryl S-300 HR	Sephacryl S-400 HR	Sephacryl S-500 HR
Fractionation range (M_r) Globular proteins	1000 to 100 000	5000 to 250 000	10 000 to 1 500 000	20 000 to 8 000 000	No data
Fractionation range (M_p) Dextrans	No data	1000 to 80 000	2000 to 400 000	10 000 to 2 000 000	40 000 to 20 000 000

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function.

Technical specifications — HiPrep prepacked columns



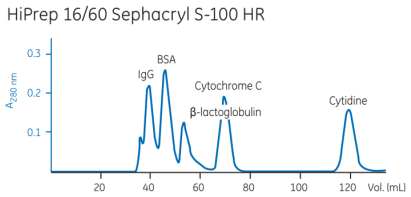
- **Max. pressure over the packed bed during operation:** 0.15 MPa, 1.5 bar, 22 psi
- **Column hardware pressure limit:** 0.5 MPa, 5 bar, 73 psi

Parameter	HiPrep™ 16/60	HiPrep 26/60
Bed dimensions	16 mm × 600 mm	26 mm × 600 mm
Approximate bed volume	120 mL	320 mL
Recommended sample volume	Up to 5 mL	Up to 13 mL
Recommended operating flow rate	0.5 mL/min	1.3 mL/min
Max. operating flow rate	1.0 mL/min	2.7 mL/min

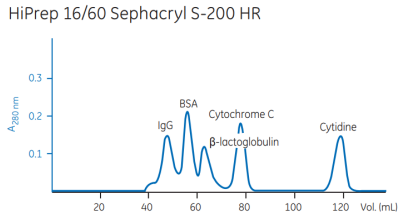
All different Sephacryl™ resins are available in both column sizes

Comparing separations on the different Sephacryl resins

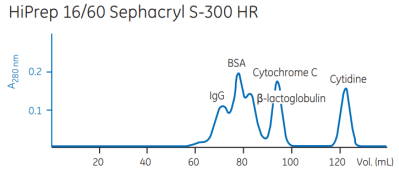
Sephacryl™ S-100 HR



Sephacryl S-200 HR



Sephacryl S-300 HR

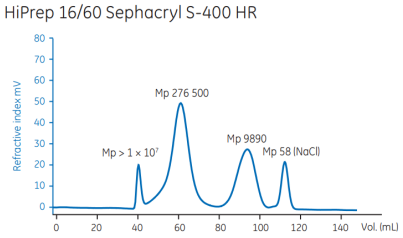


Sample: **Standard proteins**
500 µL of a mixture comprising :

IgG	(M_r 160 000)
BSA	(M_r 67 000)
β-lactoglobulin	(M_r 35 000)
cytochrome C	(M_r 12 400)
cytidine	(M_r 240)

Buffer: 50 mM sodium phosphate, 150 mM NaCl, pH 7.0
Flow rate: 0.8 mL/min
Detection: A_{280}

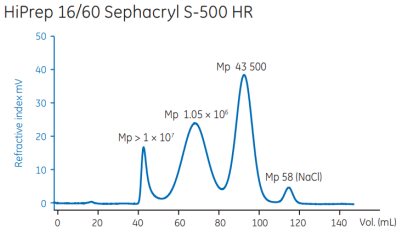
Sephacryl S-400 HR



Sample: **Dextrans**
1 mL of a mixture containing
Dextran > 1 × 10^7
Dextran 410 (M_p 276 500)
Dextran 12 (M_p 9890)

Buffer: 0.25 M NaCl
Flow rate: 0.5 mL/min
Detection: Refractive Index (RI)

Sephacryl S-500 HR



Sample: **Dextrans**
1 mL of a mixture containing
Dextran > 1 × 10^7
Dextran DXT1185K (M_p 1.05 × 10^6)
Dextran 50 (M_p 43 500)

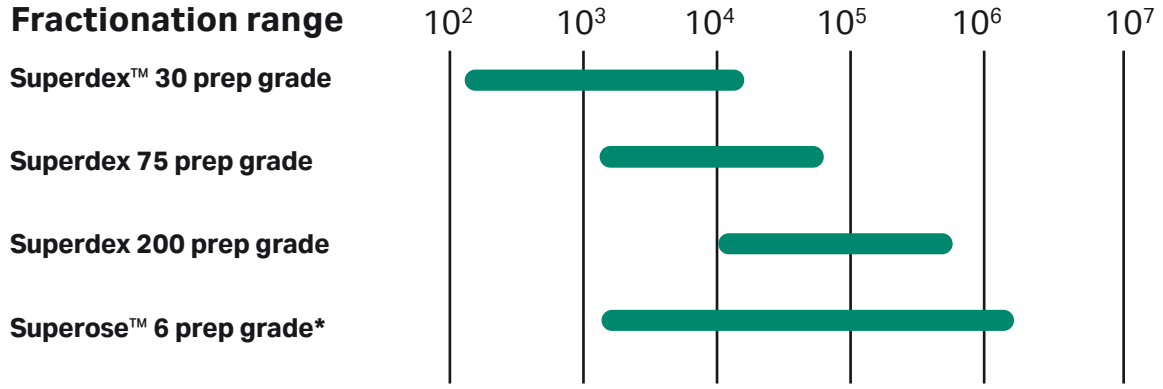
Check chromatograms to see which resin best fits with your sample.

5

HiLoad SEC columns

HiLoad Superdex prep grade and Superose prep grade[1] SEC columns deliver high resolution

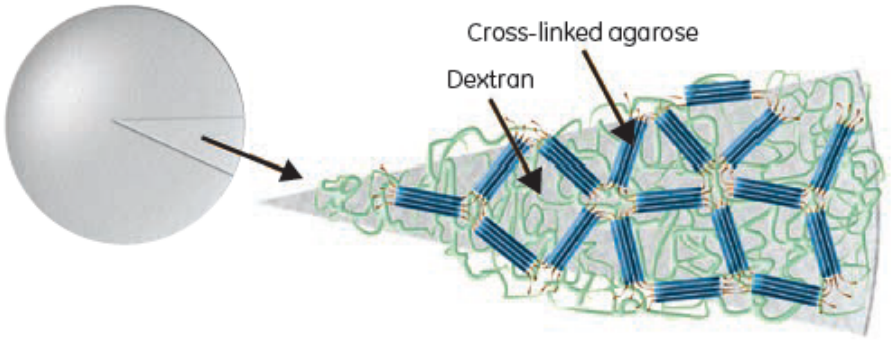
- **Four different fractionation ranges**
- **Two column dimensions**
- **Sample volumes up to 5 mL and 13 mL**



[1] Superose 6 prep grade is from Nov. 2018 available in prepacked format

For high resolution and high recovery needs

Superdex prep grade and Superose prep grade resins technical specifications

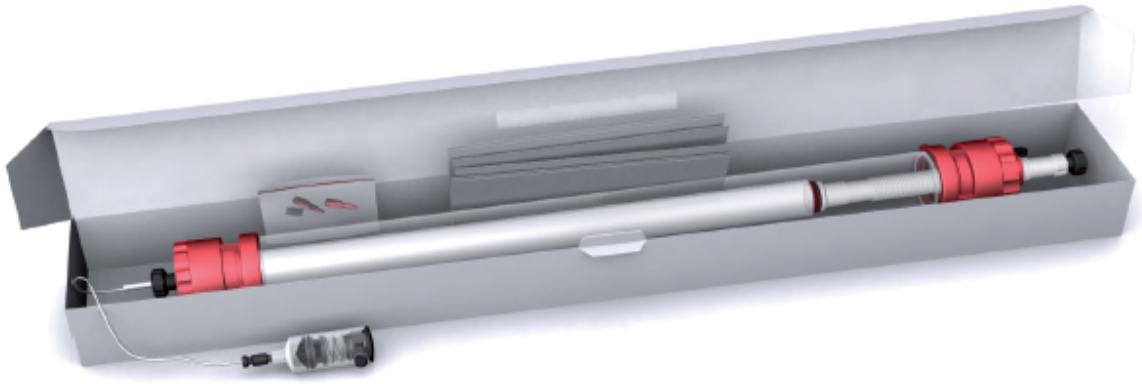


- **Matrix:** Composite of cross-linked agarose and dextran (Superdex pg)
  Composite of cross-linked agarose (Superose pg)
- **Particle size, d_{50V}[1]:** ~ 34 µm (Superdex pg); ~ 30 to 40 µm (Superose pg)
- **pH stability, operational[2]:** 3 to 12
- **pH stability, CIP[3]:** 1 to 14

Fractionation range/Resin	Superdex™ 30 prep grade	Superdex 75 prep grade	Superdex 200 prep grade	Superose™ 6 prep grade
Fractionation range (M_r) Globular proteins	< 1000	~ 3000 to 70 000	~ 10 000 to 60 000	~ 5000 to 5 000 000

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function.

Technical specifications — HiLoad prepacked columns



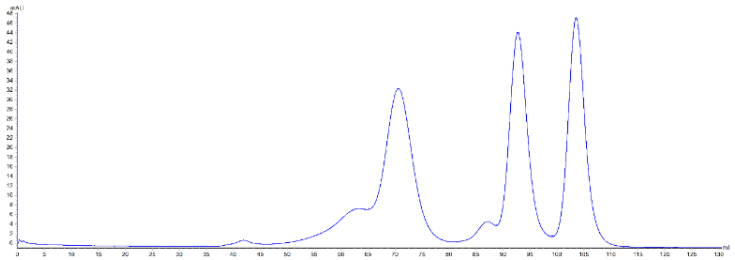
- **Max pressure over the packed bed during operation:** 0.3 MPa, 3 bar, 42 psi
- **Column hardware pressure limit:** 0.5 MPa, 5 bar, 73 psi

Parameter	HiLoad™ 16/600[1]	HiLoad 26/600[1]
Bed dimensions	16 mm × 600 mm	26 mm × 600 mm
Approximate bed volume	120 mL	320 mL
Recommended sample volume	Up to 5 mL	Up to 13 mL
Recommended operating flow rate	1.0 mL/min	2.6 mL/min
Max. operating flow rate	1.7 mL/min	4.4 mL/min

[1] HiLoad columns are called XK when sold as empty columns. Superose™ 6 prep grade in XK 26/70 is available as a custom column from CDP with code no. 90100043.

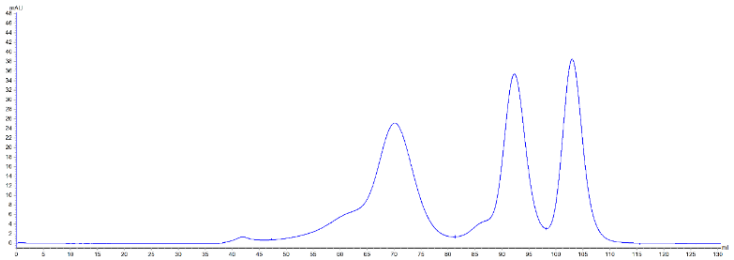
HiLoad 16/600 Superose 6 pg column — what results to expect?

Small sample volume and low flow



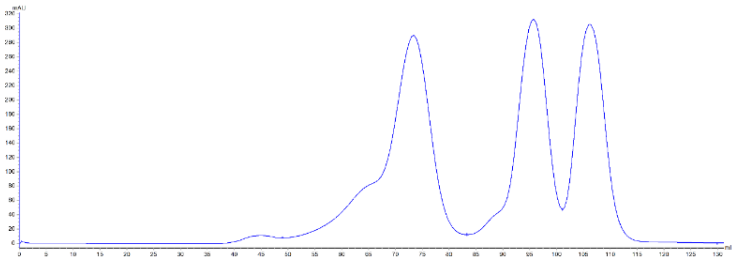
Sample volume: 0.5 mL
Flow rate: 0.8 mL/min

Small sample volume and high flow



Sample volume: 0.5 mL
Flow rate: 1.6 mL/min

Large sample volume and low flow



Sample volume: 5 mL
Flow rate: 0.8 mL/min

Protein mix (same in all three runs):

1. Thyroglobulin (M_r 669 000)
2. Ovalbumin (M_r 44 000)
3. Ribonuclease (M_r 13 700)

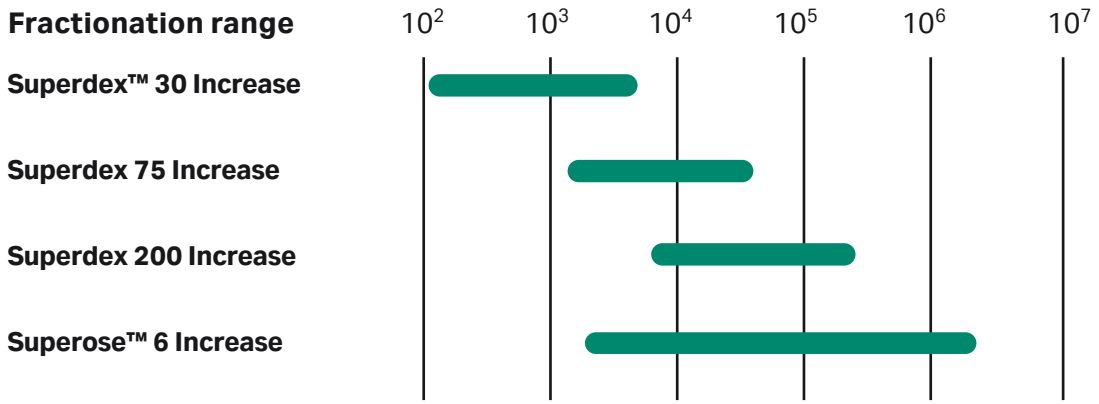
General rule: small sample volume and low flow rate gives the best resolution

6

Superdex Increase and Superose Increase columns for sample volumes < 0.5 mL

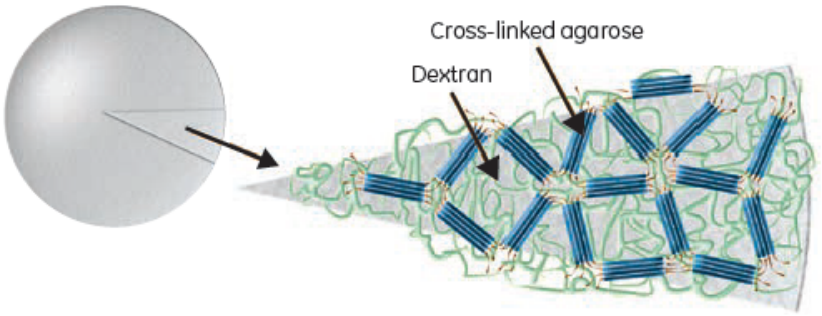
Superdex Increase and Superose Increase for highest resolution

- **Four different fractionation ranges**
- **Three standard column dimensions + dimensions on demand**
- **Sample volumes from 4 µL up to 0.5 mL**



For higest resolution and speed

Superdex Increase and Superose Increased resins technical specifications



- **Matrix:** Composite of cross-linked agarose and dextran (Superdex Increase)
  Composite of cross-linked agarose (Superose Increase)
- **Particle size, d_{50V}[1]:** ~ 9 µm
- **pH stability, operational[2]:** 3 to 12
- **pH stability, CIP[3]:** 1 to 14

Fractionation range/Resin	Superdex™ 30 Increase	Superdex 75 Increase	Superdex 200 Increase	Superose™ 6 Increase
Fractionation range (M_r) Globular proteins	~ 100 to 7000	~ 3000 to 70 000	~ 10 000 to 600 000	~ 5000 to 5 000 000

[1] Median particle size of the cumulative volume distribution.
[2] pH range where resin can be operated without significant change in function.
[3] pH range where resin can be subjected to cleaning-in-place (CIP) without significant change in function.

Technical specifications — Superdex Increase and Superose Increase standard columns

- **Typical pressure over the packed bed during operation:**
  3 MPa, 50 bar, 435 psi (10/300 and 5/150)
  2 MPa, 20 bar, 290 psi (3.2/300)
- **Column hardware pressure limit:**
  5 MPa, 50 bar, 725 psi (10/300 and 3.2/300)
  10 MPa, 100 bar, 1450 psi (5/150)

Parameter	Tricorn™ 10/300 GL	Tricorn 5/150 GL	3.2/300
Bed dimensions	10 mm × 300 mm	5 mm × 150 mm	3.2 mm × 300 mm
Approximate bed volume	24 mL	3 mL	2.4 mL
Recommended sample volume	25 to 500 µL	4 to 50 µL	4 to 50 µL
Recommended flow rate	0.8, 0.75, or 0.5 mL/min[1]	0.45 or 0.3 mL/min[2]	0.075 or 0.04 mL/min[3]
Max. operating flow rate	1.8, 1.6, 1.5, or 1.2 mL/min[4]	0.75 mL/min	0.15 mL/min

[1] 0.8 for Superdex™ 30 and 75 Increase, 0.75 for Superdex 200 Increase, 0.5 for Superose™ 6 Increase
[2] 0.45 for Superdex 75 and 200 Increase, 0.3 for Superose 6 Increase
[3] 0.075 for all Superdex Increase, 0.04 for Superose 6 Increase
[4] 1.8 for Superdex 200 Increase, 1.6 for Superdex 75 Increase, 1.5 for Superose 6 Increase, 1.2 for Superdex 30 Increase

Download data files >>

7

Superdex Increase and Superose Increase columns for larger volumes (0.5 to 8.5 mL)

Superdex Increase and Superose Increase resins in HiScale columns for larger sample volumes

We listened to you!

Now available on demand!

If you like our new generation Superdex™ Increase and Superose™ Increase columns, but need to purify larger volumes of up to 8.5 mL, we can pack the resin in HiScale™ columns.

On-demand service[1]. Contact your Cytiva representative.

[1] Custom Designed Products offer this as nonstandard products. This means that delivery could be longer than catalog products and we do not provide Instruction for use for these columns.

Technical specifications: HiScale prepacked columns

- **Max pressure over the packed bed during operation:**
  2 MPa, 20 bar, 290 psi
- **Column hardware pressure limit:**
  2 MPa, 20 bar, 290 psi

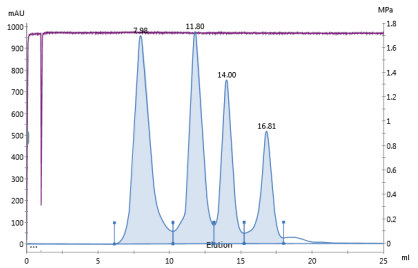
Parameter	HiScale™ 16/40	HiScale 26/40
Bed dimensions	16 mm × 400 mm	26 mm × 400 mm
Approximate bed volume	80 mL	212 mL
Recommended sample volume	Up to 3.2 mL	Up to 8.5 mL
Max. operating flow rate	~ 2 mL/min†	~ 4 mL/min†

* Max pressure over the packed bed is limited by the pressure limit of the column hardware.
† This value could differ between different resins and resin lots. Max. operating flow rate for the indivual column is stated in the documentation included with each column.

High resolution is maintained when scaling up Superdex 200 Increase with different columns formats

Standard: 10/300 GL

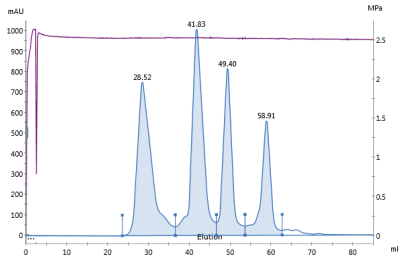
Sample: 0.5 mL
Flow rate: 0.75 mL/min
Run time: ~ 32 min



	Resolution
Thyro.	NA
Aldo.	2.25
Ovalb.	1.55
Rnase	2.17

HiScale™ 16/40

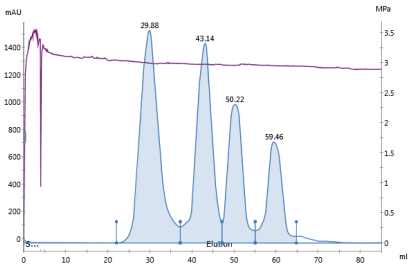
Sample: 1.6 mL
Flow rate: 2 mL/min
Run time: ~ 40 min



	Resolution
Thyro.	NA
Aldo.	2.64
Ovalb.	1.95
Rnase	2.79

HiScale 16/40

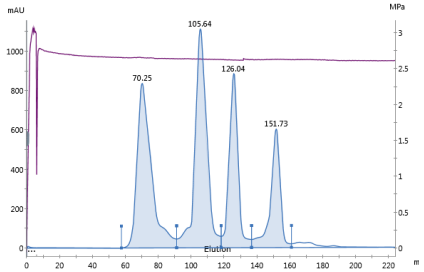
Sample: 3.2 mL
Flow rate: 2 mL/min
Run time: ~ 40 min



	Resolution
Thyro.	NA
Aldo.	1.87
Ovalb.	1.08
Rnase	1.47

HiScale 26/40

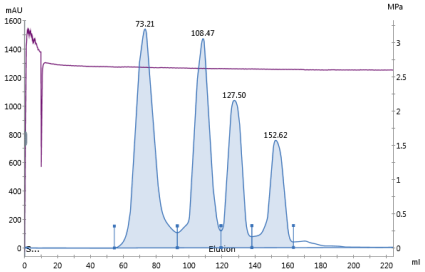
Sample: 4.25 mL
Flow rate: 4 mL/min
Run time: ~ 53 min



	Resolution
Thyro.	NA
Aldo.	2.71
Ovalb.	2.01
Rnase	2.81

HiScale 26/40

Sample: 8.5 mL
Flow rate: 4 mL/min
Run time: ~ 53 min



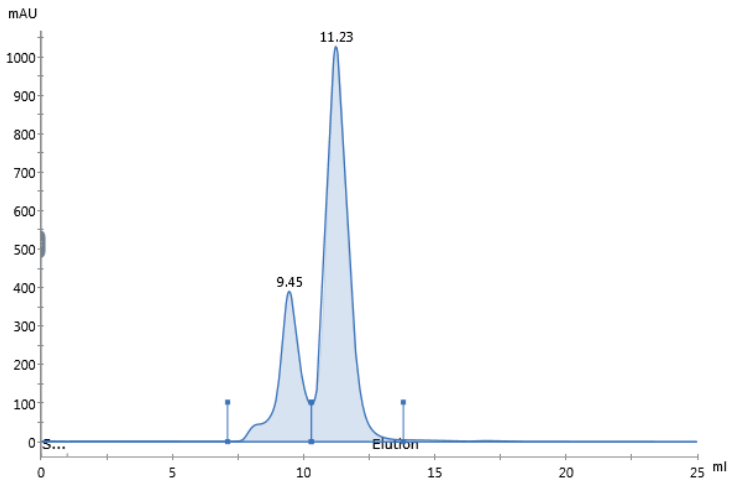
	Resolution
Thyro.	NA
Aldo.	1.97
Ovalb.	1.17
Rnase	1.62

Sample: Thyroglobulin, aldolase, ovalbumin, and ribonuclease A in PBS buffer, pH 7.4

Antibody purification with Superdex 200 Increase in different columns with maintained high resolution

Standard: 10/300 GL

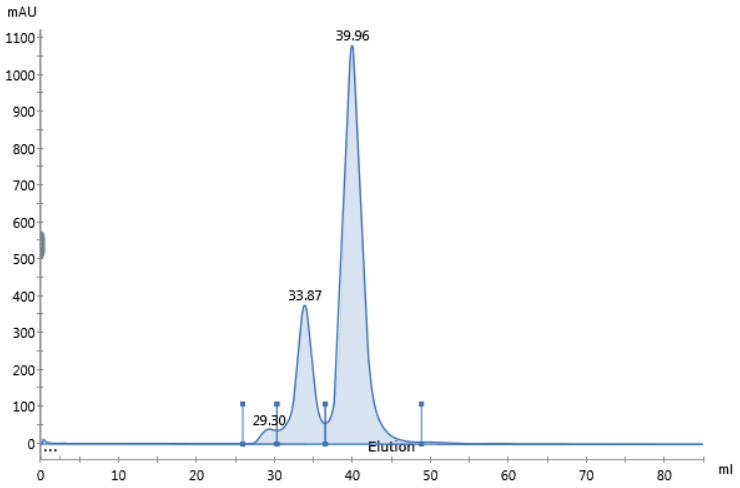
Sample: 0.5 mL IgG (2 mg/mL)



Flow rate: 0.75 mL/min

HiScale™ 16/40

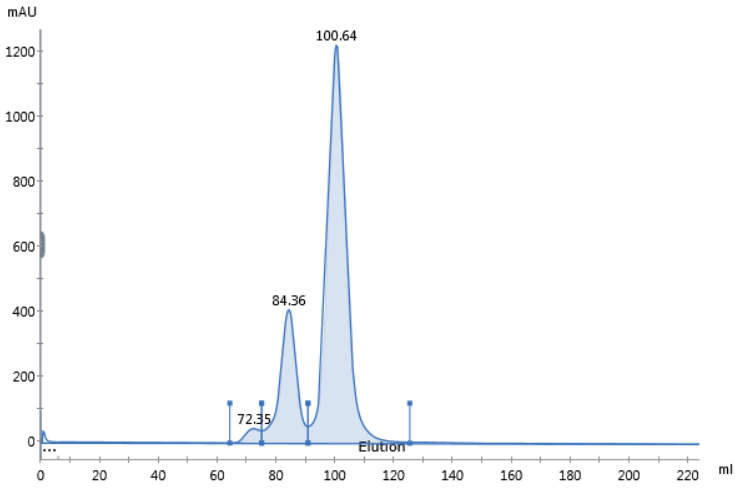
Sample: 1.6 mL IgG (2 mg/mL)



Flow rate: 2 mL/min

HiScale 26/40

Sample: 4.25 mL IgG (2 mg/mL)



Flow rate: 4 mL/min

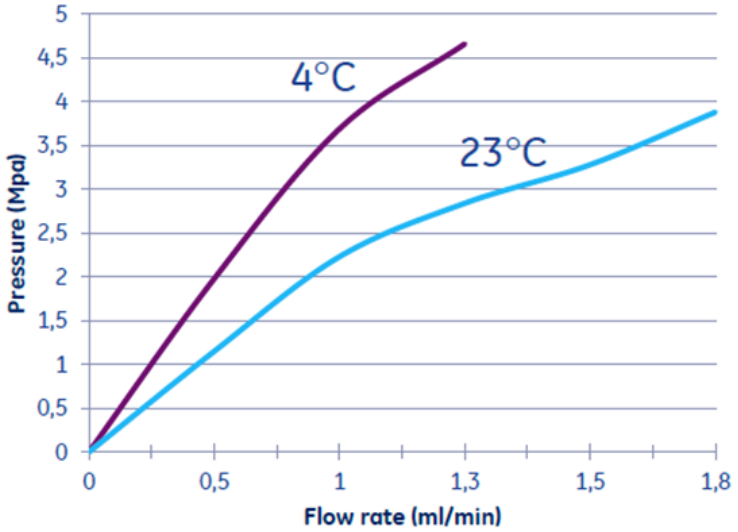
Sample*:* IgG 2 mg/mL in PBS-buffer, pH 7.4

8

Tips for successful size exclusion chromatography

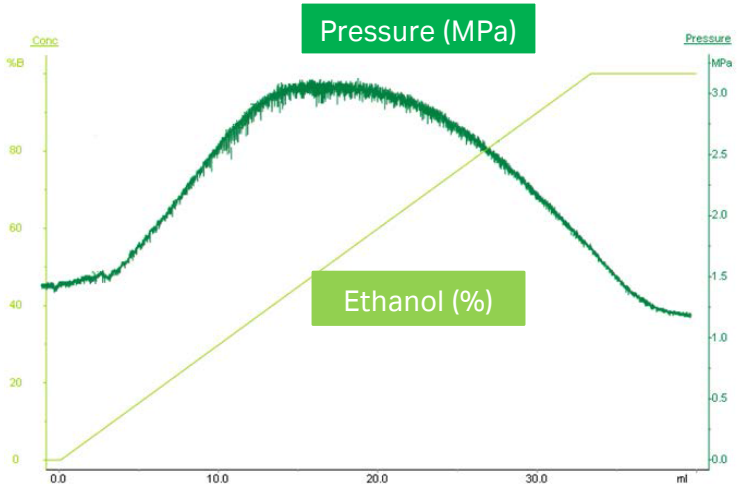
Protect the packed bed in the column — decrease flow rate when working in cold room or with viscous liquids

Low temperature increases pressure



Pressure over the column at different flow rate and temperature on Superdex™ 200 Increase 10/300 GL column in water

High viscosity increases pressure



Pressure over the column when increasing the amount of ethanol (viscosity increases up to ~ 50% ethanol) on Superdex 200 Increase 5/150 GL column

Recommendation

When working with

- viscous liquids
- or at low temperature

Our recommendation is to lower the flow rate to avoid damaging the packed bed in the column.

Save your column by cleaning with NaOH every 10 to 20 SEC cycles

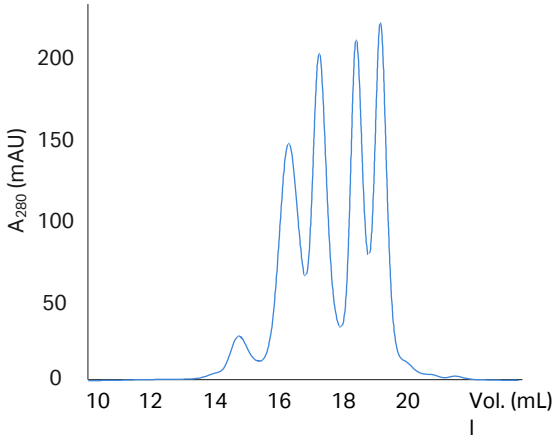
What to do?

Sodium hydroxide is a very efficient cleaning solution.

See chromatograms to the right for example of a column that could be refreshed.

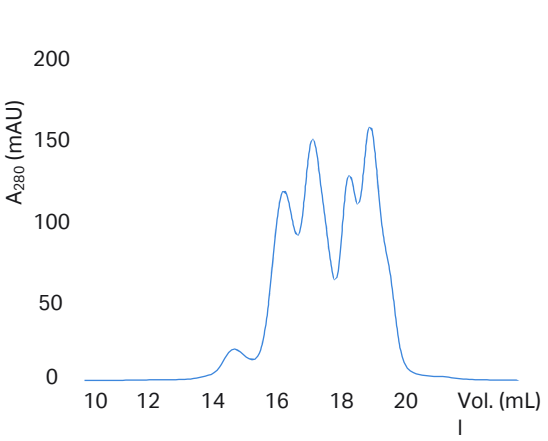
Learn more >>

Fresh column



Superose™ 6 Increase 10/300 GL

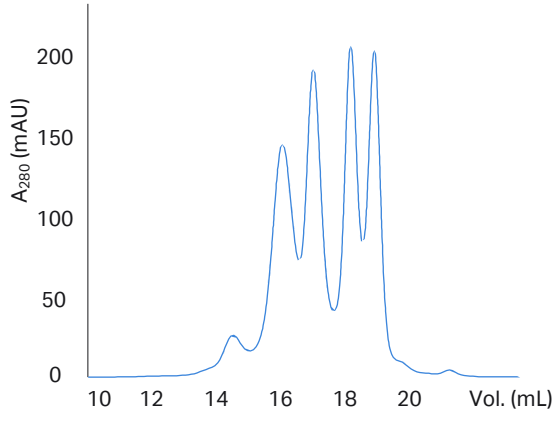
Dirty column



Column used for a very long time without cleaning

- Resolution gradually decreases
- A gap was formed resulting in this very poor resolution

Refreshed column



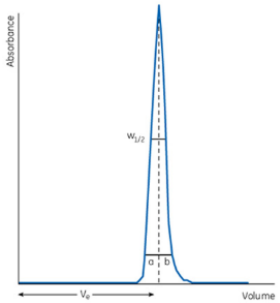
The column performance could be restored

after adjusting the adapter and NaOH cleaning.

Tips for taking care of your precious SEC columns

More on cytiva.com/ProteinResearch

Tips for maximizing your SEC column lifetime



Tips and hints for protecting your SEC column investment

READ MORE

Inspect your column to ensure you get it right

Have you ever had to deal with bubbles in the resin bed of your chromatography column? Or even a gap between the resin bed and the adapter?

READ MORE

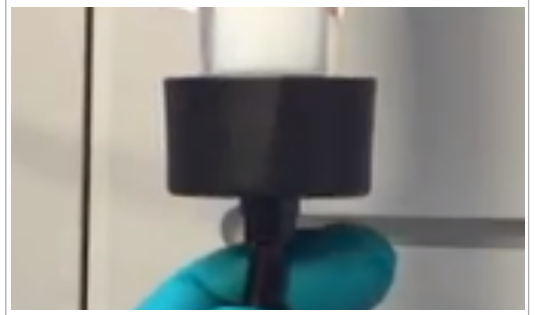
Prevent SEC columns drying out

How to connect storage device to your SEC column to protect your column during storage

WITH A SYRINGE

WITH AN ÄKTA™ SYSTEM

Excluding air from the column



Tutorial showing how to connect SEC column drop-to-drop to ÄKTA systems

WATCH NOW

9

Summary

Adding a SEC polishing step will improve your proteins' purity

SEC gives highly size homogeneous samples.

Cytiva offers a variety of SEC resins and column formats to meet your recovery, speed, and purity needs.

It is critical to regularly maintain your SEC column to ensure high performance.

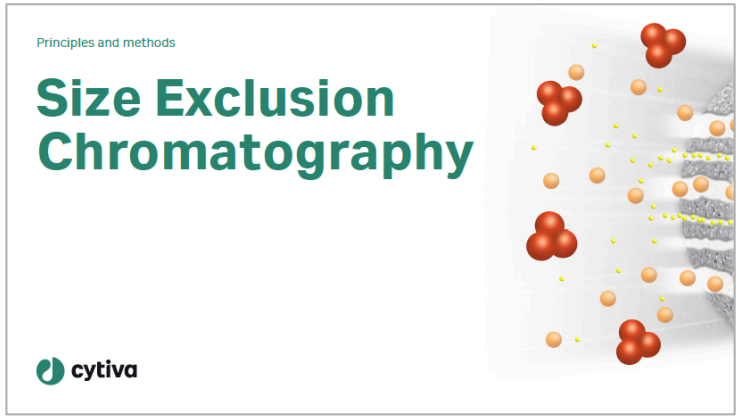
10

Useful tools to ensure successful SEC runs

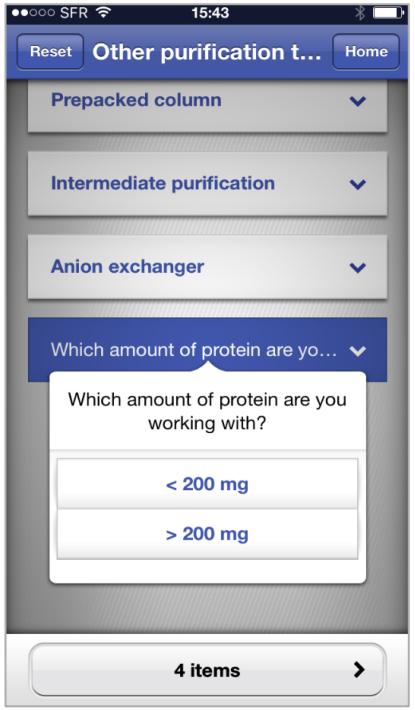
Cytiva expertise made available for you at

cytiva.com/SEC

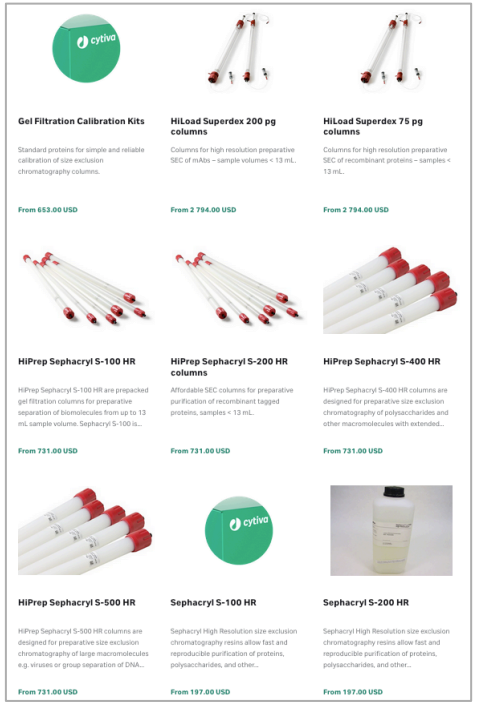
Principles and methods
Cytiva Handbook >>



Help for column selection
Interactive selector >>



Ordering information
Cytiva online shop >>



Ordering information

Columns with SEC "Increase" resins

Product code	Product name
28990944	Superdex™ 200 Increase 10/300 GL
29148721	Superdex 75 Increase 10/300 GL
29219757	Superdex 30 Increase 10/300 GL
29091596	Superose™ 6 Increase 10/300 GL
29321903*	Superose 6 Increase HiScale™ 16/40
29321904*	Superose 6 Increase HiScale 26/40
29321905*	Superdex 200 Increase HiScale 16/40
29321906*	Superdex 200 Increase HiScale 26/40
29321907*	Superdex 75 Increase HiScale 16/40
29321908*	Superdex 75 Increase HiScale 26/40

HiLoad™ columns

Product code	Product name
28989335	HiLoad 16/600 Superdex 200 pg
28989333	HiLoad 16/600 Superdex 75 pg
28989331	HiLoad 16/600 Superdex 30 pg
29323952	HiLoad 16/600 Superose 6 pg†
28989336	HiLoad 26/600 Superdex 200 pg
28989334	HiLoad 26/600 Superdex 75 pg
28989332	HiLoad 26/600 Superdex 30 pg

HiPrep™ columns

Product code	Product name
17116501	HiPrep 16/60 Sephacryl™ S-100 HR
17116601	HiPrep 16/60 Sephacryl S-200 HR
17116701	HiPrep 16/60 Sephacryl S-300 HR
28935604	HiPrep 16/60 Sephacryl S-400 HR
28935606	HiPrep 16/60 Sephacryl S-500 HR
17119401	HiPrep 26/60 Sephacryl S-100 HR
17119501	HiPrep 26/60 Sephacryl S-200 HR
17119601	HiPrep 26/60 Sephacryl S-300 HR
28935605	HiPrep 26/60 Sephacryl S-400 HR
28935607	HiPrep 26/60 Sephacryl S-500 HR

† New prepacked column, launched Nov. 2018
* These products are available on-demand. Contact your Cytiva representative

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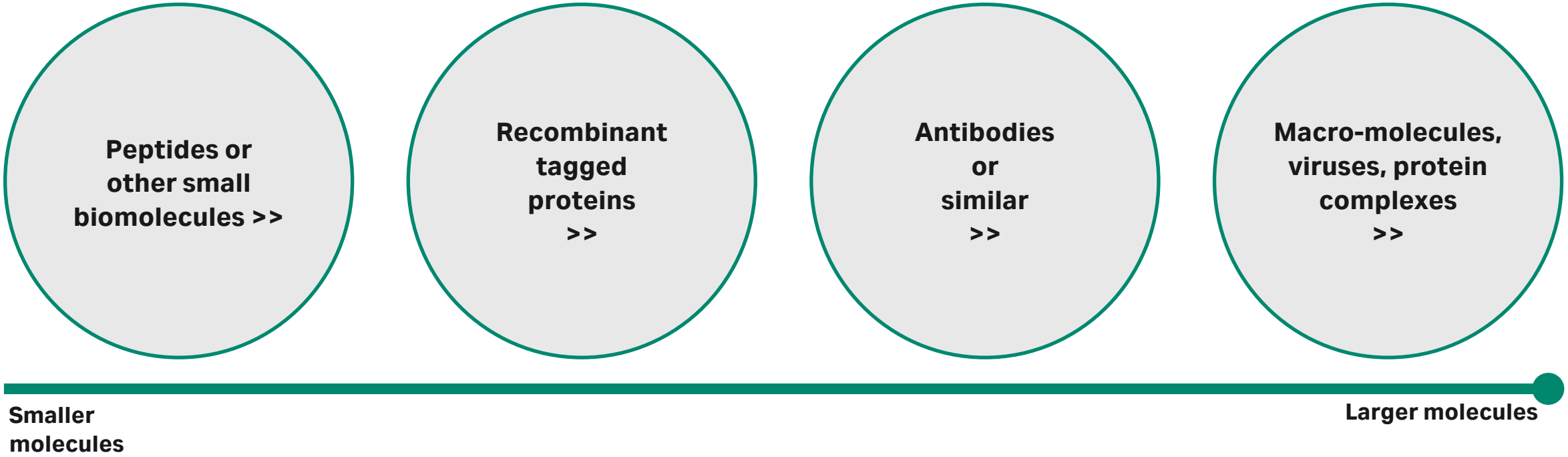
Appendix 1 — Column selection

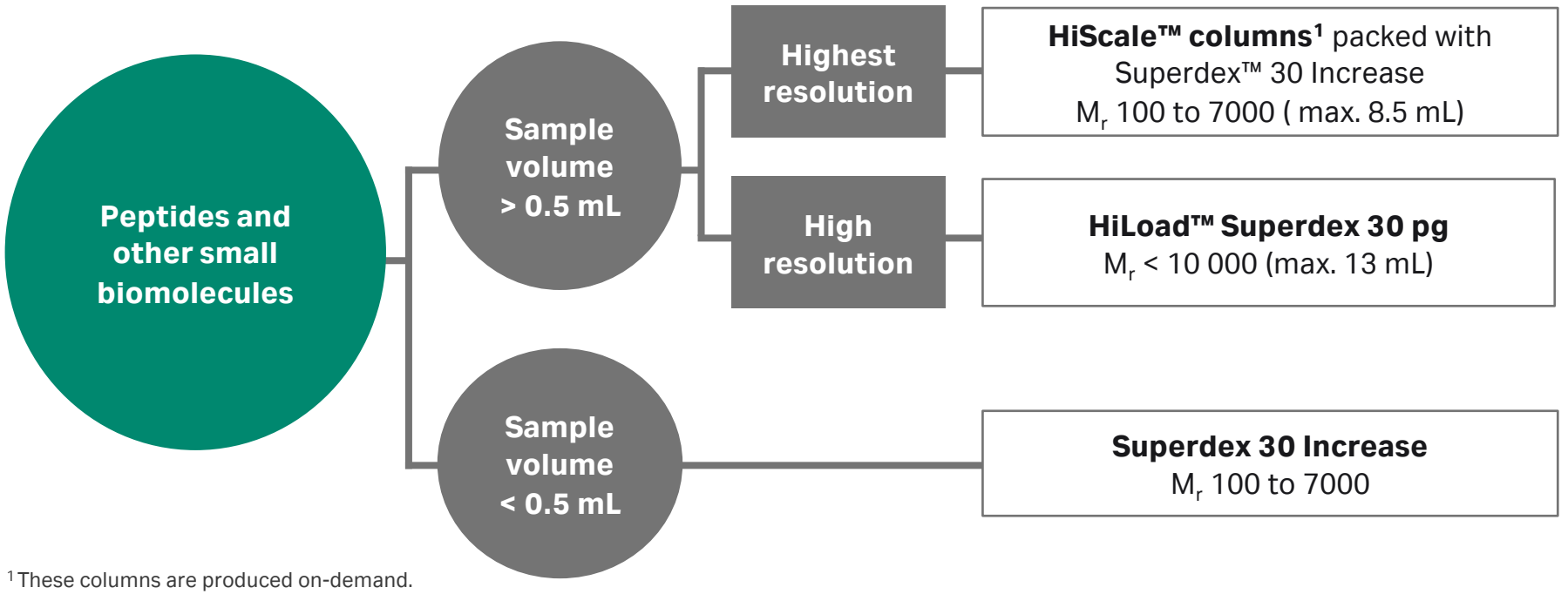
We help you to select the right SEC column!

What molecule do you need to purify?
Click on your choice.



SEC columns for peptides or other small biomolecules

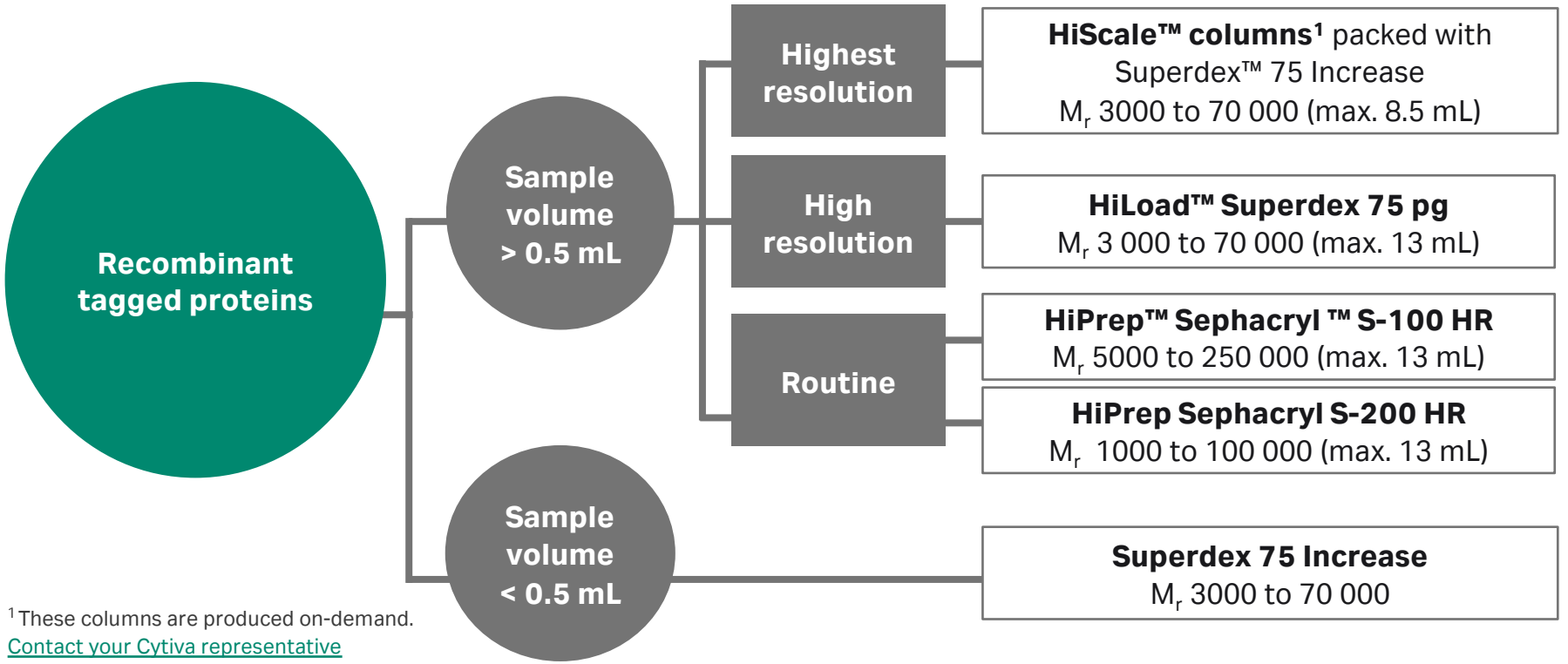
For more information, click on the column of your interest



[1] These columns are produced on-demand.
Contact your Cytiva representative

SEC columns for recombinant tagged proteins

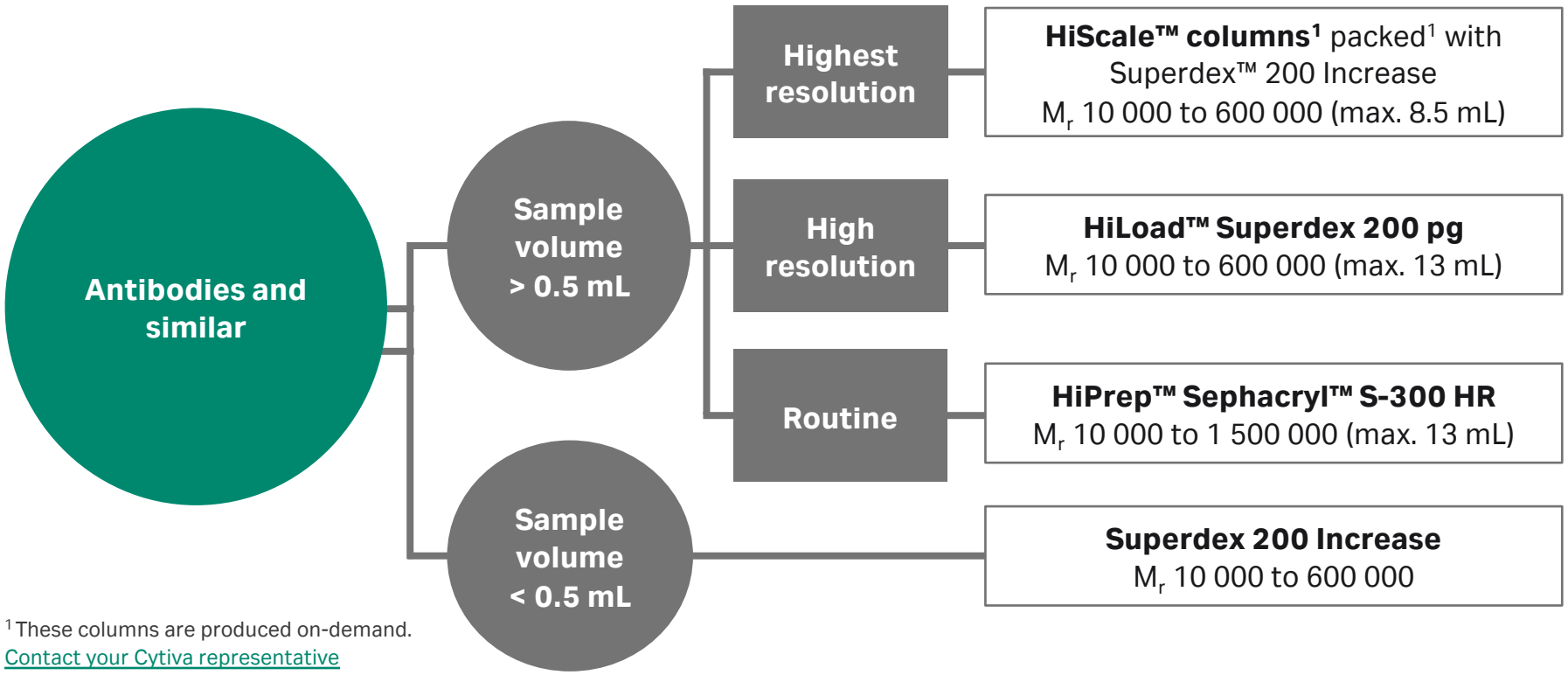
For more information, click on the column of your interest



[1] These columns are produced on-demand.
Contact your Cytiva representative

SEC columns for monoclonal antibodies or other antibodies

For more information, click on the column of your interest



[1] These columns are produced on-demand.
Contact your Cytiva representative

Macromolecules, viruses, large proteins, and protein complexes

For more information, click on the column of your interest

Macromolecules, viruses, protein complexes

Sample volume > 0.5 mL

- **Highest resolution**
  - **HiScale™ columns[1]**
    packed with Superose™ 6 Increase
    M_r 5000 to 5 000 000 (max. 8.5 mL)
- **High resolution**
  - **HiLoad™ 16/600 Superose 6 pg**
    M_r 5000 to 5 000 000 (max. 5 mL)
- **Routine**
  - **HiPrep™ Sephacryl™ S-400 HR**
    M_r 20 000 to 8 000 000 (max. 13 mL)
  - **HiPrep Sephacryl S-500 HR**
    M_r 40 000 to 20 000 000 (dextrans) (max. 13 mL)

Sample volume < 0.5 mL

- **Superose 6 Increase**
  M_r 5000 to 5 000 000

[1] These columns are produced on-demand.
Contact your Cytiva representative

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Appendix 2 — Application examples

Screening for optimal sample load of virus-like particles on HiPrep 16/60 Sephacryl S-500 HR column

Purpose of the study

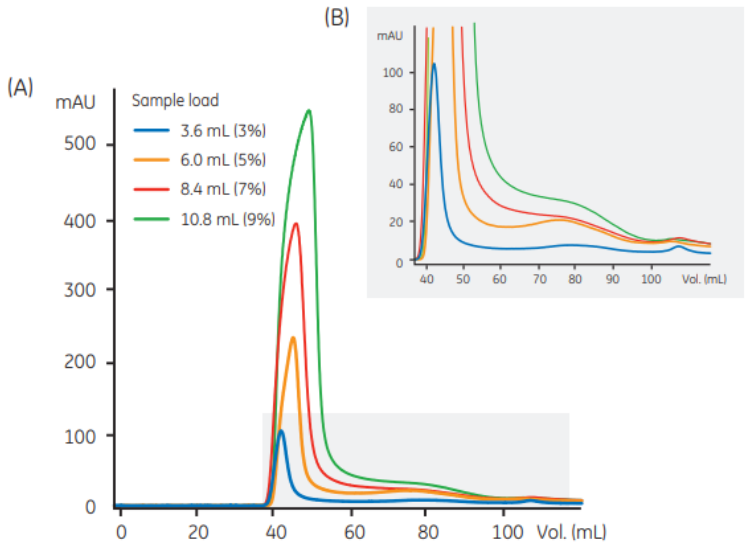
Virus-like particles (VLP)s are used as vaccines.

To increase productivity, it is important to determine the maximum amount of feed per milliliter of chromatography resin that can be loaded to give an acceptable level of purification.

The effect of increased sample load was evaluated using HiPrep™ 16/60 Sephacryl™ S-500 HR column.

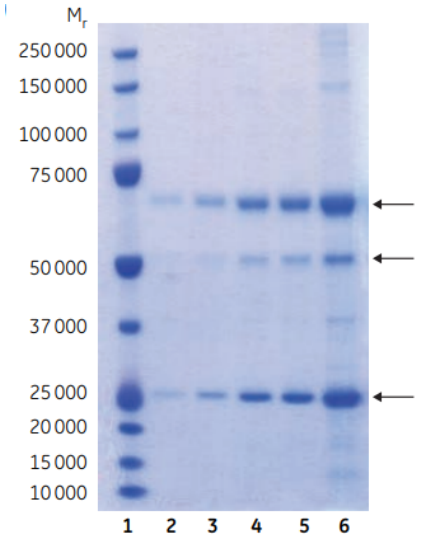
Resolution decreased with increased sample volume as expected. Product purity was analyzed by SDS-PAGE.

Sample load on SEC column



(A) Purification of a virus-like particle (VLP) by SEC using HiPrep 16/60 Sephacryl S-500 HR. Various sample volumes, previously purified on a Capto™ Q column, were loaded on the column.
(B) Enlargement of peaks presented in (A).

Purity check (SDS-PAGE)



Lanes
1. Molecular weight markers
2. Eluted pool, 3% sample load
3. Eluted pool, 5% sample load
4. Eluted pool, 7% sample load
5. Eluted pool, 9% sample load
6. Start material

SDS-PAGE analysis (reducing conditions, 4% to 12% polyacrylamide gel, Coomassie stained) of eluted pools where the arrows indicate surface proteins of the VLP (M_r 69 000, 54 000, and 27 000).

Purification of insulin using HiPrep 26/60 Sephacryl S-100 HR

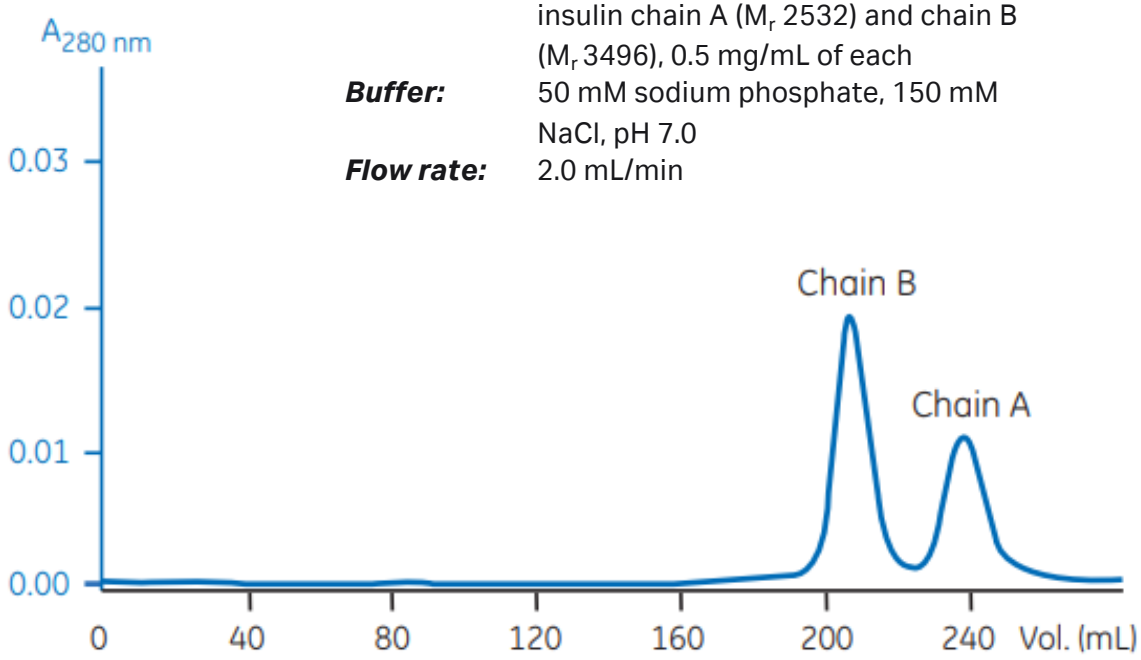
Background

Insulin consists of two chains (A and B) held together by -S-S bonds.

When these links have been broken, the two chains can, despite small differences in molecular weight, be separated by SEC.

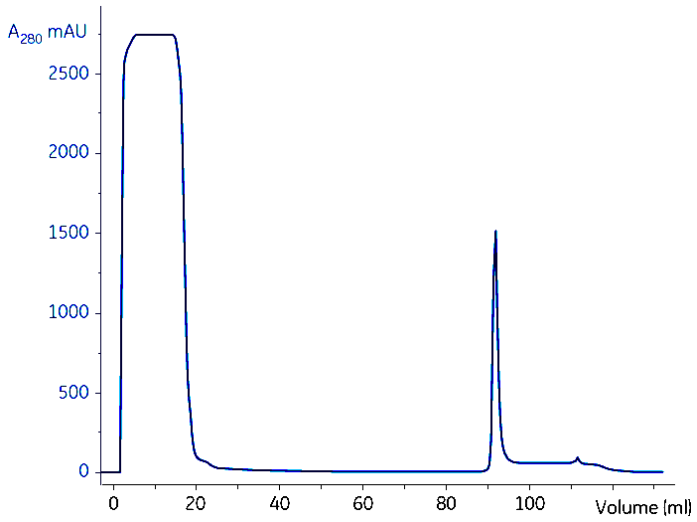
Purification of insulin

Column: HiPrep 26/60 Sephacryl S-100 HR
Sample: 1 mL of a mixture comprising bovine insulin chain A (M_r 2532) and chain B (M_r 3496), 0.5 mg/mL of each
Buffer: 50 mM sodium phosphate, 150 mM NaCl, pH 7.0
Flow rate: 2.0 mL/min



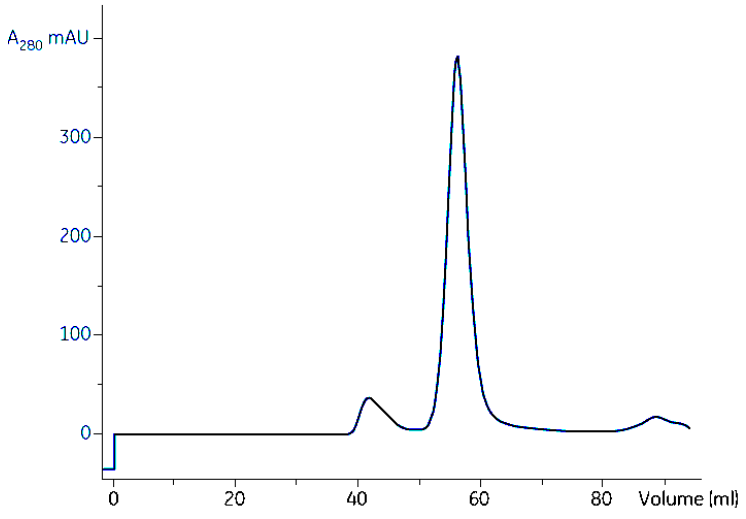
Purification of a tagged protein using HiLoad Superdex 200 pg column as polishing step

1. Capture: AC



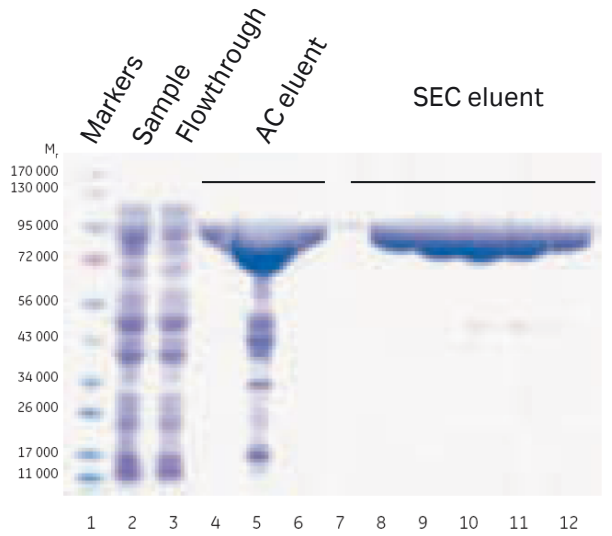
Column: MBPTrap™ HP 5 mL
Sample: 15 mL of MBP-MCAD in *E. coli* lysate, M_r ~ 85 500

2. Polishing: SEC



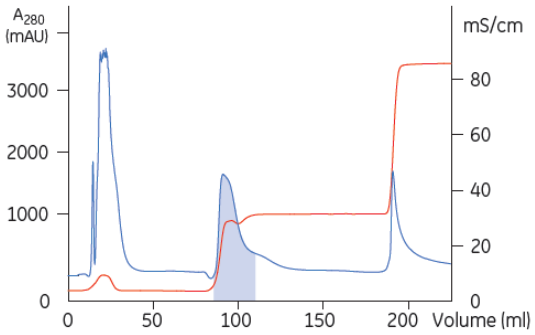
Column: HiLoad™ 16/600 Superdex™ 200 pg
Sample: 2 mL eluted fraction from AC

Purity check (SDS-PAGE)



Purification of an untagged protein using HiLoad Superdex 75 pg column as polishing step

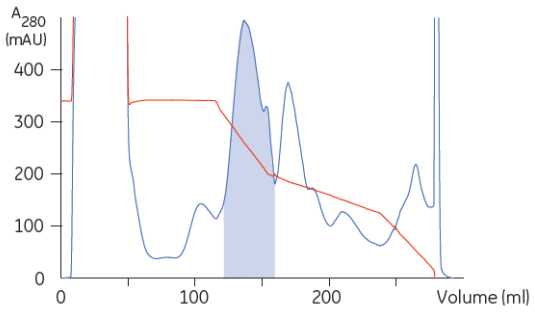
1. Capture: IEX



Column: HiPrep™ Q XL 16/10

Sample: 40 mL of clarified *E. coli* extract with DAOCS

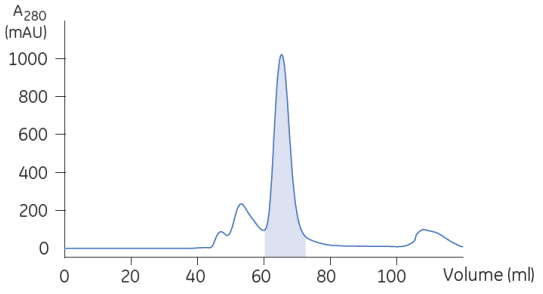
2. Intermediate purification: HIC



Column: SOURCE™ 15ISO, packed in HR column 16/10

Sample: 40 mL of DAOCS pool from IEX

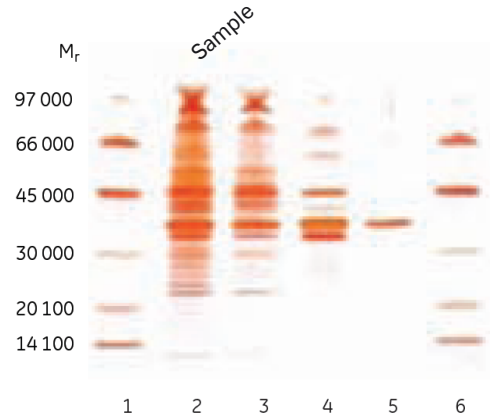
3. Polishing: SEC



Column: HiLoad™ 16/600 Superdex™ 75 pg

Sample: 3 mL of concentrated DAOCS pool from HIC

Purity check (SDS-PAGE)



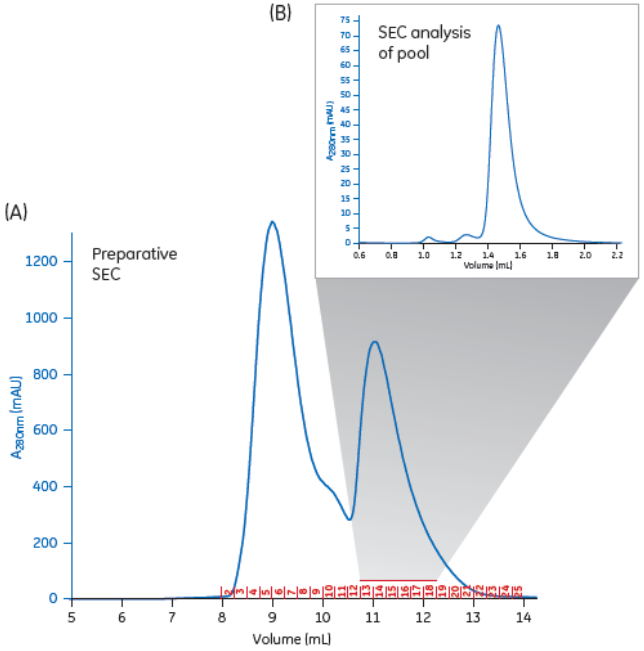
Improved purity of a his-tagged protein using Superdex 75 Increase 10/300 GL column

Purpose

A sample of a purified his-tagged protein that had oligomerized during storage and freeze-thawing was run on Superdex™ 75 Increase 10/300 GL column to remove aggregates.

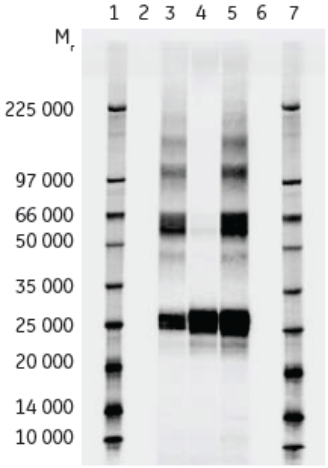
Columns:	Superdex 75 Increase 10/300 GL for preparative SEC (A) Superdex 75 Increase 5/150 GL for analysis (B)
Samples:	Concentrated, partially purified histidine-tagged protein (A) Samples of SEC fractions and from pooled peaks (B)
Sample volumes:	500 µL (A) 25 µL (B)
Buffer:	PBS
Flow rates:	0.2 mL/min (A) 0.45 mL/min (B)
System:	ÄKTA pure 25 with 2 mm UV cell to extend the linear absorbance range

Purification



The final pool (fractions marked with red bar), with target protein, contained approximately 5 mg of target protein.

Purity check



Lanes

1 and 7.	Molecular weight marker
2 and 6.	Blanks
3.	Pool of first peak (discarded) of the preparative SEC (fractions 3 to 12, diluted 1:5)
4.	Pool of the second peak of the preparative SEC (fractions 13 to 18, diluted 1:5)
5.	Start material (diluted 1:10)

Analysis of pool of both peaks from the preparative SEC purification.

SDS-PAGE separation of fluorescent prestained samples on an 8% to 18% gel.

Thank you

For local office contact information, visit cytiva.com/contact