Preparative purification of proteins with size exclusion chromatography columns
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For direct access, click on your size exclusion chromatography (SEC) topic of interest

When and why should you use SEC

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- Why use SEC in a protein purification protocol? >>

SEC tips, tools, and summary

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- Useful SEC tools >>
- Summary >>

GE SEC columns

- How to select GE SEC columns >>
- HiPrep™ Sephacryl™ columns >>
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- Superdex™ Increase and Superose™ Increase for volumes < 0.5 mL >>
- Superdex™ Increase and Superose™ Increase for volumes > 0.5 mL >>

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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

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

Largest molecules elute first

A well-packed SEC column is critical for high-resolution separations
More on SEC animation and GE handbook

### Watch the video on YouTube
The principle of gel filtration (size exclusion chromatography)

### Download the handbook
Size Exclusion Chromatography Principles and Methods

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**The principle of Gel Filtration Chromatography**

Separation in Gel Filtration Chromatography is based on the differences in sizes from biomolecules as they pass through a column packed with a chromatographic medium, which is a gel.
Why use SEC in a protein purification protocol?
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:

- 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.
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**

**Column:** HiTrap™ MabSelect™ PrismA 1 mL
**Binding buffer:** 20 mM phosphate, 150 mM NaCl pH 7.4, 50 mM sodium acetate pH 3.5
**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
**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

1These columns are produced on-demand.
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**

- LMW Start FT wash eluted 1 2 3 4 5 6 7 8 9
- HisTrap Superdex

SEC columns for polishing in purification of biomolecules | KA4628221018PP 14
How to select the most suitable GE SEC column for your protein purification
GE offers prepacked SEC columns for user convenience and reproducible results

For a broad range of biomolecules and resolutions

<table>
<thead>
<tr>
<th>Fractionation range</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superdex 30 Increase</td>
<td>(10^4)</td>
</tr>
<tr>
<td>Superdex 75 Increase</td>
<td>(10^5)</td>
</tr>
<tr>
<td>Superdex 200 Increase</td>
<td>(10^6)</td>
</tr>
<tr>
<td>Superdex 30 prep grade</td>
<td>(10^7)</td>
</tr>
<tr>
<td>Superdex 75 prep grade</td>
<td>(10^8)</td>
</tr>
<tr>
<td>Superdex 200 prep grade</td>
<td>(10^9)</td>
</tr>
<tr>
<td>Superose 6 Increase</td>
<td>(10^6)</td>
</tr>
<tr>
<td>Superose 6 prep grade</td>
<td>(10^7)</td>
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<tr>
<td>Superose 12 prep grade</td>
<td>(10^8)</td>
</tr>
<tr>
<td>Sephacryl S-100 HR</td>
<td>(10^6)</td>
</tr>
<tr>
<td>Sephacryl S-200 HR</td>
<td>(10^7)</td>
</tr>
<tr>
<td>Sephacryl S-300 HR</td>
<td>(10^8)</td>
</tr>
<tr>
<td>Sephacryl S-400 HR</td>
<td>(10^9)</td>
</tr>
<tr>
<td>Sephacryl S-500 HR</td>
<td>(10^{10})</td>
</tr>
</tbody>
</table>

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

Select your sample volume

- > 0.5 mL
  - Highest resolution
    - max. 8.5 mL
    - HiScale™ columns packed on demand\(^1\)
      with Superdex™ Increase or Superose™ Increase resin
  - High resolution
    - max. 13 mL
    - HiLoad™ prep grade columns
  - Routine
    - max. 13 mL
    - HiPrep™ Sephacryl™ columns

- < 0.5 mL
  - Superdex Increase and Superose Increase columns

For more detailed guidance, see Appendix

\(^1\)These columns are produced on-demand.

Contact your GE representative
For good resolution: HiPrep™ Sephacryl™

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

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

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

For highest resolution: HiScale™ with “Increase” resin

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

Protein mix (HiPrep Sephacryl + HiLoad Superdex)
1. Ferritin (M, 440 000)
2. Aldolase (M, 158 000)
3. Conalbumin (M, 75 000)
4. Ovalbumin (M, 44 000)
5. Carbonic anhydrase (M, 29 000)
6. Ribonuclease (M, 13 700)

Running conditions
Sample volume 0.5 mL
Flow rate 1 mL/min

HiPrep Sephacryl and HiLoad Superdex pg

Sample volume 1.6 mL
Flow rate 2 mL/min

Superdex Increase HiScale

Sample volume 1.6 mL
Flow rate 2 mL/min

Protein mix (HiScale Superdex Increase)
1. Thyroglobulin (M, 669 000)
2. Aldolase (M, 158 000)
3. Ovalbumin (M, 44 000)
4. Ribonuclease (M, 13 700)

For volumes > 0.5 mL, the choice of column depends on the resolution needed.
Data files available for download: sample volume > 0.5 mL
Click on the document of interest

HiLoad™ Superdex™ columns

HiLoad 16/600 Superose™ 6 pg

HiPrep™ Sephacryl™ columns

SEC columns for polishing in purification of biomolecules | KA4628221018PP

DOWNLOAD

DOWNLOAD

DOWNLOAD
Data files available for download: sample volume < 0.5 mL
Click on the document of interest
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

<table>
<thead>
<tr>
<th>Fractionation range</th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
<th>$10^6$</th>
<th>$10^7$</th>
<th>$10^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sephacryl S-100 HR</td>
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<tr>
<td>Sephacryl S-200 HR</td>
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<tr>
<td>Sephacryl S-300 HR</td>
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<tr>
<td>Sephacryl S-400 HR</td>
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<tr>
<td>Sephacryl S-500 HR</td>
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</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Fractionation range/Resin</th>
<th>Sephacryl S-100 HR</th>
<th>Sephacryl S-200 HR</th>
<th>Sephacryl S-300 HR</th>
<th>Sephacryl S-400 HR</th>
<th>Sephacryl S-500 HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractionation range (M_r) Globular proteins</td>
<td>1000 to 100 000</td>
<td>5000 to 250 000</td>
<td>10 000 to 1 500 000</td>
<td>20 000 to 8 000 000</td>
<td>No data</td>
</tr>
<tr>
<td>Fractionation range (M_p) Dextrans</td>
<td>No data</td>
<td>1000 to 80 000</td>
<td>2000 to 400 000</td>
<td>10 000 to 2 000 000</td>
<td>40 000 to 20 000 000</td>
</tr>
</tbody>
</table>
### Technical specifications – HiPrep™ prepacked columns

#### 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

- **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

*All different Sephacryl™ resins are available in both column sizes*
Comparing separations on the different Sephacryl™ resins

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}

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)

Check chromatograms to see which resin best fits with your sample.
HiLoad™ SEC columns
HiLoad™ Superdex™ prep grade and Superose™ prep grade¹ SEC columns deliver high resolution

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

### Fractionation range

<table>
<thead>
<tr>
<th>Fractionation range</th>
<th>10²</th>
<th>10³</th>
<th>10⁴</th>
<th>10⁵</th>
<th>10⁶</th>
<th>10⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superdex 30 prep grade</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Superdex 75 prep grade</td>
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<tr>
<td>Superdex 200 prep grade</td>
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</tr>
<tr>
<td>Superose 6 prep grade</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

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, \(d_{50V}\):**  
  ~ 34 µm (Superdex pg);  
  ~ 30 to 40 µm (Superose pg)
- **pH stability, operational:** 3 to 12
- **pH stability, CIP:** 1 to 14

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

<table>
<thead>
<tr>
<th>Fractionation range/ Resin</th>
<th>Superdex 30 prep grade</th>
<th>Superdex 75 prep grade</th>
<th>Superdex 200 prep grade</th>
<th>Superose 6 prep grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractionation range (Mr) globular proteins</td>
<td>&lt; 1000</td>
<td>~ 3000 to 70 000</td>
<td>~ 10 000 to 600 000</td>
<td>~ 5000 to 5 000 000</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HiLoad 16/600 ¹</th>
<th>HiLoad 26/600 ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed dimensions</td>
<td>16 mm × 600 mm</td>
<td>26 mm × 600 mm</td>
</tr>
<tr>
<td>Approximate bed volume</td>
<td>120 mL</td>
<td>320 mL</td>
</tr>
<tr>
<td>Recommended sample volume</td>
<td>Up to 5 mL</td>
<td>Up to 13 mL</td>
</tr>
<tr>
<td>Recommended operating flow rate</td>
<td>1.0 mL/min</td>
<td>2.6 mL/min</td>
</tr>
<tr>
<td>Max. operating flow rate</td>
<td>1.7 mL/min</td>
<td>4.4 mL/min</td>
</tr>
</tbody>
</table>

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

1 2 3

**Small sample volume and high flow**

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

1 2 3

**Large sample volume and low flow**

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

1 2 3

Protein mix (same in all three runs):
1. Thyroglobulin (M, 669 000)
2. Ovalbumin (M, 44 000)
3. Ribonuclease (M, 13 700)

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

<table>
<thead>
<tr>
<th>Fractionation range</th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
<th>$10^6$</th>
<th>$10^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superdex 30 Increase</td>
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</tr>
<tr>
<td>Superdex 75 Increase</td>
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<tr>
<td>Superdex 200 Increase</td>
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<td></td>
</tr>
<tr>
<td>Superose 6 Increase</td>
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</tr>
</tbody>
</table>

For highest 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,** \(d_{50V}^{1}: \approx 9 \mu m\)
- **pH stability, operational:** 3 to 12
- **pH stability, CIP:** 1 to 14

---

<table>
<thead>
<tr>
<th>Fractionation range/Resin</th>
<th>Superdex 30 Increase</th>
<th>Superdex 75 Increase</th>
<th>Superdex 200 Increase</th>
<th>Superose 6 Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractionation range (M_r) Globular proteins</td>
<td>~ 100 to 7000</td>
<td>~ 3000 to 70 000</td>
<td>~ 10 000 to 600 000</td>
<td>~ 5000 to 5 000 000</td>
</tr>
</tbody>
</table>

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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

**Parameter**

<table>
<thead>
<tr>
<th></th>
<th>Tricorn™ 10/300 GL</th>
<th>Tricorn 5/150 GL</th>
<th>3.2/300</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bed dimensions</strong></td>
<td>10 mm × 300 mm</td>
<td>5 mm × 150 mm</td>
<td>3.2 mm × 300 mm</td>
</tr>
<tr>
<td><strong>Approximate bed volume</strong></td>
<td>24 mL</td>
<td>3 mL</td>
<td>2.4 mL</td>
</tr>
<tr>
<td><strong>Recommended sample volume</strong></td>
<td>25 to 500 µL</td>
<td>4 to 50 µL</td>
<td>4 to 50 µL</td>
</tr>
<tr>
<td><strong>Recommended flow rate</strong></td>
<td>0.8, 0.75, or 0.5 mL/min(^1)</td>
<td>0.45 or 0.3 mL/min(^2)</td>
<td>0.075 or 0.04 mL/min(^3)</td>
</tr>
<tr>
<td><strong>Max. operating flow rate</strong></td>
<td>1.8, 1.6, 1.5, or 1.2 mL/min(^4)</td>
<td>0.75 mL/min</td>
<td>0.15 mL/min</td>
</tr>
</tbody>
</table>

\(^1\)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

#### Additional specifications:

- **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)

Download data files >>
Superdex™ Increase and Superose™ Increase columns for larger volumes (0.5 to 8.5 mL)
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 service¹. Contact your GE 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HiScale 16/40</th>
<th>HiScale 26/40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bed dimensions</td>
<td>16 mm × 400 mm</td>
<td>26 mm × 400 mm</td>
</tr>
<tr>
<td>Approximate bed volume</td>
<td>80 mL</td>
<td>212 mL</td>
</tr>
<tr>
<td>Recommended sample volume</td>
<td>Up to 3.2 mL</td>
<td>Up to 8.5 mL</td>
</tr>
<tr>
<td>Max. operating flow rate</td>
<td>~ 2 mL/min(^*)</td>
<td>~ 4 mL/min(^*)</td>
</tr>
</tbody>
</table>

\(^*\)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 individual 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

**HiScale™ 16/40**

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

**HiScale 16/40**

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

**HiScale 26/40**

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

**HiScale 26/40**

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

---

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
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

- 4°C
- 23°C

**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 >>
Tips for taking care of your precious SEC columns
More on gelifesciences.com/ProteinResearch

Tips for maximizing your SEC column lifetime
Tips and hints for protecting your SEC column investment

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?

Prevent SEC columns drying out
How to connect storage device to your SEC column to protect your column during storage

Excluding air from the column
Tutorial showing how to connect SEC column drop-to-drop to ÄKTA systems
Summary
Adding a SEC polishing step will improve your proteins’ purity

SEC gives highly size homogeneous samples.

GE 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.
Useful tools to ensure successful SEC runs
GE expertise made available for you at gelifesciences.com/SEC
# Ordering information

## Columns with SEC "Increase" resins

<table>
<thead>
<tr>
<th>Product code</th>
<th>Product name</th>
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<tbody>
<tr>
<td>28990944</td>
<td>Superdex™ 200 Increase 10/300 GL</td>
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<tr>
<td>29148721</td>
<td>Superdex 75 Increase 10/300 GL</td>
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<td>29219757</td>
<td>Superdex 30 Increase 10/300 GL</td>
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<td>29091596</td>
<td>Superose™ 6 Increase 10/300 GL</td>
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<td>29321903*</td>
<td>Superose 6 Increase HiScale™ 16/40</td>
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<td>29321904*</td>
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<td>Superdex 200 Increase HiScale 16/40</td>
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<tr>
<td>29321906*</td>
<td>Superdex 200 Increase HiScale 26/40</td>
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<tr>
<td>29321907*</td>
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## HiLoad™ columns

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<td>28989333</td>
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<td>28989331</td>
<td>HiLoad 16/600 Superdex 30 pg</td>
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<td>HiLoad 26/600 Superdex 200 pg</td>
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<td>HiLoad 26/600 Superdex 30 pg</td>
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*New prepacked column, launched Nov. 2018

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## HiPrep™ columns

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<td>HiPrep 26/60 Sephacryl S-500 HR</td>
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</tbody>
</table>

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*These products are available on-demand. Contact your GE representative.
Appendix 1—Column selection
We help you to select the right SEC column!

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

- Peptides or other small biomolecules
- Recombinant tagged proteins
- Antibodies or similar
- Macromolecules, viruses, protein complexes

Smaller molecules

Larger molecules
SEC columns for peptides or other small biomolecules
For more information, click on the column of your interest

- **Sample volume > 0.5 mL**
  - **HiScale™ columns**\(^1\) packed with Superdex™ 30 Increase Mr 100 to 7000 (max. 8.5 mL)

- **Sample volume < 0.5 mL**
  - **HiLoad™ Superdex 30 pg**
    - Mr < 10 000 (max. 13 mL)
  - **Superdex 30 Increase**
    - Mr 100 to 7000

\(^1\)These columns are produced on-demand. Contact your GE representative
SEC columns for recombinant tagged proteins

For more information, click on the column of your interest

- **HiScale™ columns**¹ packed with Superdex™ 75 Increase
  M, 3000 to 70 000 (max. 8.5 mL)

- **HiLoad™ Superdex 75 pg**
  M, 3 000 to 70 000 (max. 13 mL)

- **HiPrep™ Sephacryl™ S-100 HR**
  M, 5000 – 250 000 (max. 13 mL)

- **HiPrep Sephacryl S-200 HR**
  M, 1000 to 100 000 (max. 13 mL)

- **Superdex 75 Increase**
  M, 3000 to 70 000

¹These columns are produced on-demand. Contact your GE representative.
SEC columns for monoclonal antibodies or other antibodies

For more information, click on the column of your interest

HiScale™ columns¹ packed¹ with Superdex™ 200 Increase
Mr, 10 000 to 600 000 (max. 8.5 mL)

HiLoad™ Superdex 200 pg
Mr, 10 000 to 600 000 (max. 13 mL)

HiPrep™ Sephacryl™ S-300 HR
Mr, 10 000 to 1 500 000 (max. 13 mL)

Superdex 200 Increase
Mr, 10 000 to 600 000

¹These columns are produced on-demand. Contact your GE representative
Macromolecules, viruses, large proteins, and protein complexes

For more information, click on the column of your interest

- **Sample volume > 0.5 mL**
  - **Highest resolution**
    - HiScale™ columns
      - packed with Superose™ 6 Increase
      - Mr 5000 to 5 000 000 (max. 8.5 mL)
  - **High resolution**
    - HiLoad™ 16/600 Superose 6 pg
      - Mr 5000 to 5 000 000 (max. 5 mL)
  - **Routine**
    - HiPrep™ Sephacryl™ S-400 HR
      - Mr 20 000 to 8 000 000 (max. 13 mL)
    - HiPrep Sephacryl S-500 HR
      - Mr 40 000 to 20 000 000 (dextrans) (max. 13 mL)

- **Sample volume < 0.5 mL**
  - Superose 6 Increase
    - Mr 5000 to 5 000 000

1 These columns are produced on-demand. Contact your GE representative.
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**

![Graph showing purification and sample load](image)

(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)**

![SDS-PAGE analysis](image)

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, 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, 2532) and chain B (M, 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.

Purification

![Purification diagram]

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

Purity check

Analysis of pool of both peaks from the preparative SEC purification.
SDS-PAGE separation of fluorescent prestained samples on an 8% to 18% gel.