# Cytiva™ superSEC resin

SIZE EXCLUSION CHROMATOGRAPHY

Cytiva™ superSEC resin is a size exclusion chromatography (SEC) resin designed for the purification of large biomolecules such as exosomes — a type of extracellular vesicles — enveloped viruses, plasmids, and mRNA. Cytiva superSEC resin is part of our Custom Designed Media program and is available in 100 mL and 1 L pack sizes. Cytiva superSEC resin is an alternative Sepharose™ CL-2B, which is suitable for purifying exosomes at small scale, most commonly employing gravity flow or low flow below 15 cm/h, with pump-driven chromatography instruments.

**Key benefits include:**

- Efficient industrial-scale purification of large biomolecules based on our well-established platform of SEC resins
- Flexible process design due to large operational window of flow rates and bed heights
- Shorter chromatography cycle time and, consequently, improved productivity compared with Sepharose CL-2B
- Excellent chemical stability

## Resin characteristics

Cytiva superSEC resin is based on an agarose base matrix with good pressure-flow properties and wide pore openings to fractionate exosomes and similarly sized entities from smaller sized impurities like host cell proteins and enzymatically degraded DNA. The features of the base matrix make Cytiva superSEC resin an excellent choice for high-productivity purification of large biomolecules. The hydrophilic nature of the resin minimizes nonspecific adsorption and maximizes recovery. The main characteristics are listed in Table 1.

## BioProcess™ chromatography resins

Cytiva superSEC resin is a member of the BioProcess resins family, which includes purification resins widely used by biopharmaceutical manufacturers. Support for these products includes secure long-term resin supply.


**Fig 1.** Cytiva superSEC resin extends our platform of SEC resins. It is a high-flow alternative to Sepharose CL-2B.

**Table 1.** Main characteristics of Cytiva superSEC resin

| | |
|---|---|
| Matrix | Highly cross-linked agarose |
| Particle size* | 75 µm ($d_{50v}$) |
| Flow velocity† | < 300 cm/h, < 200 kPa, bed height 20 cm |
| Autoclavable | Yes, 20 min at 120°C in pH 7 |
| Fractionation range, peak molecular weight ($M_p$) for dextran acting as surrogate for large biological entities | Approx. $5 \times 10^4$ to $5 \times 10^6$ Da |
| pH stability (operational)‡ | pH 2–12 |
| CIP stability (short-term)¶ | pH 2–14 |
| Chemical stability | Stable in commonly used buffer solutions: 1 M sodium hydroxide, 1 M acetic acid, 8 M urea, 6 M guanidine hydrochloride, 70% ethanol, 30% isopropanol |
| Shelf life | Five years |
| Storage conditions | 20% ethanol at 4°C to 30°C |

*$d_{50v}$ is the median particle size for the cumulative volume distribution.
† Flow velocities are dependent on the column used.
‡ pH interval where the resin can be operated without significant change in function.
¶ pH interval where the resin can be subjected to cleaning in place (CIP) without significant change in function.

# Selectivity for Cytiva superSEC resin

Selectivity is a measure of the relative retention of two solutes in a column and is represented by the distance between two eluted peaks. The selectivity of an SEC resin is an inherent property of the matrix and depends on several factors including its pore size distribution. $K_D$ and $K_{av}$ are distribution coefficients describing how a molecule is distributed between the stationary phase, the resin, and the mobile phase. The coefficients represent the fraction of the resin's stationary phase that is available for diffusion of a specific molecule and provides an indication of the selectivity for a SEC resin for a given sample molecule. The $K_D$ is a distribution coefficient and $K_{av}$ is a partition coefficient. $K_{av}$ is less sensitive to variations in column preparation and column dimension than $K_D$. (see chapter "Size exclusion chromatography in theory" in Size Exclusion Handbook, Size Exclusion Chromatography, Principles and Methods (cytiva.com).

The $K_D$ distribution coefficient of Cytiva superSEC resin is determined using mixtures of dextran molecules with different peak molecular weights, $M_p$. From this quality control analysis, the $K_D$ for a dextran mixture with $M_p$ at 110 kDa is calculated and used as a release criterion for Cytiva superSEC resin. This release criterion also indicates the selectivity for larger molecules (see Fig 2).

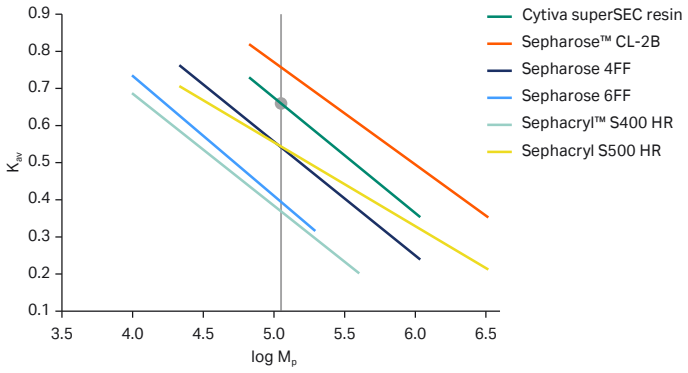


**Fig 2.** Plot illustrating the average selectivity for Cytiva superSEC resin, Sepharose CL-2B, and other SEC resins in our portfolio. The data is generated using dextrans. The vertical gray bar indicates the calculated peak molecular weight, $M_p$, used for release of Cytiva superSEC resin.

# Application: purification of exosomes from human mesenchymal stem cells (hMSCs)

hMSCs are the most common exosome producer cell type used for clinical trials. In this application we used hMSCs to produce exosome-rich cell culture supernatant (CCS). The hMSC-exosome concentration in the sample material after tangential flow filtration was $2.2 \times 10^{10}$ particles/mL (10-fold concentrated from the starting material). Fractions (4 mL) were collected from the SEC runs with

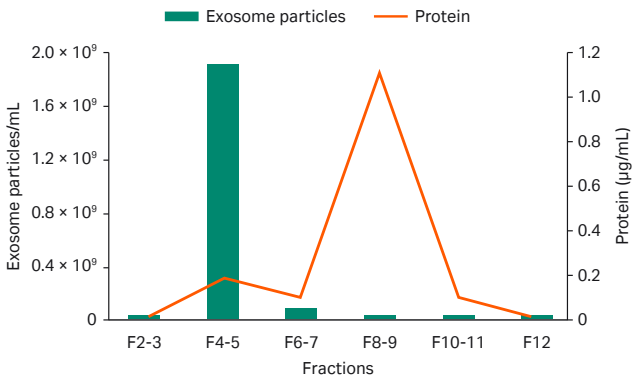


**Fig 3.** Particle concentration and protein concentration in fractions collected from purification of exosomes using Cytiva superSEC resin packed in HiScale 26/40 column at 20 cm bed height.

Cytiva superSEC resin packed in a HiScale™ 26 column at 20 cm bed height. Fractions were analyzed with nano tracker analysis and BCA assay for total protein analysis. Figure 3 shows that hMSC-exosomes were most concentrated in the early fractions and proteins eluted later. Hence, in this application Cytiva superSEC resin gives good resolution between particles and protein impurities in the exosome feed.

# Stability for cleaning

Cleaning in place (CIP) is a cleaning procedure to remove contaminants such as lipids, precipitates, or denatured proteins that might remain in the packed column after regeneration. Regular CIP also prevents the build-up of these contaminants in the chromatography bed and helps maintain the capacity, flow properties, and general performance of the resin. The frequency of CIP depends on the nature and the condition of the starting material, but one CIP cycle is generally recommended every one to five separation cycles. Furthermore, a specific CIP protocol should be designed for each process according to the type of contaminants present.

Cytiva superSEC resin withstands standard CIP solutions such as 1 M NaOH, 2 M NaCl, and 70% ethanol, or combinations thereof. The resin is also stable to commonly used aqueous buffers, ionic detergents, nonionic detergents, and polar solvents.

Regular sanitization in place (SIP) will inhibit microbial growth and maintain a high level of hygiene in the packed column. A specific sanitization protocol should be developed according to the nature and condition of the starting material. Sanitization protocols based on 0.5 to 1.0 M NaOH can be used for Cytiva superSEC resin. The resin is suitable for packing into single-use ReadyToProcess™ columns by our custom ReadyToProcess column packing service. Contact your local sales representative for more information. The ReadyToProcess columns with Cytiva superSEC resin can be presanitized with 1 M NaOH and 30% isopropanol.

## Storage

The recommended storage conditions are 20% ethanol at 4°C to 30°C. Cytiva superSEC resin is supplied as a suspension in 20% ethanol.

## Ordering information

| Product | Volume | Product code |
|---|---|---|
| Cytiva superSEC resin | 100 mL | 17553002 |
| | 1 L | 17553003 |
| HiScreen™ Cytiva superSEC columns – available via custom designed products (CDP) | 4.7 mL | 17553010 |

For other R&D columns contact customproductsls@cytiva.com.
For more information, including custom packing of ReadyToProcess columns, proceed to the Related services section, Custom Column Packing and click the hyperlink.

| Related literature | Product code |
|---|---|
| Handbook: Size-exclusion chromatography, principles and methods | CY12707 |
| Article: Exosome purification with core bead technology | CY28507 |
| App note: Purification of exosomes with TFF and SEC | CY38458 |

## Related services

Our Fast Trak™ training and education team provides high-level, hands-on training for all key aspects of bioprocess development and manufacturing. Web-based e-learnings are also available. See Biotechnology and biopharma online courses | Cytiva (cytiva.com) or reach out to your local Cytiva representative for more information.

If you want help with customized column packing this service is available. See Custom Column Packing | Cytiva (cytiva.com) or reach out to your local Cytiva representative for more information.

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