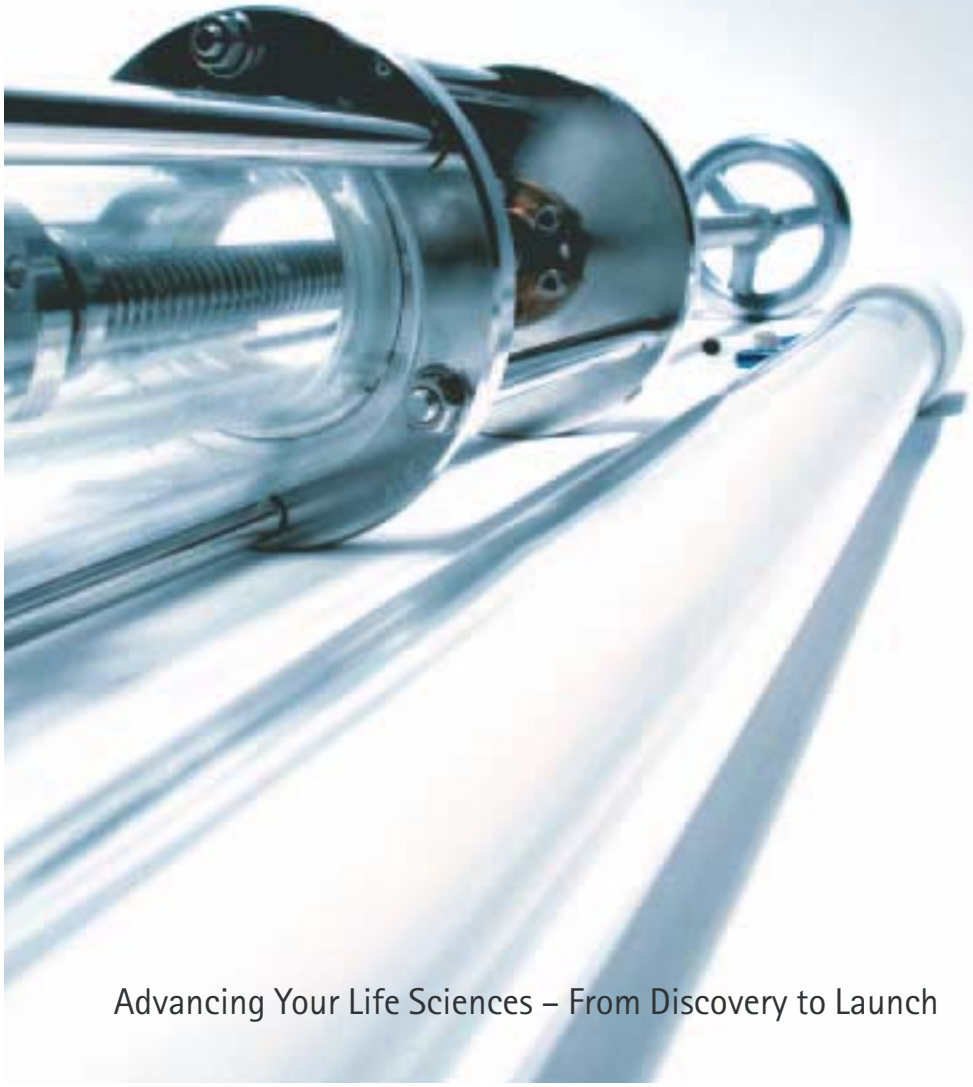
Fractogel®
EMD BioSEC

Best results in size exclusion chromatography



Advancing Your Life Sciences – From Discovery to Launch

MERCK

Fractogel® EMD BioSEC

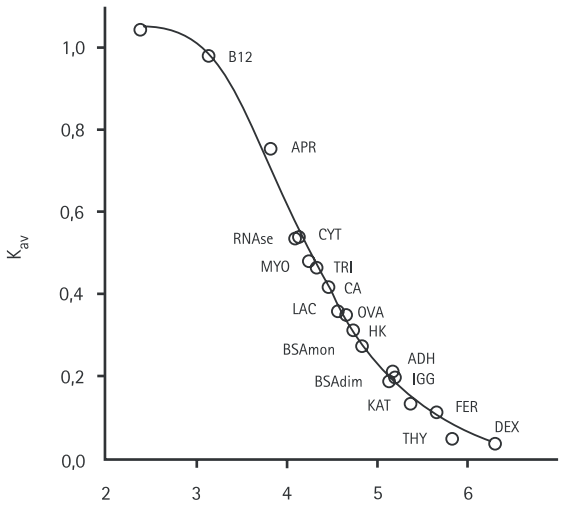
Size exclusion chromatography is the method of choice for polishing of recombinant proteins, viruses and plasma derived bio therapeutics. Fractogel® EMD BioSEC is the only stationary phase for production scale that is pressure stable and allows high performing chromatographic steps. The benefits include a shorter time to the market with simple and straightforward transfer from lab-scale to production scale columns. Thus, Fractogel® EMD BioSEC improves process economics and contributes to a safe production of biologics.

Size exclusion chromatography on Fractogel® EMD BioSEC

Fractogel® EMD BioSEC is based on Fractogel® EMD, the well established chromatographic matrix known for more than 15 years. Due to the tentacle technology used for the surface modification, the ligand density of the functional group can be chosen in such a way that the chromatographic support gives an excellent separation of proteins according to their size and shape. The dynamic pores contribute to an increased selectivity for the size-dependant migration of molecules. Fractogel® EMD BioSEC can be used for the separation of proteins in the range between 5 kDa and 1,000 kDa **(Fig. 1)**.

Due to the unique tentacle matrix, very high recoveries can be obtained using Fractogel® EMD BioSEC.
In comparison to known soft gels, the mechanical stability of Fractogel® EMD BioSEC allows for higher flow rates. This feature is very helpful during equilibration and regeneration of the columns. The pressure stability also facilitates the packing of larger columns that are sometimes used as polishing steps in the production of pharmaceutical proteins. In addition, high stability against alkali treatment enables users to set up production scale separations on Fractogel® EMD BioSEC.

Fig:1
Selectivity curve of Fractogel® EMD BioSEC (S) obtained from a column with a length of 600 mm and an inner diameter of 16 mm (121 ml bed volume). The results are derived from various standard proteins with known molecular weights. The KD-values of the individual proteins were determined by plotting their elution volumes against the logarithm of the molecular weight. The linear flow rate was 30 cm/hr with 20 mM sodium phosphate buffer (pH 7.2) containing 0.3 M NaCl as eluent.



Your advantages

high mechanical strength	►	easy to pack
high pressure stability	►	short separation times; high throughput
high selectivity	►	high purity
tentacle modification	►	excellent recoveries

Ordering information

Description	Particle size	Content	Ord. No.
Fractogel® EMD BioSEC (S)	20 - 40 µm	150 ml	1.10317.0150
Fractogel® EMD BioSEC (S)	20 - 40 µm	250 ml	1.10317.0250
Fractogel® EMD BioSEC (S)	20 - 40 µm	1000 ml	1.10317.1000
Fractogel® EMD BioSEC (S)	20 - 40 µm	5000 ml	1.10317.5000

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Amino acids; High-quality mineral salts and buffers;
Biological buffer substances; ACS grade reagents
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Column dimensions and protein load

As with all size exclusion chromatography columns, packing must be carried out very carefully to obtain optimal results. For laboratory purposes the column dimensions should range from 600 x 16 mm to 1000 x 50 mm. A standard separation is shown in **Fig. 2**. The volume of the loaded sample should not exceed 5 % of the column volume for preparative runs and no more than 1 % for analytical applications. For production scale separations, column diameters from 100 up to 300 mm and gel bed lengths up to 1200 mm can be used **(Fig. 3)**. Resolution is not correlated to the total amount of protein loaded on the column. Hence, the best separations will be obtained with more highly concentrated protein samples.

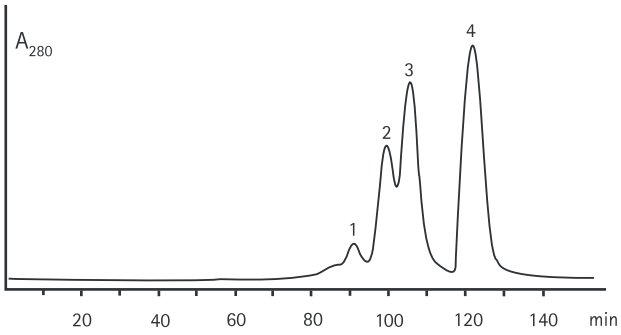

Fig. 2.
Separation of a standard protein mixture on a (600 x 16) mm Fractogel® EMD BioSEC column. The sample contains BSA (peak 1, dimer of BSA, peak 2 monomer of BSA), ovalbumin (peak 3) and cytochrome c (peak 4). 500 µl of the sample were loaded at a flow rate of 1.0 ml/min (30 cm/hr) using 20 mM sodium phosphate buffer containing 0.1 M NaCl (pH 7.2) as the eluent.

Flow rates

As a basic rule for all SEC applications, it has to be kept in mind that the flow rate significantly affects resolution. In general, running the column at a low flow rate results in higher resolution, but diffusion may occur, if the flow rate is too low. The recommended flow rates which are dependant on column diameter should not be exceeded. Good results can be obtained using a 16 mm inner diameter column and a bed height of about 600 mm. Linear flow rates of about 10 - 80 cm/hr are recommended. However, depending on the difference in size of the analytes, sometimes only a slight loss of resolution can be obtained at flow rates up to 150 cm/hr.

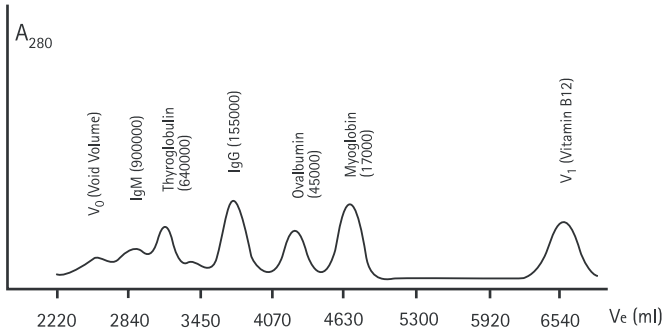

Fig. 3.
Preparative separation of standard proteins on Fractogel® EMD BioSEC (S). The length of the column was 1000 mm and the inner diameter 100 mm. The flow rate was 6.2 ml/min with 20 mM sodium phosphate buffer (pH 7.2) containing 0.3 M NaCl as eluent.

Application areas

SEC is a very mild separation method because any desirable buffer system can be used. Thus, optimal conditions with respect to the protein stability can be selected. Since no gradients are used for elution, the equipment necessary for SEC is also rather simple. In addition to protein fractionation, this method is also applicable for concurrent buffer

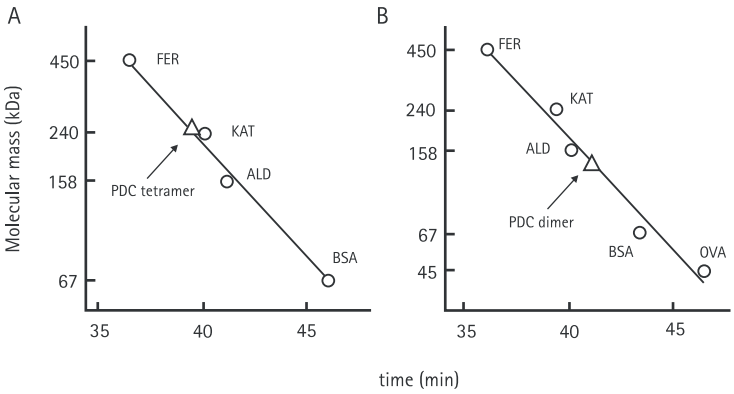

Fig. 4.
Determination of the molecular mass of of pyruvate decarboxylase (PDC) from yeast. A Fractogel® EMD BioSEC (S) column with dimension of 600 - 16 mm was equilibrated with 0.02 M Na/Mes pH 6.0 (A) and 0.02 M Na/Tricine pH 9.0 (B).

References

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exchange that may be necessary to prepare the sample for the next unit operation. For example, a protein sample can be loaded onto a SEC column after ammonium sulfate precipitation. After SEC with concomitant removal of the salt, the desired protein fraction can be loaded directly onto ion exchange or affinity chromatographic columns by selecting the appropriate buffer. As a final polishing step, monoclonal antibody oligomers can be removed by SEC, and the monomer eluted directly into formulation buffer.

Since the migration distance is correlated to the size of the molecule, the size exclusion technique can also be used for the determination of the apparent molecular weight of a protein. However, not only the size, but to a certain extent the shape of the protein is important for the retention time. Thus, calibration of the column must be performed very carefully with appropriate calibration proteins. The void volume should be determined by loading a solution of Blue Dextran (5 mg/ml). Yeast pyruvate decarboxylase provides an example of the use of size-exclusion chromatography for the estimation of the molecule's size. At low pH (0.02 M Mes buffer, pH 6), the enzyme forms catalytically active tetramers of about 240 kDa, at higher pH conditions (0.02 M Tricine, pH 9) the enzyme exists as a dimer. Both forms can be observed by SEC on Fractogel® EMD BioSEC **(Fig. 4)**.

Hands-on tips

To avoid non-specific interactions between the proteins and the matrix the buffer should contain 50 to 300 mM NaCl. Very high concentrations of salt are not suitable since protein precipitation may occur (salting out). Hydrophobic interactions at high salt concentrations may also affect the size exclusion mechanism resulting in a poor resolution or in incorrect values with respect to the estimated molecular weight of unknown proteins. SEC with Fractogel® EMD BioSEC is also a powerful tool for the final chromatographic step in many protein purification protocols. Even at production scale, SEC contributes to the removal of viruses and DNA. For the determination of the molecular weight of an unknown protein it must be noted that the actual value sometimes appears to be different from known standards. Therefore, the term "apparent" molecular weight should be used for results obtained by SEC. Notice, that, if the K_D-values are above 1, there must be nonspecific interaction between the target molecule and the matrix. In the case of K_D-values below 0 the column packing is probably damaged.

Regeneration

Regeneration of Fractogel® EMD BioSEC (S) can be achieved with 0.1 up to 0.5 M NaOH. Alternatively, sodium lauroyl-sarcosinate can be used. If necessary, the column can be washed with 20 % ethanol. The latter solution is also recommended as the storage medium.
In the presence of organic solvents, an upper column pressure drop limit of 1 bar should be not exceeded.
All equilibration and regeneration steps may be performed at high linear flow rates (for aqueous buffers or solutions up to 150 cm/hr) due to the excellent mechanical stability of the Fractogel® EMD matrix. Fractogel® EMD BioSEC (S) can also be sterilized repeatedly using an autoclave without any loss of resolution.

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