

Improved Dynamic Binding Capacity for the Purification of Large Biomolecules

Biomolecules have been of great interest to research and industry for a long time. This trend is continuing, but the targets are constantly changing and with them the requirements for chromatographic purification strategies.

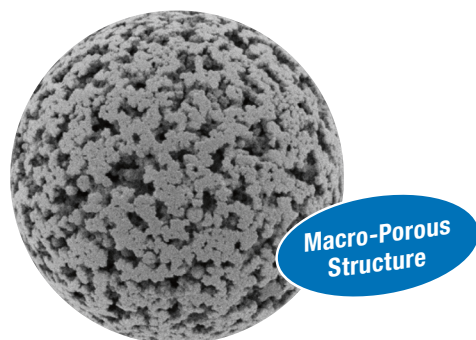
The development of new therapeutic strategies such as gene therapy is drawing attention to larger substances. But these large targets require purification techniques specially adapted to their properties.

Why Choosing the Right Process Resin is so Important

YMC offers an innovative process resin that has been developed specifically for the purification of large bio-therapeutics. Compared to other IEX resins, MacroSep IEX Q provides a macro-porous structure and an improved dynamic binding capacity (DBC) for these large molecules. The large pore size of 900 nm enables target molecules to diffuse properly to the binding sites. Only with this innovative macro-porous structure the required interaction with the stationary phase can be achieved. In addition to resins in bead format also mon-

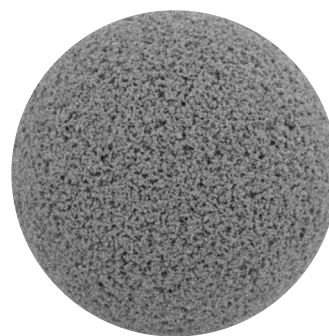
olithic stationary phases are widely used in the industry. These phases were developed to improve accessibility to the functionalities. This Technical Note demonstrates that the pore structure of MacroSep IEX Q is optimally suited for the purification of large molecules and preferable to monolithic stationary phases. The polymer-based resin is also convincing with its better separation performance, full scalability and higher robustness. Therefore, it is the perfect addition to DSP platforms for a successful and productive purification.

MacroSep IEX Q (30 µm)



Ideal choice for the purification of large biomolecules and particles such as AAV, VLPs and large nucleic acids

BioPro IEX (30 µm)



Ideal choice for the purification of smaller and mid-sized biomolecules such as proteins, antibodies and oligonucleotides

Determining the Dynamic Binding Capacity

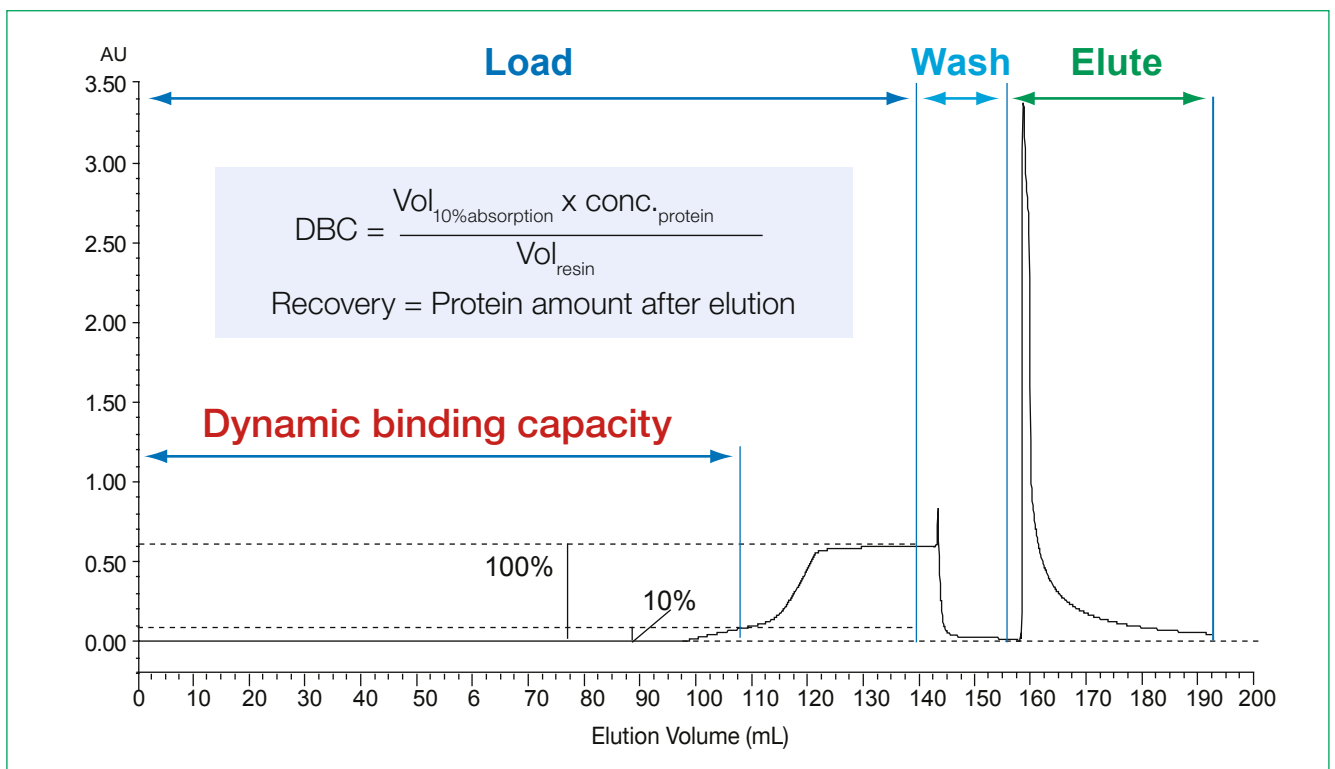
When selecting a chromatography resin for downstream processing (DSP), the focus is on high productivity. The higher the DBC of a resin, the more substance can be

loaded onto the column and consequently the higher the throughput of the process.



The DBC varies significantly between different target molecules, as it is influenced by a wide range of physicochemical and structural factors. For instance, molecular size, overall charge, and charge distribution play a critical role in determining the extent of interaction with the chromatographic matrix. In this Technical Note, we illustrate these effects using two distinct examples.

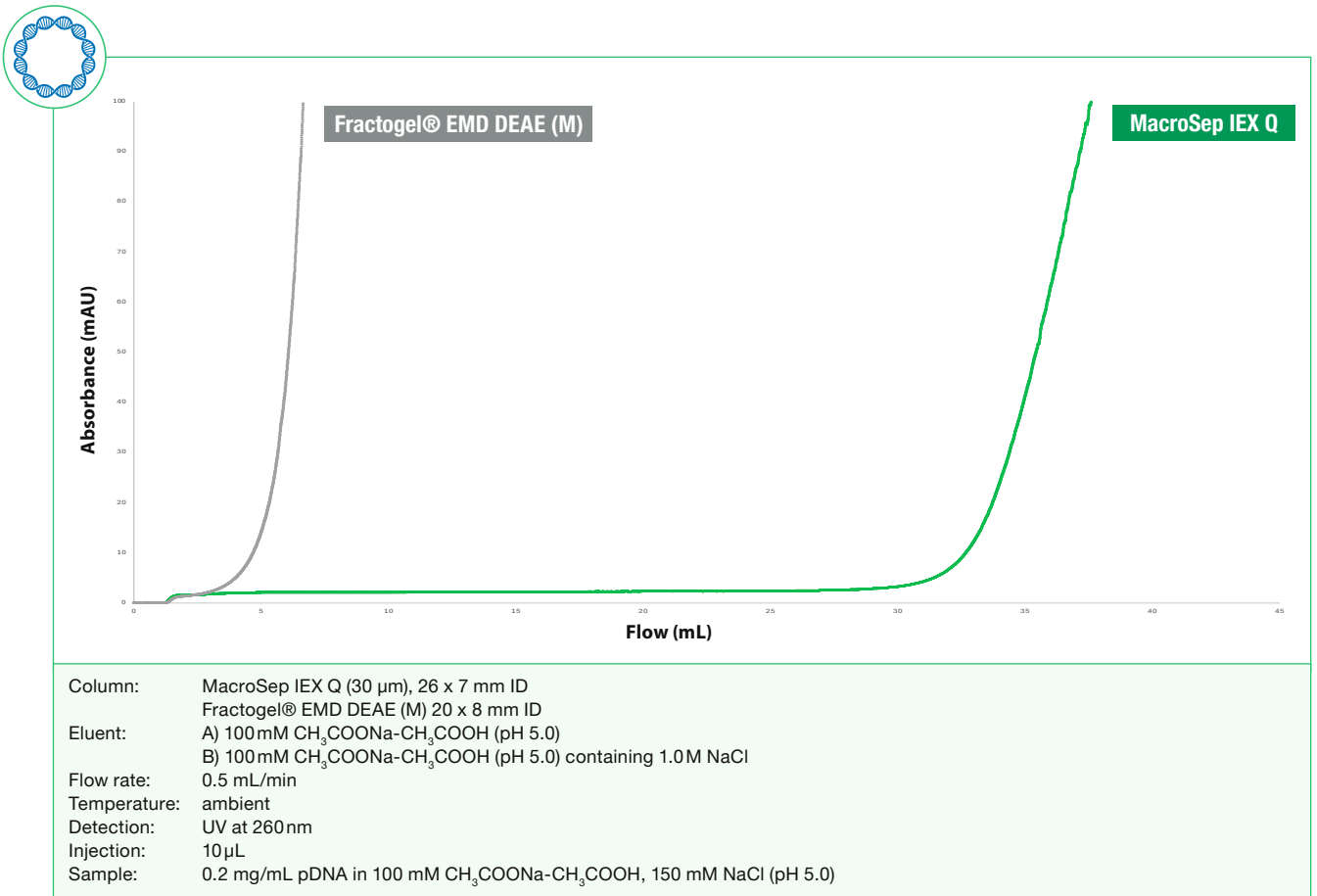
As the DBC is a real process parameter, it is determined with the help of the chromatographic set-up. For the determination, a defined amount of substance is pumped through the column and the breakthrough point of the target is measured. In a following step, the DBC per mL of resin is calculated.



Mastering the Challenge of pDNA Purification

The breakthrough curves of two different process resins for a Plasmid DNA with a size of 4.9 kbp are measured. Due to its size, resins with a standard pore size are unsuitable for purification of pDNA. The smaller

pore size prevents the diffusion of large biomolecules into the pores. As a result, they can only interact with the functional groups located on the surface and the loadability is significantly reduced.



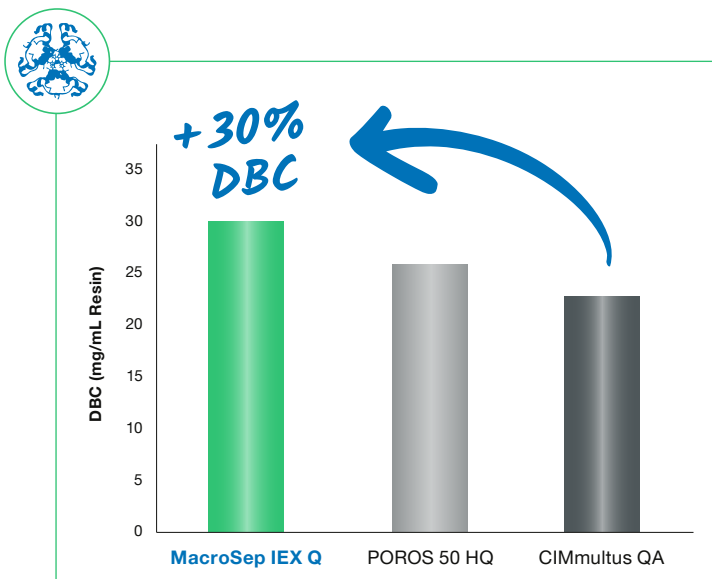
This is also shown by the results: MacroSep IEX Q shows a later breakthrough of the pDNA compared to the alternative material exhibiting a pore size of about 80 nm. Therefore, the resin from YMC enables a significantly higher loading and thus contributes to a higher productivity of the overall process.

Resin	DBC (mg/mL Resin) 10% Breakthrough
MacroSep IEX Q	7.5
Fractogel® EMD DEAE (M)	1.3

Comparative Analysis of DBC for Large Protein Complexes

Since DBC is an important parameter, it is advisable to consider it when selecting an appropriate process resin. Although the DBC varies depending on the target molecule, values obtained using representative model compounds provide a useful basis for comparison. The following overview shows a comparison of DBC values of three different AEX materials that are commonly used for the purification of large biomolecules. For

this study, the large protein complex Thyroglobulin with a molecular weight of 660 kDa was used. Based on the results MacroSep IEX Q shows the highest DBC. A larger amount of the protein complex could be bound to the resin before breakthrough was achieved. Compared to the other resins MacroSep IEX Q shows a 30% higher DBC making it the first choice resin for the purification of large biomolecules.



Resin	DBC (mg/mL Resin) Thyroglobulin MW 660 kDa
MacroSep IEX Q	30
POROS 50 HQ	26
CIMmultus QA	23

MacroSep IEX Q: The Resin of Choice for High Throughput

The dynamic binding capacity is an essential factor to increase the productivity of downstream processes. The higher the binding capacity, the higher is the maximum loadability of the target feed which consequently increases the throughput. The challenging purification of large biomolecules demands process resins that are

precisely adapted to the corresponding requirements. In a direct comparison of the specified DBC values for pDNA and Thyroglobulin, MacroSep IEX Q clearly outperforms the alternative resins used for process scale and is therefore the ideal choice for every DSP platform.

Optimise your IEX process with MacroSep IEX Q!