

PROSEP®-A High Capacity: the Throughput and Productivity Leader for Monoclonal Antibody Capture

PROSEP-A High Capacity protein A affinity medium is optimally designed for ultra high throughput capture of monoclonal antibodies (MAbs) at the process scale.

TECHNICAL BRIEF

The MAb Processing Challenge

Clinical administration of MAb therapies often involves lengthy treatment programs and relatively high doses. The resulting demand for multi-ton/yr quantities has created a need for faster, more economical production methods. A growing number of therapeutic antibodies are produced at the $\geq 10,000$ L fermentation scale, necessitating very high throughput capability at initial capture.

At the capture phase of MAb purification, the primary goals are (1) to isolate the product of interest as quickly as possible to minimize its exposure to the proteases present in the harvested culture medium, (2) to reduce the process volume to a manageable scale for subsequent steps, and (3) to maximize yield and economy. These goals are best achieved using a highly selective ligand combined with a support that is optimized for maximal throughput and productivity.

The extreme throughput and productivity requirements challenging the full-scale operation amplify a set of capture media characteristics that are not always apparent to the bench-scale researcher.

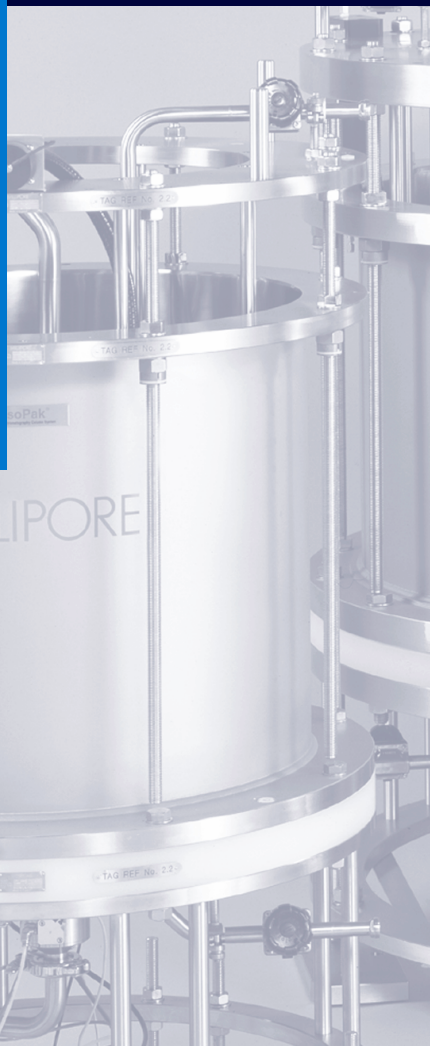
To maximize productivity ($\text{g protein} \times \text{h}^{-1} \times \text{L bed volume}^{-1}$) adsorptive media must possess high volumetric

throughput capability and high dynamic capacity. A rigid matrix clearly offers the best flow rate capability, but for this superiority to be realized, it must be combined with highly favorable mass transport of target solutes to and from available binding sites. The resulting ability to capture target IgG, wash unbound contaminants and elute the bound product quickly, while using minimal media volume, is at the basis of productivity optimization.

PROSEP-A Meets the Challenge

PROSEP-A High Capacity media is based on a porous glass matrix that is fully incompressible yet highly porous, with a very high percentage of large, open-ended, interconnected pores. Its open pore structure allows very rapid mass transport, resulting in very high dynamic capacity for IgG. Its high dynamic capacity is maintained at flow rates ranging beyond those possible with state-of-the-art agarose or polymeric matrices.

The following summarizes the attributes and performance characteristics of PROSEP-A media which enable the very high productivity levels and economy needed for large scale capture chromatography of this class of biotherapeutics.



Volumetric Throughput

The absolute rigidity of the PROSEP-A porous glass matrix enables operation at >1000 cm/h without the media particle deformation and bed compression experienced by even the newest generation of hardened agarose media. Pressure vs. flow data are presented in Figure 1.

Dynamic Binding Capacity

The large, interconnected pores of the PROSEP-A base matrix enhance mass transfer and thereby enable IgG capture at very high dynamic capacity even at very high flow rates.

Binding capacity determinations often focus on total mass loaded at 10% breakthrough. However, in actual processes, one maximizes product yield by loading to sub-saturating levels, therefore the truly process-practical approach is to establish working capacity using 1% breakthrough. As illustrated in Figure 2, IgG breakthrough occurs later (at greater mass load) and with a sharper profile for PROSEP-A media than for agarose media. The result is higher capacity at 1% breakthrough.

A plot of capacity vs. residence time (Figure 3) shows the comparatively higher IgG capacity of PROSEP-A vs. agarose at 1% breakthrough at velocities which result in residence times below 3 minutes.

Pressure/Flow Curves

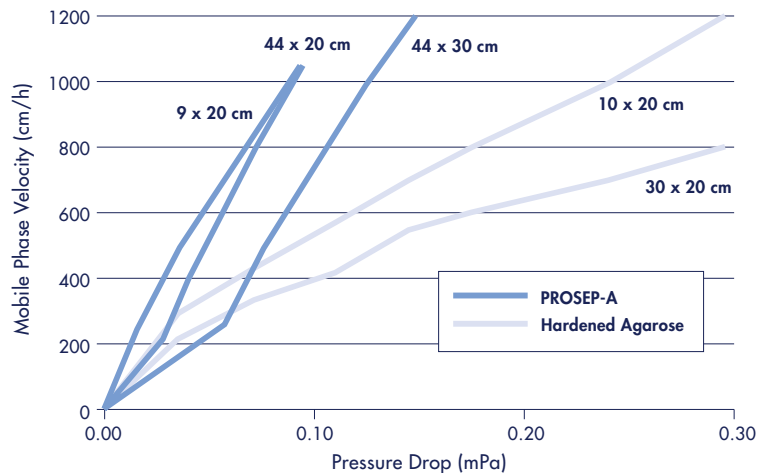


Figure 1: Pressure/flow curves for PROSEP-A media and hardened, highly cross-linked, agarose.

Breakthrough Curve Comparison

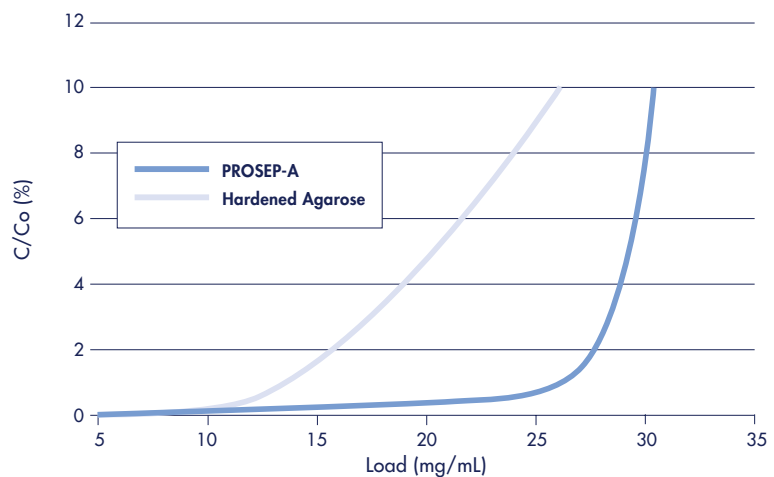


Figure 2. Human polyclonal IgG breakthrough curves, PROSEP-A vs. hardened agarose media at 500 cm/h at a 19 cm bed height.

Productivity

The combination of superior flow properties and excellent mass transfer enables IgG capture using PROSEP-A at velocities as much as 50% faster than any other commercialized capture media.

The ability to load, wash and elute from PROSEP-A at flow rates >500 cm/h, yields volumetric productivity ($\text{g} \times \text{h}^{-1} \times \text{L bed volume}^{-1}$) and areal productivity ($\text{g} \times \text{h}^{-1} \times \text{L bed volume}^{-1}$) that both surpass levels possible with state-of-the-art agarose media.

The ability to use PROSEP-A media at bed heights exceeding 20 cm, enables the use of smaller, less-costly columns, thus providing yet another means of increasing areal productivity and lowering costs beyond levels possible with other media.

Rapid mass transfer also enhances the efficiency of the post-load wash and elution steps. This, combined with the high flow rate capability of PROSEP-A media, permits faster cycling with reduced buffer consumption, resulting in additional productivity gain vs. conventional media.

Process Flexibility

A common operational goal of the capture step is complete product recovery from a single fermentation batch within an 8-hour work shift. Rapid cycling is one method by which product capture and recovery can be accomplished with reduced chromatography media volume.

An example of process optimization using PROSEP-A media is illustrated in Table 1. By decreasing bed volume, increasing flow velocity, and adding one extra cycle, significant process economy is obtained while accomplishing complete product capture within the same time frame as a conventional hardened agarose. The choice of column geometry in this example provides additional savings due to the use of lower cost column hardware and less plant area.

Dynamic Capacity vs. Residence Time

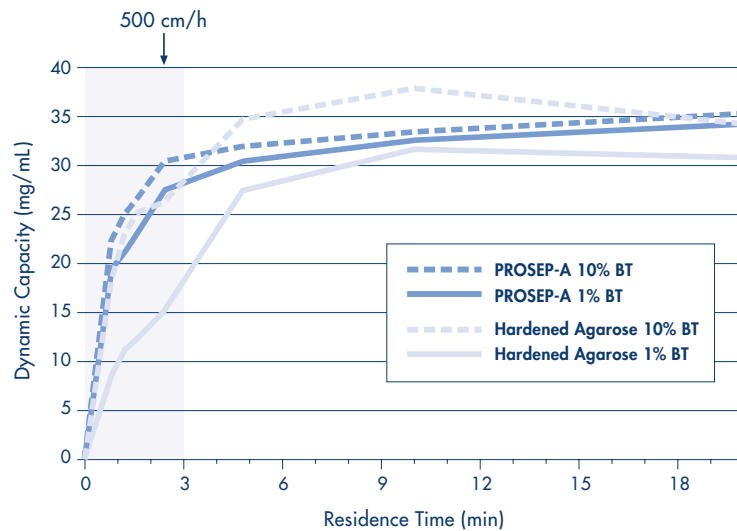


Figure 3. Dynamic capacity vs. residence time, PROSEP-A vs. hardened agarose media (bed height = 19 cm). Feed: 1 mg/mL human polyclonal IgG.

Typical Process Optimization

	Hardened Agarose	PROSEP-A
Feed Volume (L)	10,000	10,000
Feed IgG Concentration (g/L)	1	1
Loading Velocity (cm/h)	500	750
Elution/Regeneration Velocity (cm/h)	500	1050
Column Diameter (cm)	80	60
Bed Height (cm)	20	30
Bed Volume (L)	100	85
Dynamic Binding Capacity (g/L)	20	20
Number of Cycles	5	6
Buffer Usage Ratio*	X	0.9X
Volumetric Productivity ($\text{g} \times \text{h}^{-1} \times \text{L}^{-1}$)	12.9	15.1
Areal Productivity ($\text{g} \times \text{h}^{-1} \times \text{cm}^{-2}$)	0.3	0.4
Total Time (h)	7.8	8.0

Table 1. Typical process optimization using PROSEP-A.

* The buffer usage ratio is based on number of cycles and bed volume and assumes approximately 15% fewer bed volumes buffer required at post-load wash, elution, and post-elution regeneration/sanitization steps for PROSEP-A media (vs. agarose media).

Areal Productivity

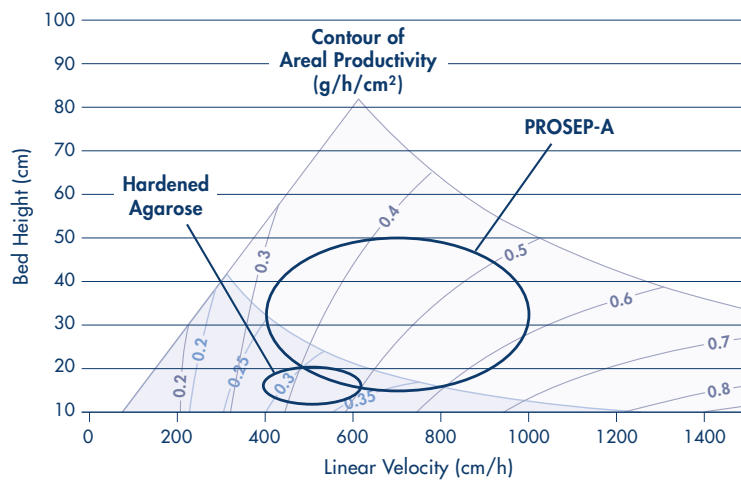


Figure 4. Areal productivity at various bed heights and flow velocities. Ovals delineate functional ranges of bed height and velocity for each media type.

A comparison of the possible operating ranges for PROSEP-A and hardened agarose media (Figure 4) provides an illustration of the wide flexibility in process parameters made possible by the unique properties of PROSEP-A. Process flexibility can reduce the need for replacing existing equipment when processing needs change. As shown above, it can also provide better overall process economy.

Summary

PROSEP-A is the most effective capture tool to address the therapeutic MAb producers' expanding needs for production speed, capacity and economy.

To learn more about the benefits of PROSEP-A High Capacity Media and other Millipore Chromatography products, please contact your local Millipore technology specialist.

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