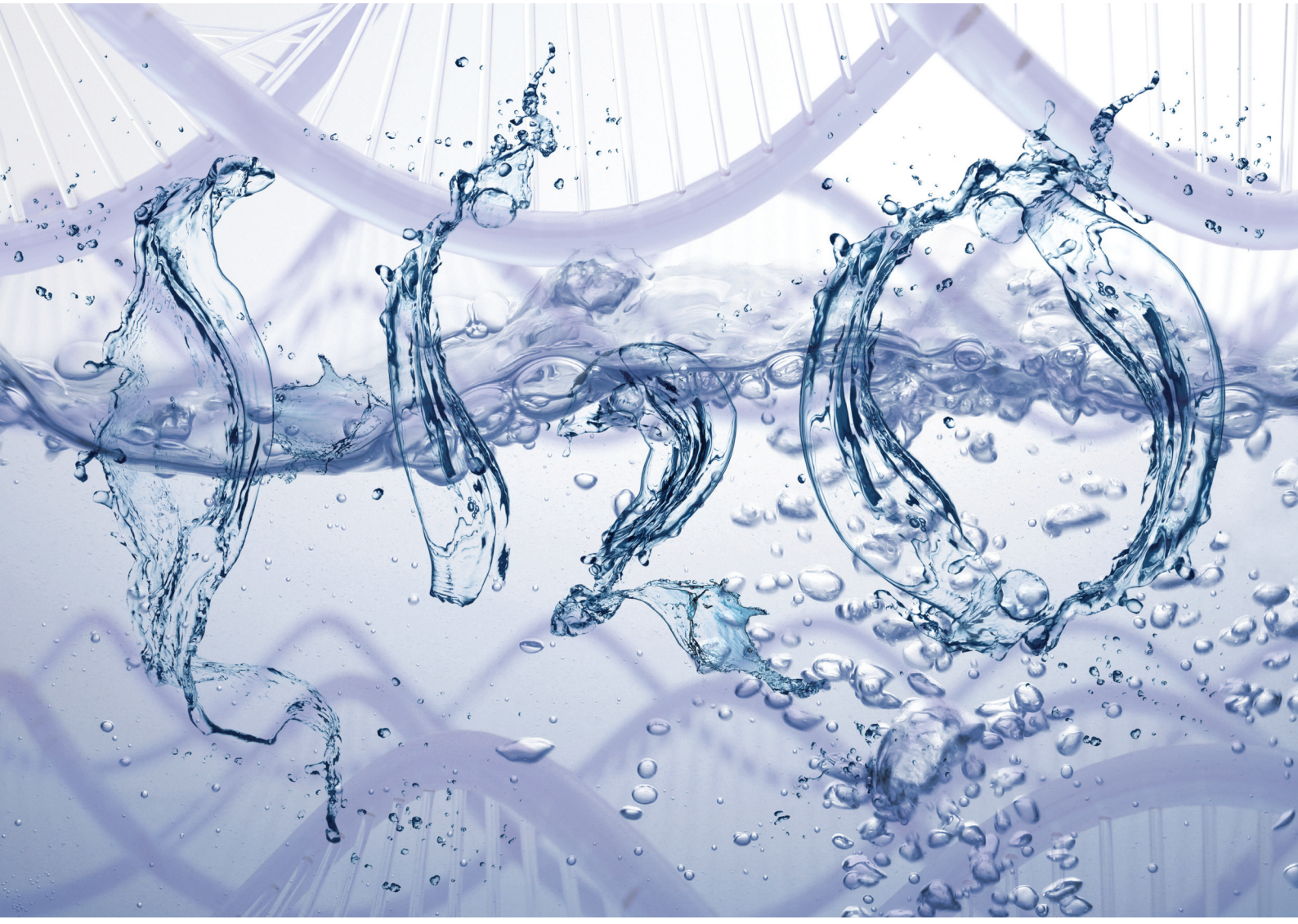
TOSOH

Toyopearl GigaCap® Resins for Reducing Downstream WFI Consumption



• Toyopearl GigaCap CM-650M • Toyopearl GigaCap S-650M • Toyopearl GigaCap Q-650M

TOSOH BIOSCIENCE

Introduction

Advances in upstream expression have significantly increased bioreactor protein titers. Many of today's processes have their expressed biologics in the 2.5-3.5g/L concentration range. Several reports indicate concentrations greater than 10g/L have been achived.

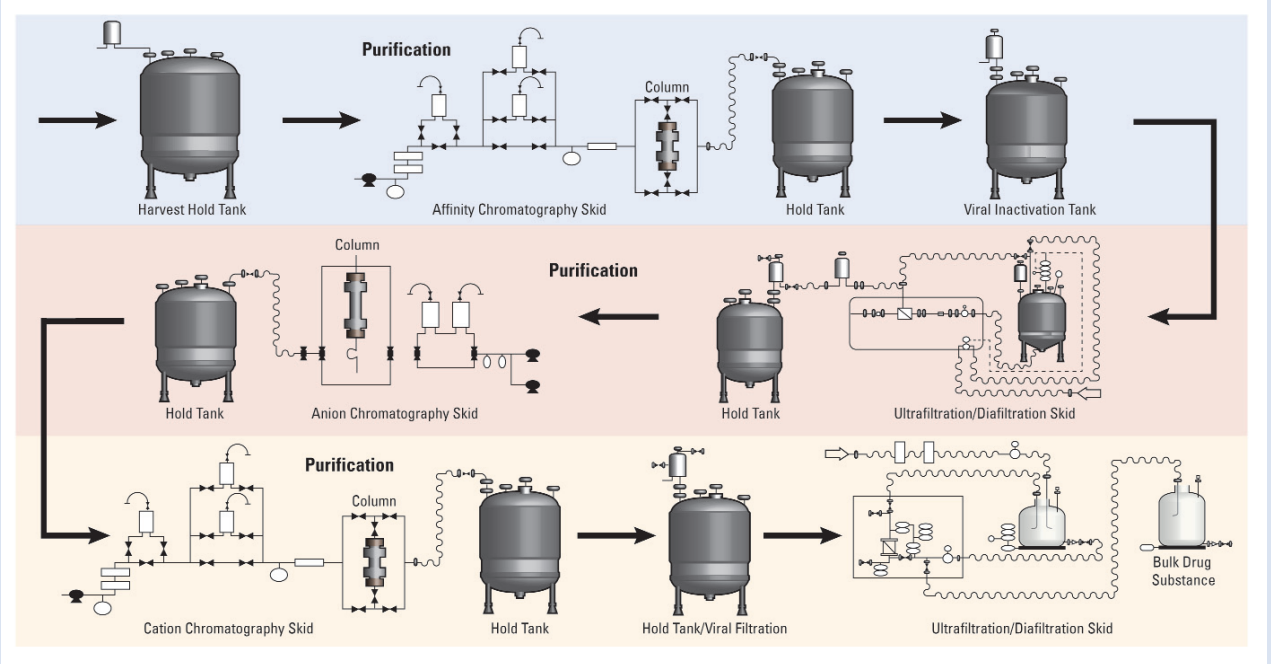
By optimizing their expression systems, upstream engineers can increase the quantity of active biologic produced in their pre-existing bioreactors. For downstream purification, the given equipment and its plant footprint may not be readily able to handle the increased biomass originating upstream.

A critical issue is the proportionality of target titer in the bioreactor to the volume of WFI (water for injection) needed for purification. If a bioreactor in a given process is producing a target at a 1g/L titer and downstream needs 10,000L of WFI to process it, then the same bioreactor with an improved titer of 3g/L will require 30,000L of WFI to process the increased protein mass. In some cases, 25,000L of feedstock may require 125,000L+ of WFI per process cycle (includes affinity, cation, and anion chromatography steps as well as UF/DF) (Figure 1).

The existing downstream plant footprint becomes a constraining factor as these higher volumes of water are demanded. The number of manufacturing cycles will therefore need to be expanded. Existing tanks/bags (Figure 2) for buffer preparation and in-process hold steps may be increased in size or number. Inline buffer dilution could be used, but the increased volume of WFI needed would remain at an elevated level. At some point, larger chromatography columns could be incorporated, or resins changed to higher capacity versions, to accommodate the increased kg/yr requirements.

The bottleneck forms as titers increase. At some point a new plant or new production process may be justified.

Figure 1: Typical downstream unit operations



Toyopearl GigaCap resins can reduce WFI consumption through high DBC

When chromatographic resins are screened for the capture or concentration step, the first performance parameter evaluated is dynamic binding capacity (DBC) for the target molecule (Table 1). Higher resin binding capacity allows more feedstock biomass to be processed per liter of resin. When considering several resin candidates with equivalent final purity and yield, the one with the highest capacity is selected. Having a smaller resin column volume also normally leads to reduced buffer/WFI consumption for CIP, re-equilibration steps, and later downstream steps.

However, all of the benefits to be derived by reducing the amount of WFI needed for the capture step can be undone for further downstream steps if the selected resin's target elution peak tails.

Figure 2: Downstream container modules

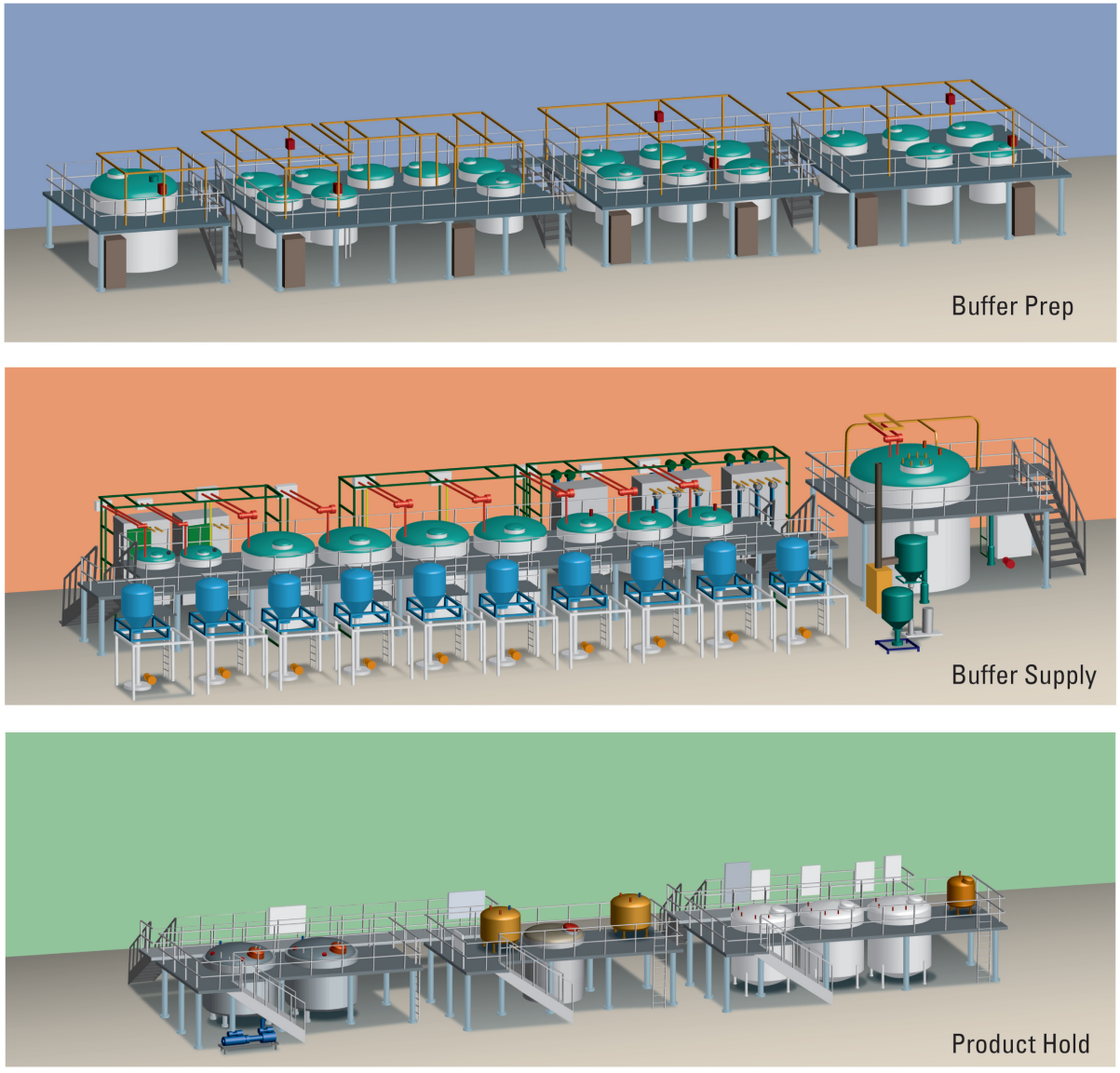


Table 1: Toyopearl GigaCap resins dynamic binding capacities (DBC)

Resin	Ligand	pKa	Loading conductivity (mS/cm) (up to*)	DBC (g/L) 212 cm/hr, 6mm ID x 4cm
Toyopearl GigaCap S-650M	-R-SO_3^-	2.3	6	145[1] (polyclonal hIgG)
Toyopearl GigaCap CM-650M	-R-COO^-	4.7	11	100[2] (polyclonal hIgG)
Toyopearl GigaCap Q-650M	-R-$N(CH_3)_3^+$	12.2	6	173[3] (BSA)

* suggested guideline only

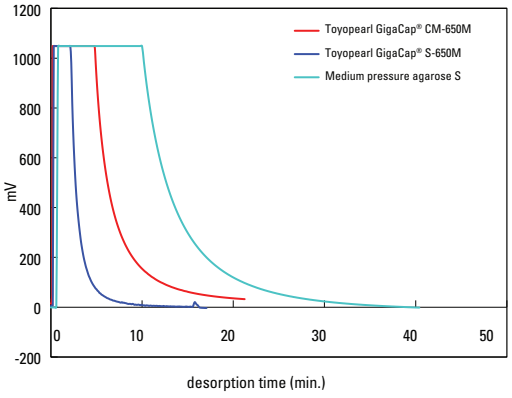
[1] 0.1mol/L acetate buffer pH 4.7
[2] 50mmol/L acetate buffer pH 4.7
[3] 50mmol/L Tris-HCl

Toyopearl GigaCap resins minimize peak tailing and decrease WFI consumption

When purity and yield are equivalent among the screened resins, selecting the resin with the highest capacity makes sense for the capture step. The resin's design, however may be such that it has poor mass transfer properties or non-specific binding interactions between the target and the resin's backbone. This causes the target elution peak to tail (Figure 3), requiring the use of more WFI buffer for elution, and resulting in larger pool volumes and lower titer fractions. This could ultimately impact subsequent processing steps.

For instance, if one resin tails and elutes X grams of target in 20CVs and a second resin (perhaps even with a lower DBC) elutes the same X grams of target in 5CVs, the second resin elutes the target in 75% less WFI volume for downstream processing. If the next downstream unit operation is a flow through chromatographic step or ultrafiltration/diafiltration step (Figure 1), the smaller elution volume reduces their in-process costs. In some cases, a lower DBC resin with the best elution properties may be selected because of its overall effect on the downstream economics of the process, even if this means a slightly larger column may be needed for the capture step.

Figure 3: Peak tailing comparison



	Elution Volume (mL)	Recovery (%)
Medium pressure agarose S	38	96
Toyopearl GigaCap® S-650M	8	96
Toyopearl GigaCap® CM-650M	20	96

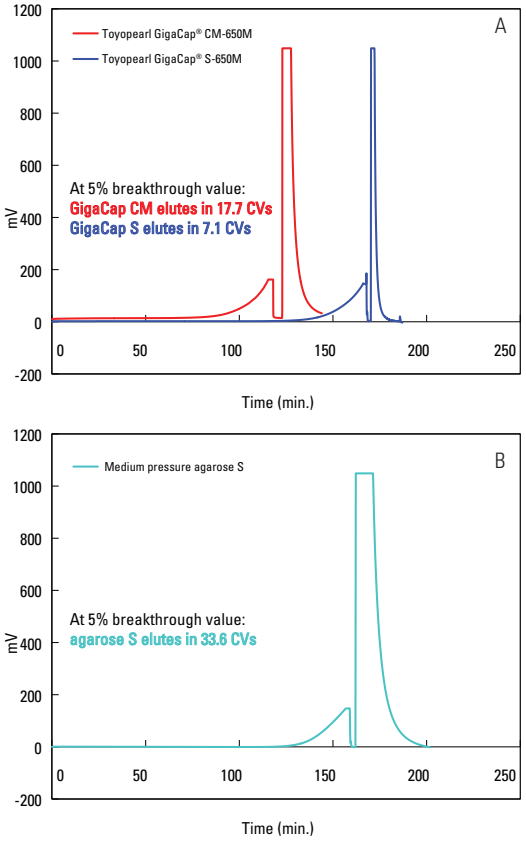
Conditions:
Column size: 6mm ID x 40mm height
Sample: polyclonal human IgG (1mg/mL)

1. Toyopearl GigaCap® CM-650M
Loading buffer: 0.05mol/L sodium acetate buffer (pH4.7)
Loading linear velocity: 212cm/hr
Elution buffer: 0.05mol/L sodium acetate buffer (pH4.7) + 1.0mol/L NaCl

2. Toyopearl GigaCap® S-650M , medium pressure agarose S
Loading buffer: 0.1mol/L acetate buffer (pH4.7)
Loading linear velocity: 212 cm/hr
Elution buffer: 0.1mol/L acetate buffer (pH4.7) + 1.0mol/L NaCl

Elution linear velocity: 424cm/hr
Detection: UV@280nm

Figure 4: Breakthrough and elution profile comparison-cation exchanges resins



See conditions used in Figure 3.

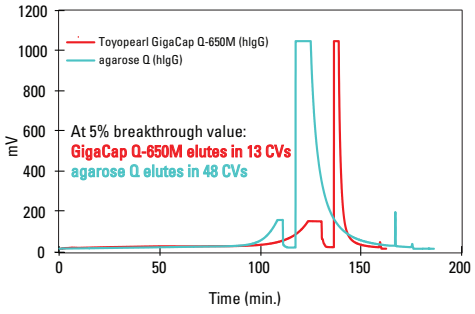
Note: The experimental conditions were selected to show the effect of peak tailing on elution CV for the above resins. Production CVs may be different.

Toyopearl GigaCap CM-650M and Toyopearl GigaCap S-650M resins

Figure 4 shows the full binding and elution curves for Toyopearl GigaCap CM-650M resin, Toyopearl GigaCap S-650M resin, and a competitive medium pressure agarose cation exchange resin.

Figure 3 shows the elution volume of the medium pressure agarose resin as 38mL and the elution volume for Toyopearl GigaCap S-650M resin as 8mL. In the agarose resin, the mAb elutes in 5X the buffer volume of the Toyopearl resin resulting in a significantly lower titer product pool. Note that for this set of experimental conditions the breakthrough curves indicate a slightly higher capacity for the Toyopearl GigaCap S-650M resin (Figure 4A) than the agarose resin (Figure 4B) and similar recoveries.

Figure 5: Breakthrough and elution profile comparison-anion exchange resins



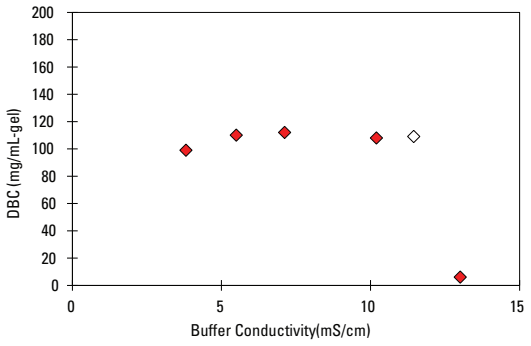
Toyopearl GigaCap Q-650M resin

The binding curves for Toyopearl GigaCap Q-650M resin, an anion exchange resin, and a medium pressure agarose Q resin are shown in Figure 5. In this case, the agarose resin elutes in 3X the buffer volume of Toyopearl GigaCap Q-650M resin. Again, the Toyopearl GigaCap resin has less tailing and a more concentrated target in a lower elution pool volume than the agarose resin.

Minimizing WFI needed for feedstock dilution

Newer resins are engineered to maintain their DBC at increasing feedstock conductivities. Resins such as Toyopearl GigaCap CM-650M can tolerate conductivities up to 10-11mS/cm (Figure 6) while maintaining its capacity at 90-100mg-mAb/mL resin. This also reduces the amount of WFI needed for feedstock dilution and loading.

Figure 6: Toyopearl GigaCap CM-650M loading conductivity tolerance



Conditions:
Dynamic binding capacities were determined at 10% breakthrough
Column size: 6mm ID x 40mm height
Sample: polyclonal human IgG (1mg/mL)

Loading buffer: 50, 75, 100, 150, 200mmol/L sodium acetate buffer (pH 4.7)
Loading buffer: (white symbol) 15mmol/L sodium acetate buffer (pH 4.7)+100mmol/L NaCl
Loading linear velocity: 212cm/hr
Elution buffer: Loading buffer + 0.5mol/L NaCl

Elution linear velocity: 212cm/hr
Detection: UV@280nm

ToyoScreen GigaCap process development columns

Toyopearl GigaCap ion exchange resins are available in prepacked columns for resin screening:

ToyoScreen GigaCap CM-650M
ToyoScreen GigaCap S-650M
ToyoScreen GigaCap Q-650M

They are produced in 1mL and 5mL column volumes and can be used to determine both dynamic binding capacity and protein elution volumes. This data is helpful when comparing with competitive resins, also available in prepacked columns.

The amount of WFI reduction experienced resin to resin will depend on the target molecule's chromatographic properties and the optimum gradient for each media evaluated.

A selection of our more popular Toyopearl ion exchange resins includes prepacked columns for the three GigaCap resins plus Toyopearl SuperQ-650M and Toyopearl Q-600C AR (alkaline resistant) medias. These are also available in 1mL or 5mL column volumes.

Please see the ordering information for these products.

Summary

The data in this brochure demonstrates the improved elution dynamics of the polymeric Toyopearl GigaCap ion exchange resins when compared to commercial agarose based resins. These properties result in significantly less tailing in the elution process with up to 75% less buffer volume required for the target fractions.

When the resins' new elution properties and minimal peak tailing are combined with their higher binding capacities, improvements can be achieved in some or all of the following steps in existing processes:

Using the same resin bed volume

- Less WFI needed for buffer prep and supply
- Up to 75% less WFI needed for elution*
- Smaller hold tanks for downstream process steps

Reduced bed volume because of higher DBC

- Smaller resin volumes
- Less buffer for elution
- Less buffer needed for purification, CIP, re-equilibration

The use of Toyopearl GigaCap resins can significantly contribute to the reduction of downstream purification bottlenecks.

*Results may vary depending on the target molecule, gradient conditions, and resin selectivity.

Ordering information

Toyopearl GigaCap S-650M bulk media

Part #	*Product description*
21833	Toyopearl GigaCap S-650M, 100mL
21834	Toyopearl GigaCap S-650M, 250mL
21835	Toyopearl GigaCap S-650M, 1L
21836	Toyopearl GigaCap S-650M, 5L
21837	Toyopearl GigaCap S-650M, 50L

Toyopearl GigaCap Q-650M bulk media

21854	Toyopearl GigaCap Q-650M, 100mL
21855	Toyopearl GigaCap Q-650M, 250mL
21856	Toyopearl GigaCap Q-650M, 1L
21857	Toyopearl GigaCap Q-650M, 5L
21858	Toyopearl GigaCap Q-650M, 50L

Toyopearl GigaCap CM-650M bulk media

21946	Toyopearl GigaCap CM-650M, 100mL
21947	Toyopearl GigaCap CM-650M, 250mL
21948	Toyopearl GigaCap CM-650M, 1L
21949	Toyopearl GigaCap CM-650M, 5L
21950	Toyopearl GigaCap CM-650M, 50L

ToyoScreen GigaCap process development columns

21868	ToyoScreen GigaCap S-650M, 1mL x 6 ea.
21869	ToyoScreen GigaCap S-650M, 5mL x 6 ea.
21859	ToyoScreen GigaCap Q-650M, 1mL x 6 ea.
21860	ToyoScreen GigaCap Q-650M, 5mL x 6 ea.
21951	ToyoScreen GigaCap CM-650M, 1mL x 6 ea.
21952	ToyoScreen GigaCap CM-650M, 5mL x 6 ea.
21396	ToyoScreen IEC Mix Pack, 1mL x 6 grades x 1 ea. (GigaCap Q-650M, SuperQ-650M, Q-600C AR, GigaCap CM-650M, GigaCap S-650M, SP-550C)
21397	ToyoScreen IEC Mix Pack, 5mL x 6 grades x 1 ea. (GigaCap Q-650M, SuperQ-650M, Q-600C AR, GigaCap CM-650M, GigaCap S-650M, SP-550C)

TOSOH BIOSCIENCE

TOSOH Bioscience LLC
3604 Horizon Drive, Suite 100
King of Prussia, PA 19406
Orders & Service: (800) 366-4875
Fax: (610) 272-3028
www.tosohbioscience.com
email: info.tbl@tosoh.com

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