PALL Life Sciences

Product Note

USD 2591[1]

Q and S HyperCel™ Sorbents

High Productivity Ion Exchangers for Protein Capture and Separations

Product Description and Application Overview

*Filtration. Separation. Solution.*SM

Introduction

Q and S HyperCel sorbents are industry-scalable chromatography ion exchangers designed for high productivity protein capture and intermediate separation steps. Applications include purification of recombinant proteins, monoclonal and polyclonal antibodies, plasma derivatives, vaccines or other biopharmaceuticals.

Q and S HyperCel sorbents are manufactured at large scale and meet the needs of industrial users and regulatory authorities. A Regulatory Support File (RSF) is available to assist users in the development of validation procedures. These sorbents offer:

- Distinctive selectivities with more target/contaminant separation options
- High dynamic binding capacity at short (2 minutes) residence time
- Excellent flow rate properties for fast feedstock processing
- Enhanced process economics

Product Description

Q and S HyperCel sorbents are composed of a rigid cellulose matrix that has excellent flow properties and generates low backpressure, compatible with the needs of manufacturing-scale protein production. Q and S HyperCel sorbents are available in a variety of packaging configurations as well as convenient 1 mL and 5 mL prepacked PRC columns designed for fast method optimization, selectivity screening or small preparative work. Q and S HyperCel sorbents are supplied in 1 M NaCl containing 20% ethanol as a slurry or as a moist cake.

Q and S HyperCel sorbents have a chemical stability that ensures simple clean-in-place (CIP) and storage. For standard CIP, 0.5 to 1 M NaOH treatment is recommended, while long-term storage in 10 to 100 mM NaOH is possible.

Table 1

Main Properties of Q and S HyperCel Sorbents

	Q HyperCel Sorbent	S HyperCel Sorbent
Particle Size	60 – 90 µm	60 – 90 µm
Ionic Groups	99 – 138 µeq/mL	59 – 84 µeq/mL
Dynamic Binding Capacity[1]	160 – 198 mg/mL	134 – 190 mg/mL
Working Pressure[2]	< 1.2 bar (17 psi)	< 1.3 bar (19 psi)
Working pH	1 – 13	
Cleaning pH	1 – 14	
Recommended Cleaning Conditions[3]	0.5 – 1 M NaOH	

[1] Q HyperCel sorbent: determined using a 5 mg/mL BSA in 50 mM Tris-HCl, pH 8.5 at 2 minute residence time. S HyperCel sorbent: determined using a 5 mg/mL hIgG in 50 mM sodium acetate, pH 4.5 at 2 minute residence time.

[2] Measured at 1,000 cm/h in a Pall LRC column (1.5 cm ID x 20 cm height) in above mentioned buffers.

[3] Injection of 5 column volumes (CV) of 0.5–1 M NaOH, 1 hour contact time.

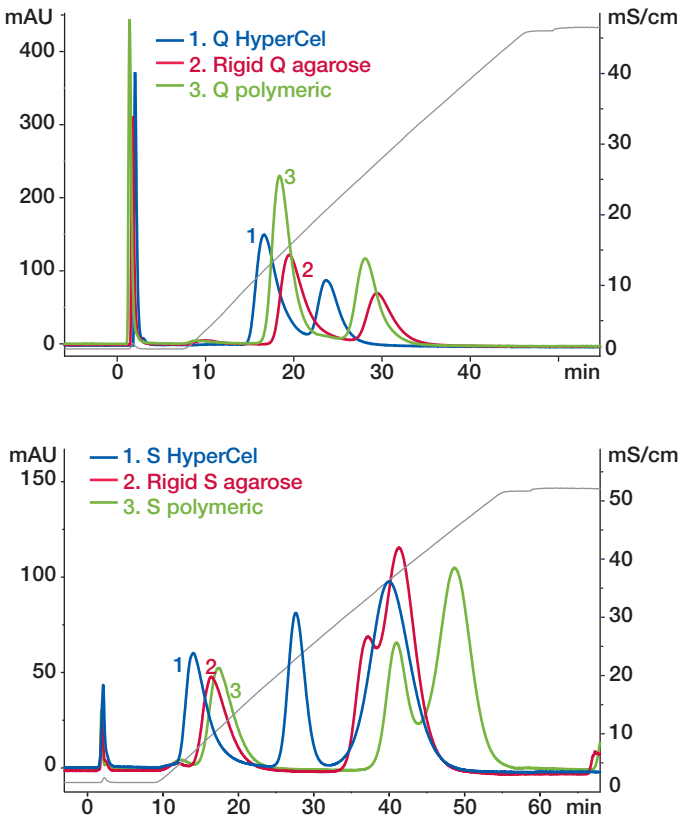
Features and Benefits

Distinctive Protein Selectivity and Salt Sensitivity

Chromatography sorbent selectivity is a key parameter to discriminate between the target protein and contaminants in the feedstock. Screening of sorbent selectivity is critical and should be done at early stages of process development. Due to the difference in the bead structure and the specific ionic charge density, Q and S HyperCel sorbents have different salt sensitivity and selectivity for proteins than other conventional and new generation ion exchangers. The chromatograms in Figure 1 illustrate the differences in selectivity between Q and S HyperCel sorbents and commercially available anion and cation exchangers.

Figure 1

Separation of a Protein Mix on Q and S HyperCel Sorbents and Comparison with a Highly Cross-linked Agarose, and Polymeric Q and S Ion Exchangers



Protein Mix: Q sorbents: Cytochrome C, Human Transferrin, Bovine Serum Albumin; S sorbents: Cytochrome C, Lysozyme, Ovalbumin.

Due to their moderate ionic charge density, Q and S HyperCel sorbents give efficient ligand utilization while minimizing the concentration of salt required to desorb bound proteins (see examples 1 and 4 in Applications

section). Bound proteins are eluted under low salt conditions, which means that less subsequent processing is required for the next downstream processing step, e.g., buffer exchange, dilution or diafiltration. Binding and elution in low salt conditions may also contribute to a better separation between target protein and contaminants (the target protein could be eluted while strongly bound contaminants remain on the column).

High Dynamic Binding Capacity for Enhanced Process Productivity: Smaller Columns, Lower Buffer Requirements, Faster Operations

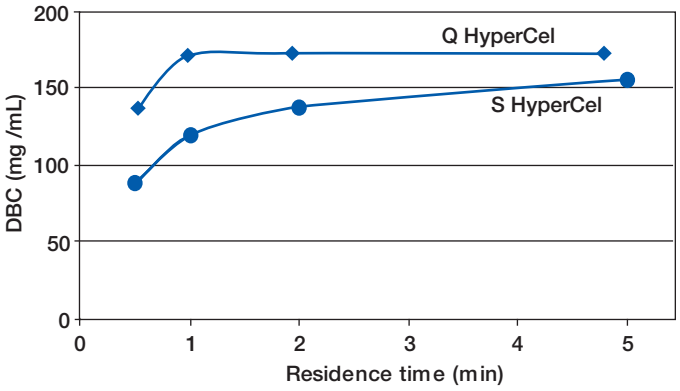
As shown in Figure 2 and Table 2, Q and S HyperCel sorbents have very high dynamic binding capacities (DBC) for proteins at short residence times (2 or even 1 minute).

Combined with excellent flow characteristics at low backpressures (Figure 3), large volumes of feedstock can be processed quickly, increasing the overall process throughput, and limiting the risk of protein degradation.

High binding capacity facilitates operation using columns of moderate volume and footprint, allowing further reduction in buffer-volume requirements, and leading to equipment savings and reduced investment costs for sorbents. Binding pH and feedstock conductivity have an important influence on the dynamic binding capacity of both Q and S HyperCel sorbents; the highest capacities are usually obtained with feedstock of conductivity around or below 5 mS/cm.

Figure 2

Dynamic Binding Capacity vs. Residence Time of Q and S HyperCel Sorbents



DBC for BSA for Q HyperCel sorbent and for hIgG for S HyperCel sorbent, determined at 10% breakthrough in 50 mM Tris-HCl buffer, pH 8.5 for Q HyperCel sorbent, in 50 mM sodium acetate buffer, pH 4.5 for S HyperCel sorbent. Column: LRC column of 1 cm ID x 10 cm length.

Table 2

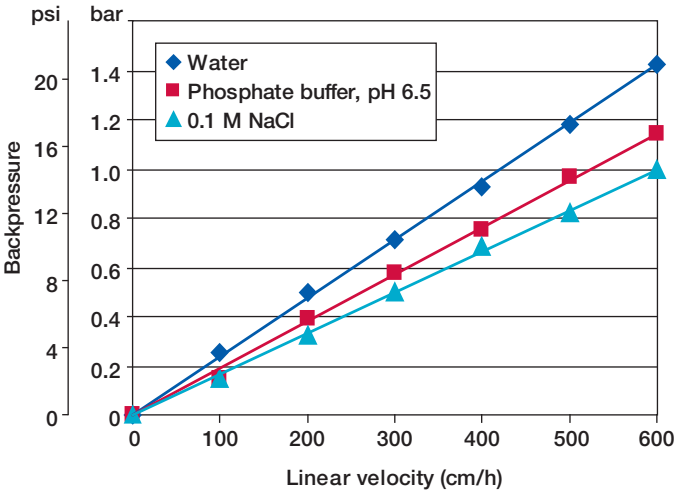
Binding Capacity of Q and S HyperCel Sorbents

	Q HyperCel Sorbent[1]	S HyperCel Sorbent[2]
At 300 cm/h (2 min RT)	168 mg/mL	148 mg/mL
At 600 cm/h (1 min RT)	152 mg/mL	112 mg/mL

[1] DBC at 10% breakthrough for a 5 mg/mL BSA solution in 50 mM Tris-HCl, pH 8.5.
[2] DBC at 10% breakthrough for a 5 mg/mL hIgG solution in 50 mM sodium acetate, pH 4.5.
RT = Residence Time

Figure 3

Pressure vs. Flow Rate of Q HyperCel Sorbent



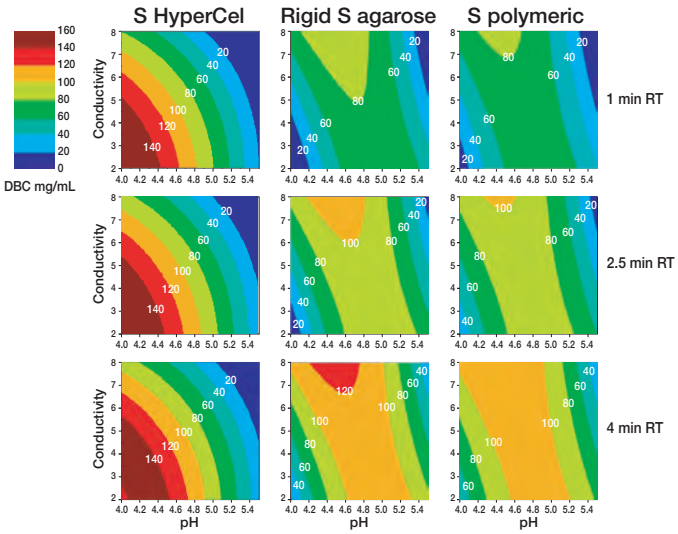
Column: 40 cm ID x 20.8 cm bed length.

A Design of Experiments (DoE) study using a central composite face-centered model (MODDE◆ software from Umetrics) was used to explore the influence of various pH (4.0 to 5.5), conductivities (2 to 8 mS/cm) and residence times (1 to 4 minutes) on the DBC for model proteins. Bovine serum albumin (BSA) was used for Q HyperCel sorbent and commercially available anion exchangers (data not shown), while polyclonal human IgG was employed for S HyperCel sorbent and other cation exchangers.

Data in Figure 4 shows the impact of pH and conductivity on DBC for polyclonal human IgG on S HyperCel sorbent. The contour plots achieved for rigid agarose S and polymeric S sorbents are clearly very different from those obtained with S HyperCel sorbent. The DBC decreases with extremes of pH and conductivity, while the optimal DBC is achieved at intermediate pH (4.6 to 5.2) and moderate conductivity (6 mS/cm).

Figure 4

Influence of Binding pH and Feedstock Conductivity on DBC of S HyperCel Sorbent for Polyclonal hIgG



Dynamic binding capacity for polyclonal human IgG on S HyperCel sorbent, rigid S agarose sorbent and S polymeric sorbent at various residence times (RT), (1 to 4 min), pHs (4.0 to 5.5) and conductivities (2 to 8 mS/cm).

Excellent Flow Properties and Easy Packing/Unpacking

Q and S HyperCel sorbents are very easy to pack and unpack in laboratory, pilot and production-scale columns, and show excellent flow properties, compatible with the requirements of advanced production processes. The sorbents can be packed in 100 mM NaCl buffers or in water for simple operations. For example, a 1.5 cm ID x 22 cm bed length column can be operated at 1,000 cm/h with less than 1.5 bar (22 psi) backpressure. Similarly, a 40 cm ID x 21 cm bed height column at 500 cm/h (compression factor 1.2) will generate only around 1 bar (15 psi) backpressure in buffer (see Figure 3).

Selectivity Screening Using PRC Prepacked Columns of 1 mL and 5 mL

Rapid screening and condition optimization can be achieved using the 1 mL PRC column. Once the appropriate chemistry is selected, the conditions of use can be optimized in a 5 mL PRC column by doubling the height. Two 5 mL columns can be connected in series to increase the column bed height to 20 cm, and more closely model real conditions in pilot scale or for scale-down applications (see Pall Application Note USD 2718). Columns of 1 mL can also be connected in series.

Figure 5

PRC Columns, 1 mL and 5 mL

Application Examples

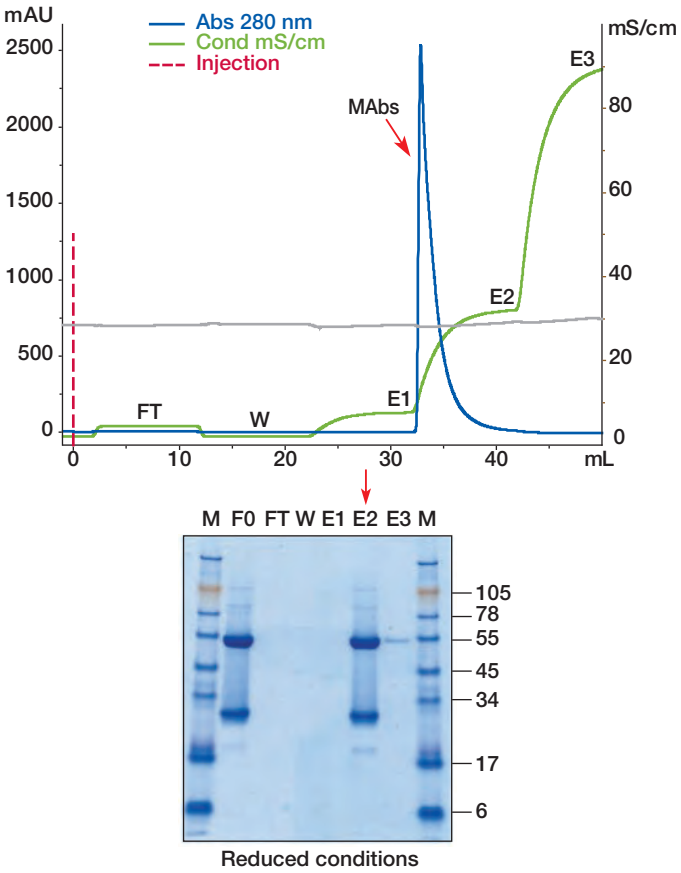
Application 1. Intermediate Purification of a Monoclonal Antibody (MAb) from NS0 Cell Culture on S HyperCel Sorbent

Sample Courtesy of Dr. Gideon Slama, CureTech, Israel.

After clarification, an NS0 cell culture supernatant was loaded on an MEP HyperCel sorbent prepacked column for IgG capture. Further to this capture step, the IgG purity was estimated to be 93% by size exclusion chromatography (SEC). The eluate of the MEP HyperCel sorbent column was adjusted to pH 4.5 and diluted 2-fold (conductivity 4 mS/cm), and loaded on a prepacked column of S HyperCel sorbent (0.5 cm ID x 5 cm length, 1 mL). Figure 6 shows the stepwise elution of the MAb by three steps of 50 mM, 300 mM and 1 M NaCl (pH 4.5). Results in Table 3 show that S HyperCel sorbent recovered the MAb with ~99% purity and ~97% recovery in 300 mM NaCl. Comparison with another cation exchanger (Pall CM Ceramic HyperD® F ion exchange sorbent, data not shown) shows that S HyperCel sorbent efficiently recovered the antibody with less salt than CM Ceramic HyperD sorbent (300 mM NaCl compared to 600 mM), illustrating the salt sensitivity and lower retentivity of the S HyperCel sorbent.

Figure 6

Intermediate Purification Step on S HyperCel Sorbent (Stepwise Elution)



PRC column 5 mm I.D. x 50 mm length (1 mL S HyperCel sorbent).
Equilibration in 50 mM sodium acetate, pH 4.5.
F0: MEP eluate, pH 4.5 (4 mS/cm)
FT: Loading of 10 mL of MEP HyperCel sorbent eluate (RT = 4 minutes)
W: Wash in 10 CV of 50 mM sodium acetate, pH 4.5 (buffer A)
E1: Buffer A + 50 mM NaCl, pH 4.5 (5 mS/cm)
E2: Buffer A + 300 mM NaCl, pH 4.5 (30 mS/cm)
E3: Buffer A + 1 M NaCl, pH 4.5

Table 3

Intermediate Purification of MAb from NS0 Cell Culture on S HyperCel Sorbent

Fraction	Quantity (mg)	IgG Purity (%)	IgG Recovery (%)
Load with the MEP HyperCel sorbent column eluate	10.5	95.1	Not applicable
Wash with 50 mM sodium acetate, pH 4.5	0	0	0
Elution with 50 mM NaCl	0	0	0
Elution with 300 mM NaCl	9.9	98.6	97.5
Elution with 1 M NaCl	0	0	0

Application 2. Contaminant Removal from MAb by Intermediate Polishing on S HyperCel Sorbent Following Capture on Mixed-mode MEP HyperCel Sorbent

Sample Courtesy of Dr. Jean-François Depoisier, NovImmune, Switzerland.

Data presented in Table 4 show a good clearance of HCPs and aggregates, and illustrate the complementary selectivities of S HyperCel sorbent following MAb capture by mixed-mode chromatography on MEP HyperCel sorbent.

An undiluted pool from MEP HyperCel sorbent was applied to S HyperCel sorbent at pH 4.8 at a conductivity of 6.8 mS/cm. The S HyperCel sorbent achieved a high degree of HCPs and aggregates removal. Antibody light chains present in the MEP eluate were also efficiently removed by the S HyperCel sorbent step. A capacity of 87 mg/mL of antibody was obtained (conductivity 6.8 mS/cm, RT 2.5 min.).

Applications 1 and 2 suggest an easy combination of S HyperCel sorbent as a second purification step following capture on MEP HyperCel mixed-mode sorbent.

Table 4

Contaminant Removal from MAb by Intermediate Polishing on S HyperCel Sorbent Following Capture on Mixed-mode MEP HyperCel Sorbent

Fractions	Feedstock[1]	Elution Pool[2]	Run 1	Run 2
Residence Time (min)	–	–	2	2
DBC (mg/mL)	–	–	87 (6.8 mS/cm)	> 97 (3.3 mS/cm)
Recovery (%)	100	–	ND	93
HCP (ppm)	28,230	296	66	45
Aggregates (%)	2.2	0.5	0.4	0.3
Light Chain (%)	1.3	3	0	0.4

[1] Followed by Step 1 – Chromatography on MEP HyperCel mixed-mode sorbent
[2] Followed by Step 2 – Chromatography on S HyperCel ion exchange sorbent
ND = Not determined

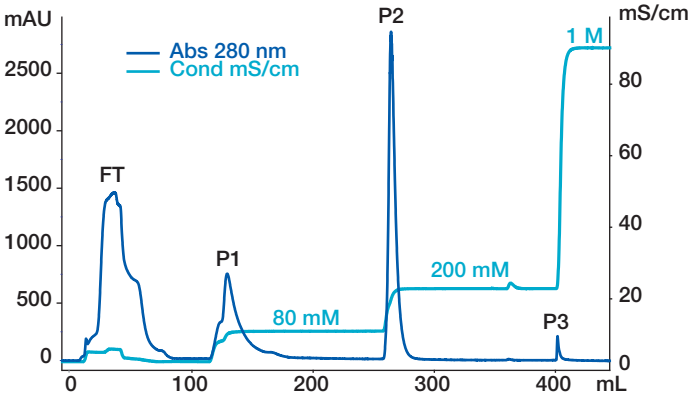
Application 3. 1500-fold Scale-up of a Purification Step on Q HyperCel Sorbent

Data presented in Figure 7 shows a direct 1500-fold scale-up from laboratory to production scale of the pre-purification of β-lactalbumin from microfiltered bovine whey on Q HyperCel sorbent. For methods development and optimization, the sample was directly loaded on a Pall LRC column of 1 cm I.D. equilibrated in 20 mM sodium phosphate, pH 6.8, with a residence time of 3 minutes

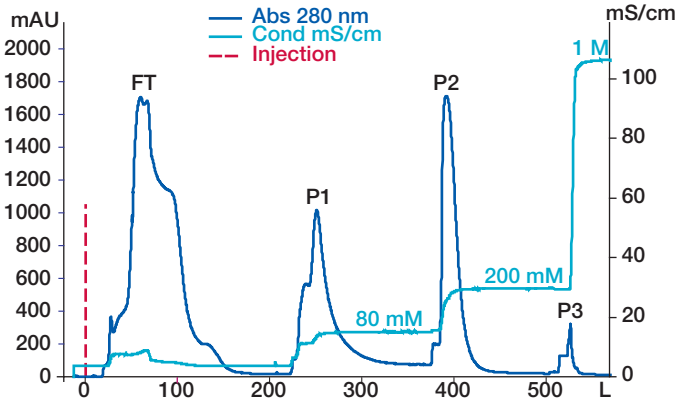
(300 cm/h). A stepwise elution was carried out using 10 CV of 80 mM NaCl in equilibration buffer, followed by 200 mM and 1 M NaCl in the same buffer. Fraction analysis by SDS-PAGE, size exclusion chromatography (SEC) and Bradford assay (not shown) indicates that the first elution peak (P1) contained mainly β-lactalbumin with purity around 91% (SEC) and a recovery of 90%. The second elution peak eluted at 200 mM NaCl (P2) contained mainly β-lactoglobulin, and the 1 M NaCl step cleared off some remaining impurities (P3). The conditions optimized on the 1 cm ID column were transferred to a Pall Resolute® 400 (40 cm I.D.) column containing 21.3 L of Q HyperCel sorbent. Residence time was kept constant (3 minutes). Data evidenced a 1500-fold scale-up with high purity (87%) and recovery (89%) of the target protein, achieved in less than 3 hours for processing of 42 L of crude feedstock.

Figure 7

1500-fold Scale Up of the Chromatography of Sweet Whey Proteins Using Q HyperCel Sorbent at 300 cm/h (3 min Residence Time)



Pall LRC 10/80-200 column,1 cm ID x 18.1 cm length (14.2 mL)



Pall Resolute 400 Column, 40 cm ID x 17 cm length (21.3 L)

Application 4. Purification of Recombinant GFP from *E. coli* Lysate on Q HyperCel Sorbent

Figure 8 shows the purification of recombinant GFP (Green Fluorescent Protein) on Q HyperCel sorbent (Figure 8a). rGFP has a molecular mass of 29.1 kDa and a pI of 6.1, and can be conveniently monitored by UV absorbance at 490 nm. 100 µL of crude rGFP from *E. coli* lysate were loaded on a PRC column of 5 mm ID x 50 mm bed height (1 mL) equilibrated in 50 mM Tris-HCl, pH 8.5. Elution was performed at 1 mL/min with a gradient up to 0.5 M NaCl. A comparison was done with Pall Q Ceramic HyperD F sorbent (Figure 8b). Data shows that rGFP was eluted from the Q HyperCel sorbent column with a conductivity of 13.5 mS/cm, showing a different selectivity and a lower retentivity than the Q Ceramic HyperD F sorbent column; this may contribute to a better purity of the protein isolated on Q HyperCel sorbent in this case. The same purification using step elution (Figure 9a) shows that rGFP could be completely eluted with only one step of 0.1 M NaCl on Q HyperCel sorbent, when two steps of 0.1 M and 0.2 M NaCl were necessary to recover the rGFP from Q Ceramic HyperD F sorbent (Figure 9b).

Figure 8a

Purification of rGFP from E. coli Lysate (Gradient Elution) – Q HyperCel Sorbent

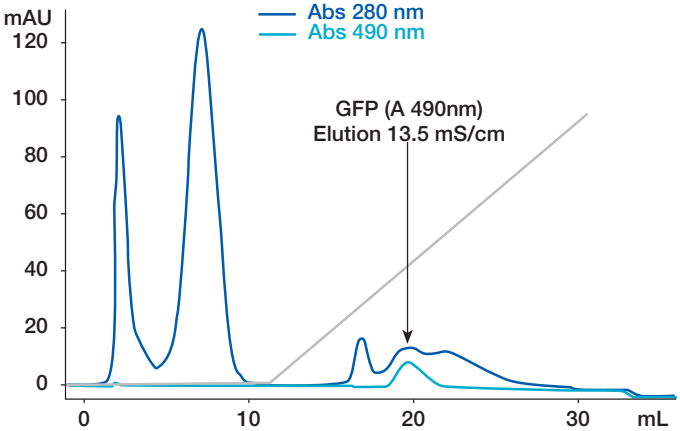


Figure 8b

Purification of rGFP from E. coli Lysate (Gradient Elution) – Q Ceramic HyperD F Sorbent

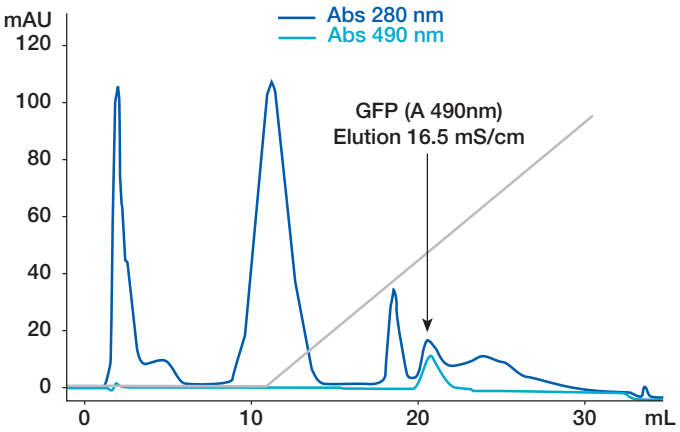


Figure 9a

Purification of rGFP from E. coli Lysate (Step Elution) – Q HyperCel Sorbent

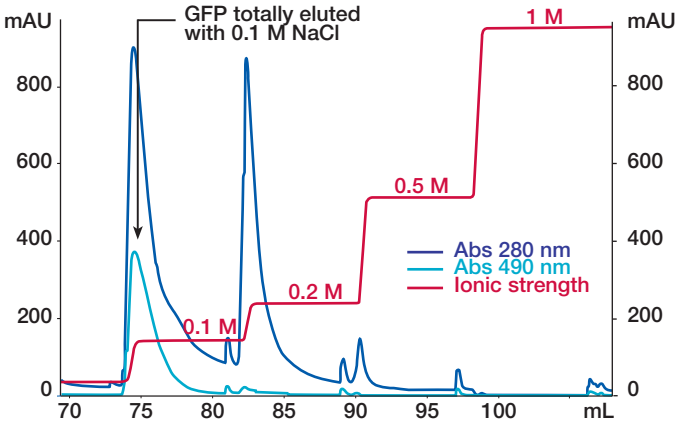
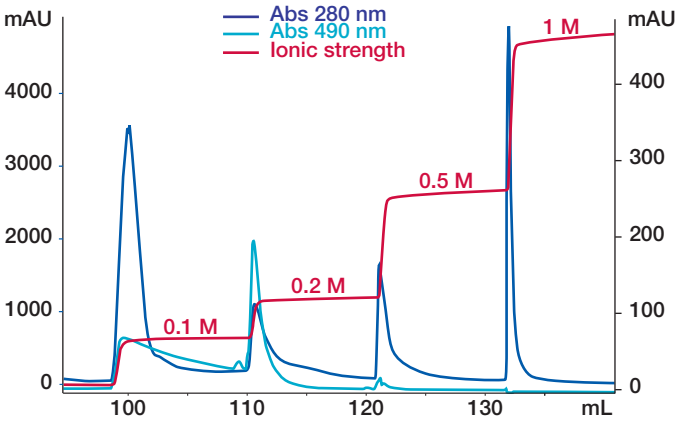


Figure 9b

Purification of rGFP from E. coli Lysate (Step Elution) – Q Ceramic HyperD F Sorbent



Ordering Information

Size	Product Number	
	Q HyperCel Sorbent	S HyperCel Sorbent
5 mL	20196-012	20195-013
25 mL	20196-024	20195-025
100 mL	20196-036	20195-037
1 L	20196-048	20195-049
5 L	20196-050	20195-051
10 L	20196-062	20195-063
1 mL PRC Prepacked Column, 5 mm ID x 50 mm	PRC05X050QHCEL01	PRC05X050SHCEL01
5 mL PRC Prepacked Column, 8 mm ID x 100 mm	PRC08X100QHCEL01	PRC08X100SHCEL01

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