BioSepra® Q & CM HyperZ™

Q & CM HyperZ™ Ion Exchange Sorbents

High Productivity Expanded Bed Adsorption Chromatography.

- Direct capture from crude, unclarified feedstock.
- Limited dilution required for efficient binding.
- Small bead produces more rapid binding and higher capacity.
- High density allows operation with higher flow rates and higher biomass loads.
- Outstanding chemical resistance.

Table 1: HyperZ™ Sorbents Main Properties.

	Q HyperZ	CM HyperZ
Average particle size (µm)	75	75
Nature of ionic groups	Quaternary amine (Q)	Carboxymethyl (CM)
Ionic capacity (µeq/ml)	100-130	100-180
Particle size distribution	40-105	40-105
Mean particle density	3.2 g/ml	3.2 g/ml
Binding capacity (mg/ml)	80 (BSA)*	~ 50 (hu IgGs)**
Degree of expansion (H/H_0)	2 at 300 cm/h	2 at 300 cm/h
Chemical stability	Stable in all commonly used aqueous buffers and also 2M NaOH, 1 M HCl, 20 % ethanol, 6 M guanidine-HCl	
Working pH range	4-13	4.13
Cleaning pH	1-14	1-14
Packaging	Dry form (2 g ~ 1 ml)	Dry form (2 g ~ 1 ml)

(*) Breakthrough capacity at 10% using 5 mg BSA/ml in 50 mM Tris-HCl buffer, pH 8.6, 150 mM NaCl.
(**) Breakthrough capacity at 10% using 5 mg hu IgG/ml in 50 mM sodium acetate buffer, pH 4.7, 150 mM NaCl.

Expanded bed adsorption (EBA) chromatography is a technology ideally suited for rapid processing of large volumes of crude biological feedstocks and for the purification of plasmids, IgG and various recombinant proteins. Rapid, direct capture of target proteins by expanded bed adsorption offers considerable operational benefits:

- Significantly reduces process cycle time by eliminating post-fermentation clarification,
- Improves product quality and yield,
- Reduces total capture-step costs by 20-40%.

Existing ion exchange sorbents for EBA have not been designed for optimal productivity in industrial operations. Protein binding typically requires significant feedstock dilution, unacceptably increasing process volumes and load times. In addition, these sorbents are incompatible with high biomass loads (>10% ww/v); exceeding these levels can only be achieved by a reduction in operating flow rates, substantially increasing process cycle duration.

BioSepra® Q and CM HyperZ™ are members of a new family of high-density sorbents from Ciphergen, specifically designed for high productivity expanded bed chromatography and efficient capture of biomolecules directly from crude, unclarified feedstocks in a single pass operation.

CIPHERGEN®

Higher biomass and flow rate without sacrificing capacity.

The HyperZ™ product range has been designed to deliver the broadest useful linear flow range possible of any commercial expanded bed sorbents. Due to a high charge density in its hydrogel moiety, the CM HyperZ™ sorbent has unique protein binding properties in the presence of salt. This provides significant benefits at process scale, like avoiding or limiting the dilution of large volumes of feedstream.

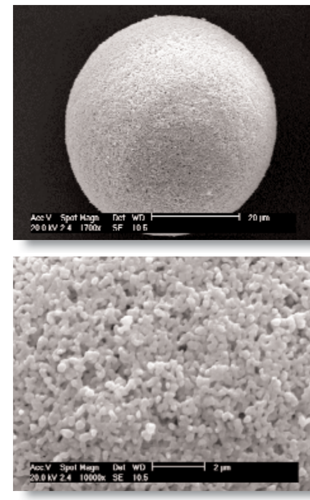
• High density particles: Faster flow rates.

Figure 2 shows the hydrodynamic behavior of Q and CM HyperZ™ sorbents, and a standard profile for an SP EBA sorbent: ~2 times higher linear flow velocities can be used with CM HyperZ™ without risk of particle elutriation. Typically, bed expansions of 2-3 are obtained with HyperZ™ sorbents at linear velocities of 300 cm/h. Under similar conditions, conventional EBA sorbents, which have lower densities and larger particle sizes, show much higher degrees of bed expansion, leading to a particle elutriation or upper frit clogging. 75 µm average particle size also improves mass transfer as well as resolution when the sorbent is used in packed bed mode.

• Higher biomass loads.

CM HyperZ™ can operate in the presence of high biomass loads while maintaining reasonable (2-3) bed expansion rates (see

Figure 1. Structure of HyperZ sorbents.



Scanning electron micrograph image of HyperZ bead and surface prior to introduction of the hydrogel.

Figure 3). This is of critical importance when highly concentrated feedstocks need to be processed directly.

Figure 4 shows that linear velocities exceeding 300 cm/h are obtained at bed expansions of 2-3, even with very high density cell culture (12 x 10^6 cells/ml).

• Scale up: Use any commercially available EBA column and improve productivity.

HyperZ sorbents can be easily packed in any type of EBA column and offer substantial benefits of bed stability. Figure 5 shows an example of the excellent packing consistency, from 5 cm to 30 cm I.D. commercially available columns.

Figure 2. Comparison of hydrodynamic behavior in the presence of 9% glycerol.

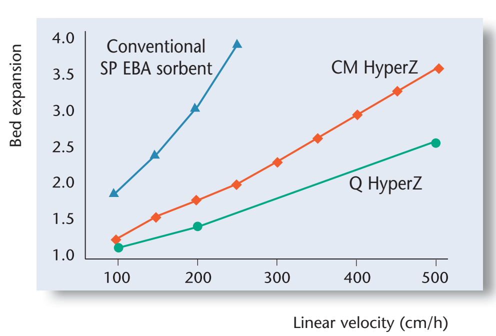


Figure 3. Bed expansion as a function of biomass.

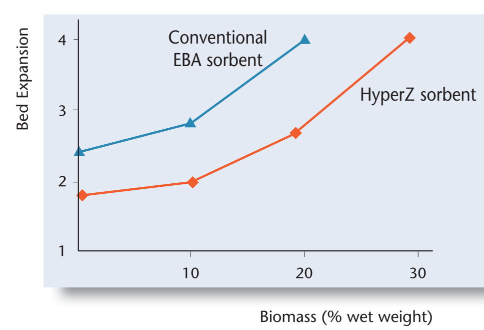
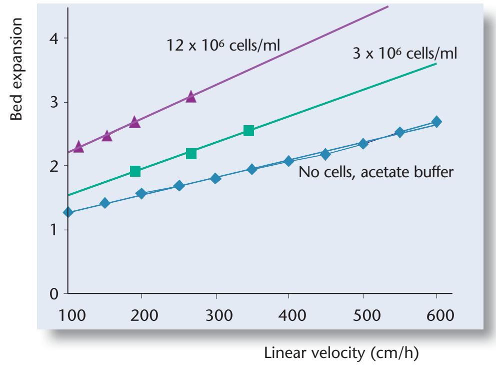
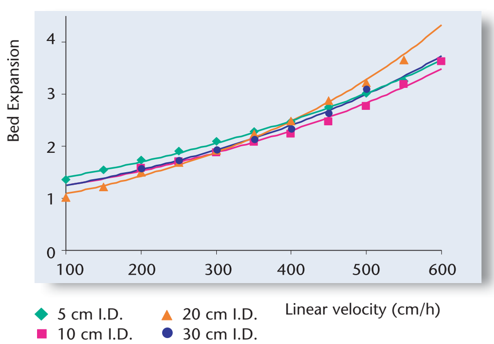


Figure 4. CM HyperZ expansion in the presence of different mammalian cell concentrations.



Column:1 cm I.D., 10 cm settled bed height CM HyperZ sorbent. Sample: crude high density hybridoma cell culture diluted with equilibration buffer (50 mM acetate / 5 mM citrate, 75 mM NaCl, pH 4.7).

Figure 5. Scale-up with Q and CM HyperZ sorbents.



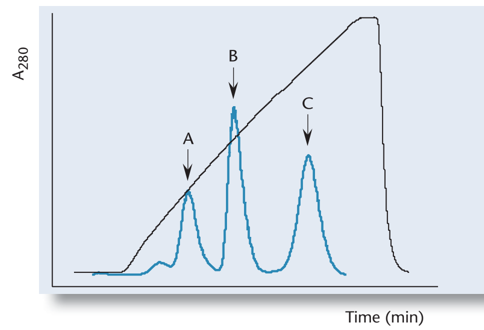
20 cm settled bed height. Conditions: 50 mM acetate / 5 mM citrate, pH 4.5 or 50 mM Tris-HCl, pH 8.6.

Maximum flexibility: Packed bed or Expanded Bed, in any commercially available column.

Establishing and maintaining a basic purification process and choice of chromatography sorbents throughout development, pilot studies and production can significantly reduce process re-optimization time and effort during scale-up.

HyperZ™ sorbents provide the ultimate in process flexibility. With clarified feedstocks, HyperZ™ can be used in conventional packed bed mode for process optimization, scale-up and even in very large-scale production settings. The relatively small particle size of HyperZ® (75 µm, to be compared to 200 µm for conventional EBA sorbents) increases efficiency and resolution of the separation when the sorbent is used in a regular packed bed column (Figure 6), while still operating at low back-pressure.

Figure 6. Efficient separation of a mixture of proteins in packed bed mode using CM HyperZ sorbent.



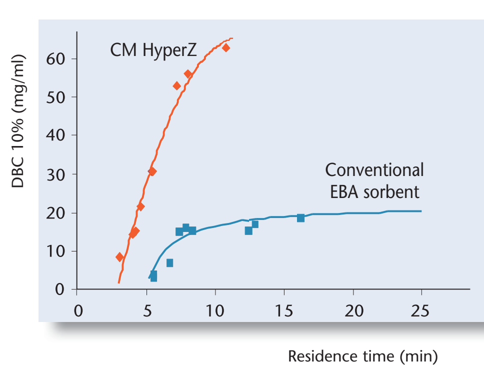
Column: 1.1 cm I.D., 8 cm packed bed height. Sample: 1 ml load (15 mg/ml ovalbumin (A), 3 mg/ml cytochrome c (B) and 5 mg/ml lysozyme (C)). Conditions: Linear gradient 50 mM acetate, pH 4.5, 0-120 mM NaCl.

Short residence times, high dynamic binding capacity: Increase throughput.

HyperZ™ are composite sorbents which represent an extension of the well-known HyperD® family of enhanced diffusion sorbents. These unique sorbents are composed of a high-capacity hydrogel polymerized within the pores of a rigid inert bead. The hydrogel moiety has a very high number of ionic binding sites. Proteins diffuse quickly within the hydrogel, and are rapidly "captured" by these highly accessible charges.

Residence time gives a broad measure of productivity and sorbent performance, independently of column geometry. Figure 7 illustrates that short residence times are needed for protein capture on CM HyperZ™. Only a modest reduction of the dynamic binding capacity (DBC) is observed if residence time is decreased from 10 min. to 5 min., in contrast to conventional EBA sorbent.

Figure 7. Dynamic capacity vs. residence time.



Sample: 5 mg/ml hu IgG in 50 mM acetate / 5 mM citrate, 150 mM NaCl, pH 4.5. Studied performed in packed bed columns.

With Q HyperZ™ (data not shown), residence times of 2-5 min. are also sufficient to reach the operation capacity of the sorbent.

As illustrated in Figures 8 and 9, CM HyperZ™ is particularly well suited to direct IgG capture from crude feedstock: routinely, capacities from 25 to 60 mg/ml of IgG are obtained, even in the presence of moderate salt (150 mM NaCl, or conductivity from 14 to 19 mS/cm).

With Q HyperZ™, average capacities of 80 mg/ml of BSA are obtained in a Tris pH 8.6 buffer at 300 cm/h.

CM HyperZ™: An IgG Capture tool, with minimal feedstock dilution.

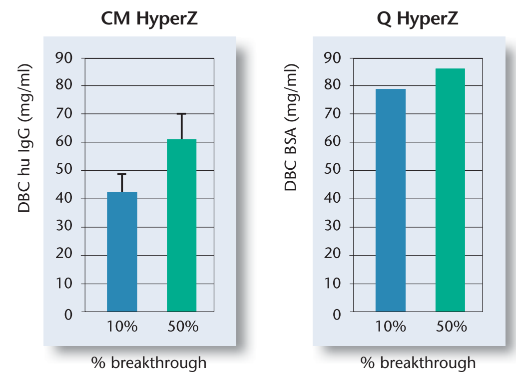
With conventional cation exchange sorbents, significant feedstock dilution (2-5 fold) with water or low-salt buffer is required to achieve reasonable protein binding capacity. These dilution steps significantly contribute to processing time, cost and product quality. CM HyperZ™ can be used for the direct harvest of proteins from undiluted or minimally diluted feedstock, resulting in shorter cycle time and lower cost.

Figure 9 shows the adsorption performance of CM HyperZ™ for hu IgG measured at pH 4.5 in various salt conditions. Remarkably high capacities are observed at relatively high salt concentration (> 60 mg/ml with 150 mM NaCl).

Q HyperZ™ : A new tool for anion exchange EBA.

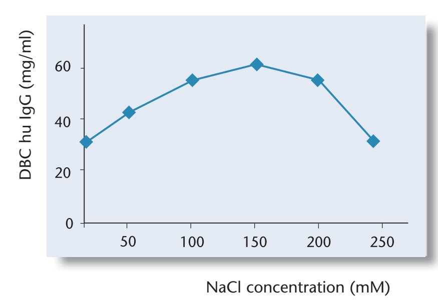
Expanded bed anion exchange adsorption has been used to purify natural and recombinant proteins and enzymes from various sources, including *E.coli* fermentation, unclarified crude yeast homogenates, plasma and milk from transgenic animals. The broad use of EBA anion exchange has been limited by the fact that animal cells as well as cell debris of yeast homogenates or DNA tend to be adsorbed by Q or DEAE functional groups, resulting in fouling and decrease of sorbent capacity. Recent work (EBA 2002 meeting) suggests that special care given to cell disruption methods could improve the results.

Figure 8. HyperZ sorbent dynamic capacity.



1 cm I.D. EBA column, 11 cm settled bed height. Linear velocity: 300 cm/h (2X bed expansion). Equilibration buffer:
- CM HyperZ: 50 mM acetate / 5 mM citrate, 150 mM NaCl, pH 4.5. Sample: 5 mg/ml hu IgG.
- Q HyperZ: 50 mM Tris-HCl, pH 8.6. Sample : 5 mg/ml BSA.

Figure 9. Direct capture of hu IgG in the presence of salt using CM HyperZ sorbent.



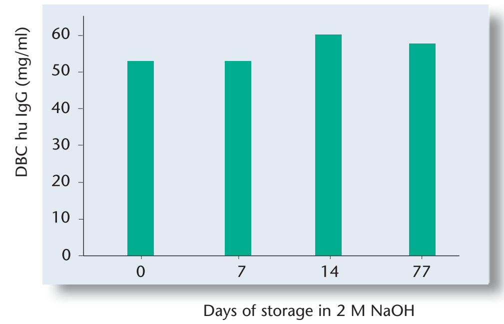
1.1 cm I.D. column, 8 cm packed bed height. Equilibration buffer: 50 mM acetate / 5 mM citrate, pH 4.5. Adjusted in NaCl as indicated. Linear flow rates: 100 cm/h. Dynamic binding capacities recorded at 10% breakthrough. Sample: 5 mg/ml hu IgG in equilibration buffer.

Increased sorbent life time due to outstanding chemical resistance.

The HyperZ™ product range is designed for use in expanded bed operations where maximal sorbent fouling can occur. Q and CM HyperZ™ sorbents have been engineered to withstand very harsh clean-in-place treatments. Long term storage data of CM HyperZ™ is shown in Figure 10.

Other experiments (data not shown) have demonstrated that continuous storage of HyperZ™ in hot solutions of NaOH (1 M at 55°C) for several days does not impact sorbent performance. Agents such as 20% ethanol, 30% isopropanol or mixtures (20% ethanol/1 M NaOH) or acidic mixtures can also be used for regeneration and cleaning.

Figure 10. CM HyperZ sorbent long-term stability to NaOH.



Capacity at 10% breakthrough hu IgG determined at 100 cm/h in packed bed columns. Sample: 5 mg/ml hu IgG in 50 mM acetate / 5 mM citrate, 150 mM NaCl, pH 4.5.

• Application 2. Purification of recombinant endostatin on CM HyperZ™ after method optimization on CM ProteinChip® Arrays.

Courtesy of Dr. Joseph Shiloach, NIDDK, NIH, Bethesda, MD (USA)

Figure 11 (upper part) shows a chromatographic purification of recombinant endostatin from a crude *P. pastoris* fermentation extract. The binding and elution conditions were first optimized on a CM ProteinChip® Array (data not shown). The "on-chip" optimized conditions were transferred on a CM HyperZ™ sorbent : adsorption of endostatin was done at pH 5.0, and desorption achieved by a two-step gradient of NaCl (200 and 800 mM). Linear flow rate was 300 cm/h. Collected fractions

Applications.

Typical applications for CM and Q HyperZ™ sorbents include :

- Direct capture of IgG and IgG fragments from a variety of feedstocks.
- Purification of intracellular and extracellular enzymes.
- Purification of recombinant proteins from *E. coli* or yeast fermentation.
- Recombinant proteins from transgenic milk or plant extracts.
- Potential alternative to packed bed ion exchange sorbents.

• Application 1. Protein capture in the presence of high *E. coli* biomass.

Table 2 shows comparative capture of lysozyme from *E. coli* lysate at 2.5% and up to 11.5% ww/v concentrations. In contrast to the conventional EBA sorbent (SP agarose), CM HyperZ™ tolerates biomass loads up to 11.5%, without significant decrease of the linear flow. Acceptable bed expansion can be maintained and process time decreased. Recovery >80% and purity of >65% were achieved in a single pass.

Figure 11. Capture of endostatin from P. pastoris fermentation broth on CM HyperZ sorbent and fraction analysis using the ProteinChip® technology.

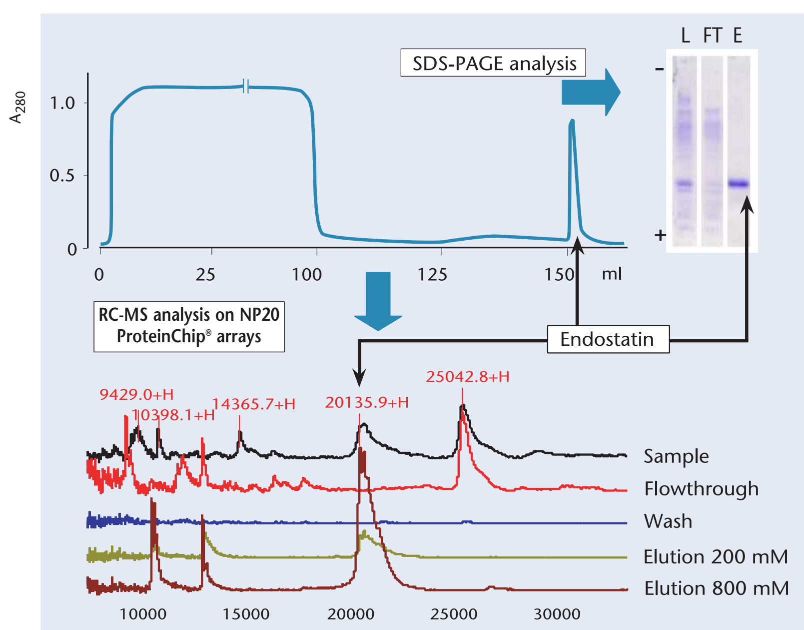


Table 2: Purification of lysozyme from *E. coli* lysate.	CM HyperZ 11.5% biomass	CM HyperZ 2.5% biomass	SP Agarose 11.5% biomass	SP Agarose 2.5% biomass
Loading time (28 CV)	180 min at 120 cm/h	83 min at 260 cm/h	350 min at 65 cm/h	228 min at 100 cm/h
Estimated lysozyme binding capacity (mg/ml)	19	25	11	23
Estimated recovery (%)	68	89	62	84

1 cm I.D. column, 13 cm settled bed height. Equilibration: 50 mM acetate/5mM citrate, 50 mM NaCl, pH 4.5; Elution: same buffer + 1 M NaCl.

were analyzed (lower part of figure) by SELDI-TOF-MS (Surface Enhanced Laser Desorption-Ionization, combined to Time-of-Flight Mass Spectrometry), using the ProteinChip® reader. CM HyperZ™ demonstrated a fast and efficient capture of the protein, with a 10X concentration factor in one step.

- **Application 3. Direct Capture of Murine IgG_1 from Hybridoma Cell Culture**

Figure 12 shows a typical monoclonal antibody purification, without feedstock dilution. The Mouse monoclonal IgG_1 was concentrated 30X in a single step, with purity >85% (GPC) and 95% recovery.

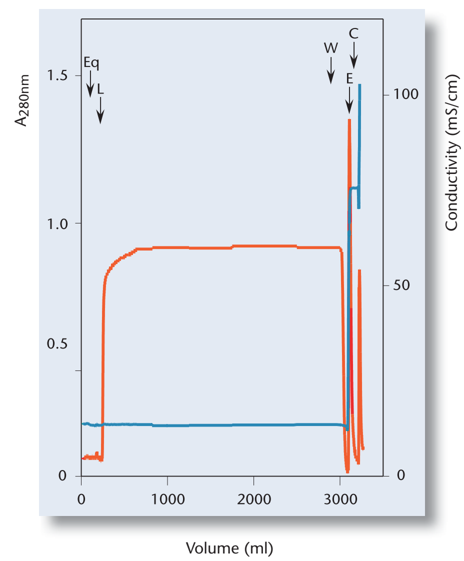
- **Application 4. Purification from unclarified yeast lysate at 20% ww/v biomass loads.**

Courtesy of M. Jahanshahi & A. Lyddiatt, Biochemical Recovery Group, University of Birmingham (U.K.)

Figure 13 shows the purification of cytochrome c from unclarified lysates of baker yeast prepared by wet-milling of cell paste suspended in buffer.

The upper part of the figure shows the Dynamic Binding Capacity per ml of CM HyperZ™ at C/C_0=0.1 (C and C_0 represent the cytochrome c concentration in the effluent and the feedstock respectively).

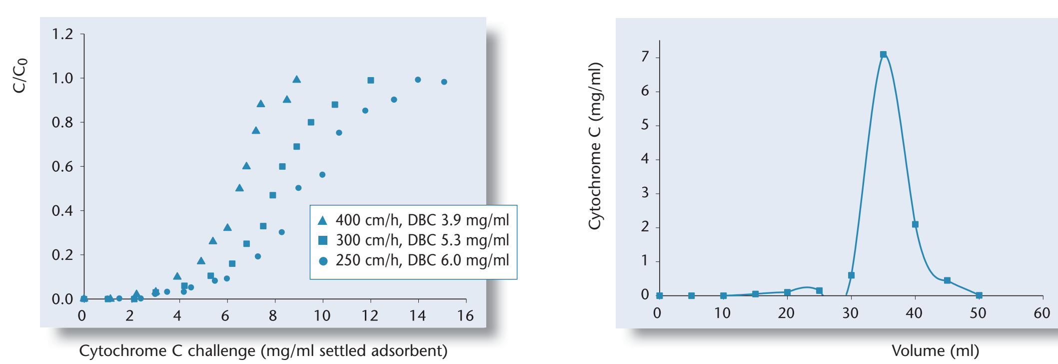
Figure 12. Purification of mu IgG_1 from hybridoma serum-free medium supernatant on CM HyperZ sorbent.



1 cm I.D. x 15 cm height column. Equilibration: 50 mM acetate, pH 4.5, 120 mM NaCl; Flow rate: 300 cm/h (2.5X bed expansion in presence of cells). Sample: 3 L, undiluted, hybridoma cell culture supernatant. (1x10[6] cells/ml) adjusted to pH 4.5. Conductivity: 18 mS/cm. Elution: 50 mM acetate, pH 4.5, 1 M NaCl in expanded bed mode.

Capacities, flow rates and low elution volumes demonstrate excellent performance of CM HyperZ™ in the presence of very high biomass.

Figure 13. Effect of linear flow rate on dynamic binding capacity of CM HyperZ sorbent in presence of 20% biomass.



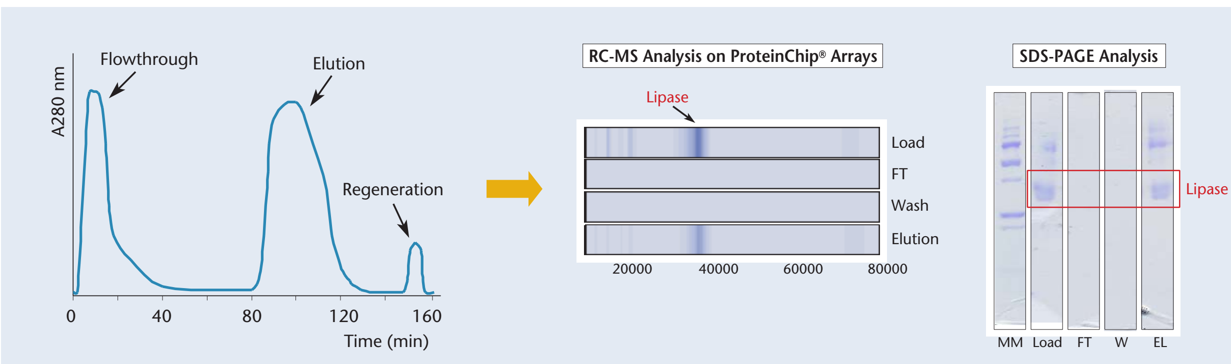
1 cm I.D. column, 20 cm bed height. Sample: Unclarified yeast lysate. 20% (wet weight) biomass. Cytochrome C represents only 1-2% of total protein present in sample. Binding: 10 mM Tris-HCl, pH 7.5. Elution: 60 mM NaCl, 50 cm/h in packed bed. > 90% recovery of bound cytochrome C in all studies. Typical elution volumes: 1.3-1.5 CV. Concentrations of lysates are expressed as the frozen wet weight of original undisrupted cells, relative to the suspension volume (ww/v). Cytochrome c was quantified by spectrophotometric assay.

• Application 5. Purification of a lipase from *Y. lipolytica* on Q HyperZ™.

Courtesy of Prof. R.M. Willemot, INSA Toulouse, France.

Figure 14 shows direct capture of a recombinant lipase from a crude clarified fermentation broth of *Yarrowia lipolytica*. Adsortion of the enzyme was performed at pH 9.0 with diluted sample for reduction of the ionic strength below 6 mS/cm. Elution was peformed by use of an NaCl linear gradient from 0 to 1 M in one hour. Flow rate was 300 cm/h. Collected fractions analyzed both by SDS-PAGE and RC-MS (Ciphergen Biosystems) indicate that lipase (# 36kDa) was efficiently captured on Q HyperZ™ sorbent.

Figure 14. Capture of lipase from Y. lipolytica fermentation broth on Q HyperZ sorbent.



0.7 ml Q HyperZ packed bed column; Equilibration: 50 mM Tris-HCl , pH 9.0 ; Sample (2 ml) is diluted 3 times; Linear flow rate 300 cm/h; Elution in 50 mM Tris-HCl, 1 M NaCl, pH 9.0 at 150 cm/h; Regeneration: 1 M NaOH.

Large-scale manufacturing, Regulatory and Validation support

HyperZ™ sorbents are manufactured at large-scale under ISO 9001 at the BioSepra Process Division of Ciphergen. Regulatory support documents and plant audits are available on request.

References

1. Trinh, L., et al., *Bioseparation 9* (2000) 223.
2. Voute, N., et al., *IJBC Vol. 5/1* (2000) 49.
3. Voute, N. and Boschetti, E., *Bioseparation 8* (1999) 115.
4. Ciphergen Process Proteomics Product Note LPN PN702-001: "Q, S, DEAE, CM Ceramic HyperD® ion exchange sorbents" (01/2002).

Ordering Information

Product	Cat. No.	Size	
Q HyperZ™	21012-010	50 g	(~25 ml)
	21012-020	250 g	(~125 ml)
	21012-030	1 kg	(~500 ml)
	21012-040	4 kg	(~2 L)
	21012-050	10 kg	(~5 L)
CM HyperZ™	21011-010	50 g	(~25 ml)
	21011-020	250 g	(~125 ml)
	21011-030	1 kg	(~500 ml)
	21011-040	4 kg	(~2 L)
	21011-050	10 kg	(~5 L)

2 grams are approximately equivalent to 1 ml.
Custom packaging available on request.

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About BioSepra® Products & Services

Ciphergen Biosystems develops, manufactures and markets BioSepra® process chromatography sorbents that greatly simplify protein purification development and significantly improve biopharmaceutical manufacturing productivity.

Over the past 25 years, BioSepra® chromatography products & services have earned an outstanding reputation for product innovation and technical support. Our expanded R&D sorbent program, new ISO 9001 manufacturing plant and recently launched MEP HyperCel™ and CM HyperZ™ sorbents represent our latest commitment to the biopharmaceutical industry.

With the acquisition of BioSepra® products & services, Ciphergen has been able to combine chromatography development expertise with SELDI-based ProteinChip® technology to set in motion an entirely new approach to protein purification called Process Proteomics. This new approach combines the previously separate operations of purification optimization and protein analysis. This single-step, on-chip approach dramatically accelerates and simplifies purification development and analysis. The future of Process Proteomics begins with Ciphergen.

These products are for laboratory research use only and are not intended for human or animal diagnostic, therapeutic, or other clinical uses, unless otherwise stated. The information contained in this brochure are subject to change without notice.

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