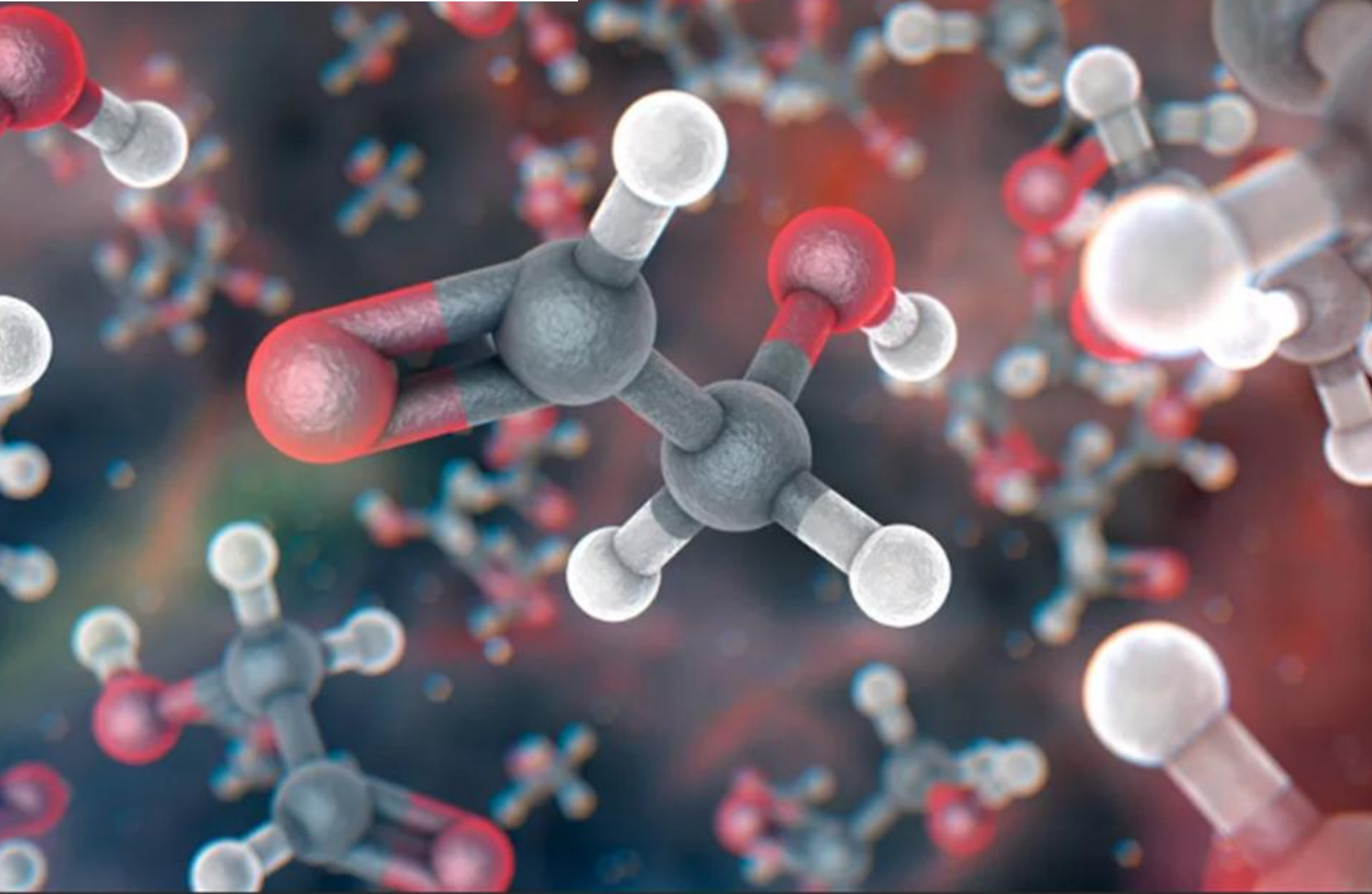


ThermoFisher
S C I E N T I F I C



POROS™ chromatography resins

Thermo Scientific™ POROS™ chromatography resins are designed for both analytical and process-scale bioseparations. They enable high-resolution separations combined with high capacity and robust chemical stability.

POROS bioprocessing resins are based on high-performance chromatography resins—combining high resolution, high capacity, and high speed—for process-scale bioseparations. These rigid, robust particles enable high-resolution separations with double to triple the throughput of conventional fast-flow gels. They are easy to handle and pack and offer outstanding cleanability. POROS bulk chromatography resins are used throughout the industry, offering high-performance, high-throughput process-scale bioseparations.

POROS® have compatibility and stability to salt buffers. Sorbents POROS® includes various cations and anion exchangers, and sorbents for affinity chromatography of specific proteins.

Sorbents POROS® are supplied pre-packaged analytical and preparative columns of various sizes and in the form of a bulk material with a nominal particle size of 20 and 50 µm



Leading Capabilities for Every Step of Your Workflow

Upstream

Downstream

Cell line development and media optimization



Mixing, cell culture and fermentation



Harvest and collection



Purification



Bulk storage and final fill



QC & Analytics

- Core competencies in ligand and resin development POROS™ and Capture Select™ chromatography resins;

- Affinity ligand production capabilities since 1997 in Naarden, the Netherlands - upstream vessels ranging from 10 L- 15,000 L;

- Resin manufacturing facilities in Bedford MA, USA - successful track record in resin development and commercialization for more than two decades

- Dedicated pilot plant facility;
- Scale-up to commercial lot sizes of 250 L.



POROS™ chromatography resins



CaptureSelect™ affinity ligands and resins



Transfer assemblies



Large volume liquids



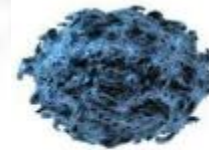
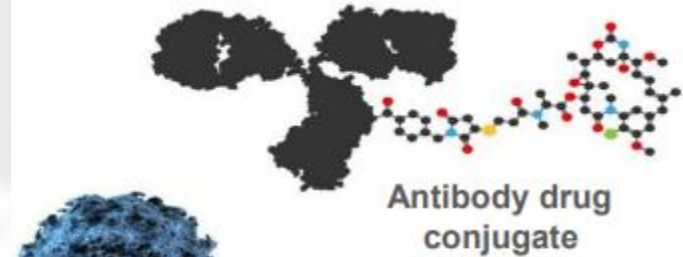
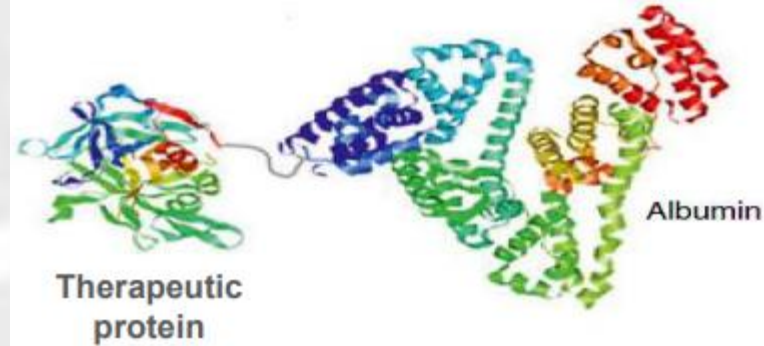
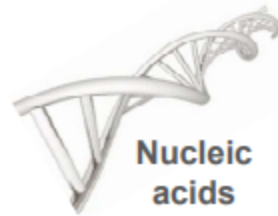
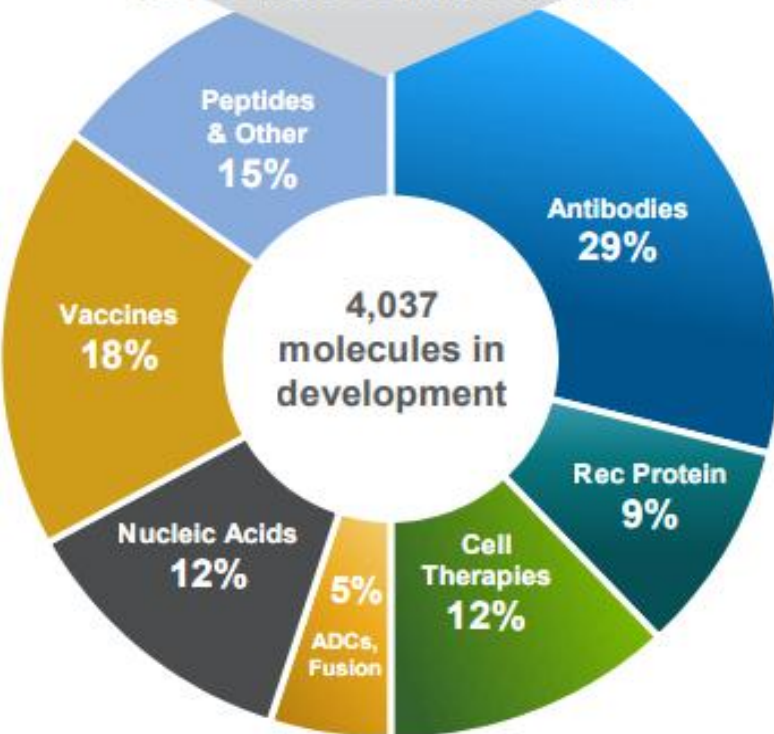
Host Cell DNA, Host Cell Protein and Protein A Quantitation



Growing Diversity of Biological Molecules in Development

2016 Global Biologics Preclinical to Registered

Less than 30% are MAbs



New molecule modalities lead to new purification challenges driving a need for additional tools

BioProduction Purification Solutions

CAPTURE

Affinity resins

- CaptureSelect™ affinity resins
 - Unique, tunable affinity ligands / resins
 - High purity in a single step

Non-Affinity capture resins

- POROS™ High performance IEX and HIC resins

POLISH

POROS Ion Exchange resins

- High performance
- Cation and Anion exchange

POROS Hydrophobic Interaction resins

- Selectivity through a wide range of hydrophobicity
- High performance

Cell Culture Clarification

Capture Chromatography

Viral Inactivation

Polish Chromatography

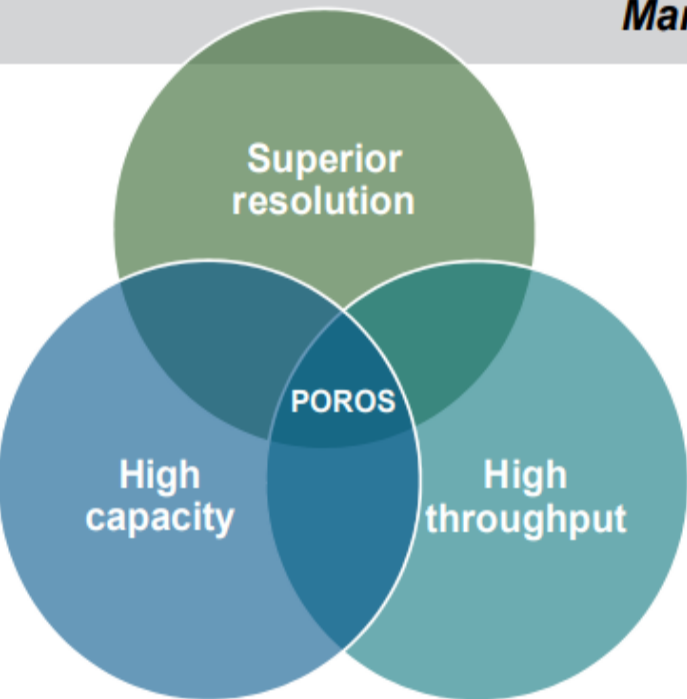
Viral Filtration

Formulation

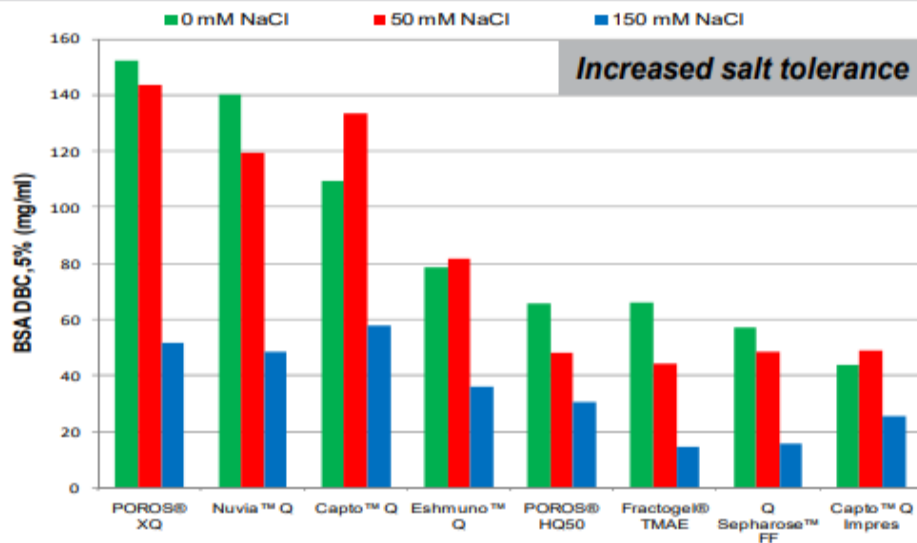
CaptureSelect & POROS; two technologies addressing today's challenges in the purification workflow

POROS Chromatography resin features simplify downstream process design

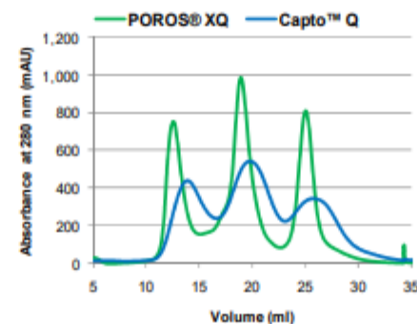
Many factors influence downstream process development



*Excellent separation of closely related product forms
Decreased process times
Lower cost of goods*

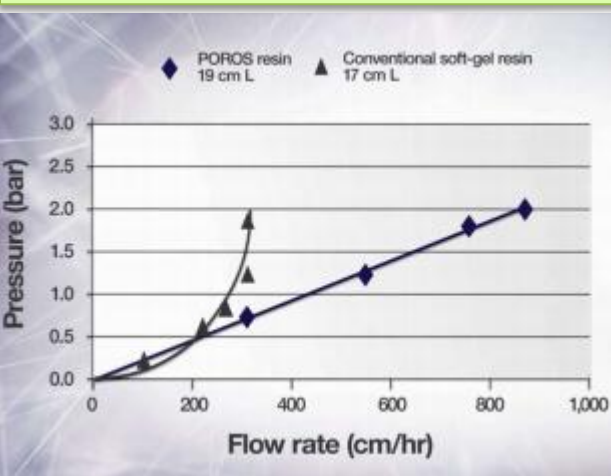


Superior resolution

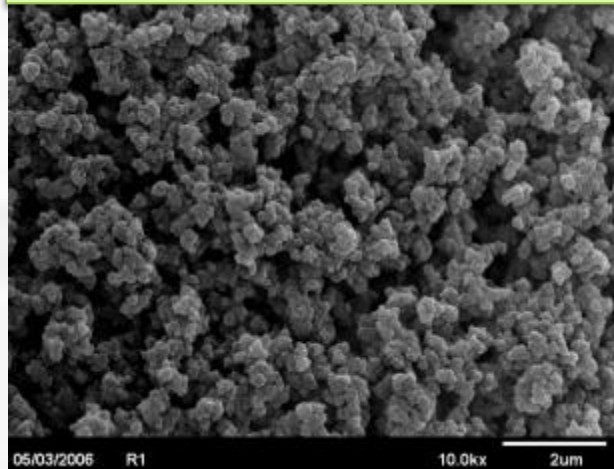


The unique features of the POROS bead

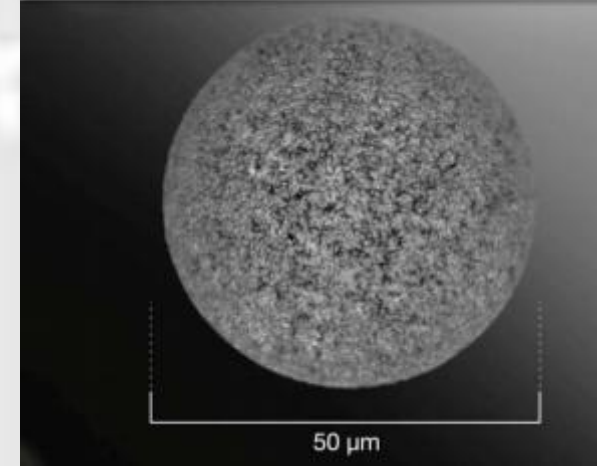
Linear pressure flow curve



Large throughpores



50 micron bead size



Polystyrene-Divinylbenzene Backbone:

- Rigid, incompressible;
- Easy handling ;
- Highly robust and chemically stable;
- Linear and predictable performance;

Large through-poresond product:

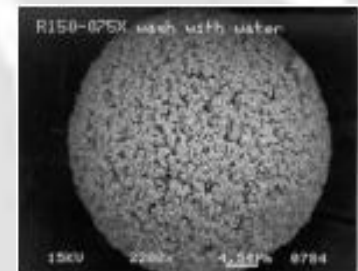
- Increased surface area;
- Diffusion is no longer rate limiting ;
- Improved mass transfer;
- More efficient purification

50 micron bead size:

- Superior resolution;
- Improved capacity;
- Excellent pressure-flow properties;
- Fully scalable;

Product manufacturing at scale

- **CaptureSelect Ligands**
 - **Proprietary affinity ligands :**
- ISO9001 certified manufacturing facility (Netherlands);
- Ligand production in yeast since 1997;
- Upstream vessels: 10 L- 15,000 L ;
- Cleanroom environment for final purification;
- Ligand production capabilities at kg's/batch;
- Completely animal origin-free production process;
- Security of supply through 2nd source ThermoFisher Vilnius site
- **Resin offering**
 - ***POROS™ chromatography resin***
 - High performance rigid, 50 micron polystyrene-divinylbenzene, resin;
 - Fully scalable, batch size up to 250 L;
 - Quality system, ISO 9001:2008 & ISO 13485:2003 Certified ;
 - ***OEM Agarose***
 - Batch sizes 6 L- 300 L, current affinity batch size 6L-90L;
 - Quality system, ISO 9001 certified.



Quality documentation

• **All CaptureSelect products which are suitable to be used in GMP processes are being manufactured under the industry appropriate quality standards:**



- ISO 9001:2008 certified;
- Guidance for Industry; Monoclonal Antibodies Used as Reagents in Drug Manufacturing (FDA; CDER and CBER, March 2001);
- Guideline on development, production, characterization and specifications for monoclonal antibodies and related products (EMA);
- To some extent: GMP guidelines/regulations (e.g. ICH, 21CFR, Rules and guidance for Pharmaceutical Manufacturers and Distributors 2002);
- **The products come with Regulatory Support Files, supported by both EMEA and FDA regulations, for regulatory filings;**
- **Regulatory support files can be accessed on-line after CDA;**
- **For all products dedicated ligand specific leakage ELISAs are available;**

Bioproduction Purification Products and Services

Bioprocess Affinity Resins

- **ANTIBODY-BASED THERAPEUTICS**

KappaXL

FcXL

CH1-XL

MabCapture™ A Select

- **PROTEIN THERAPEUTICS**

FSH

HSA

hCG

tPA

TSH

hGH

C-tagXL

- **VIRAL VECTORS**

Heparin

AAV8, AAV9, AAVX

Affinity solutions from screening to final manufacturing

Bioprocess Non-Affinity Resins

- **ANION EXCHANGE**

XQ

HQ50

D50

PI50

- **CATION EXCHANGE**

XS

HS50

- **HYDROPHOBIC INTERACTION**

Ethyl

Benzyl

Benzyl Ultra

- **REVERSED PHASE**

OLIGO R3

Resins for bioprocessing at any scale

Services

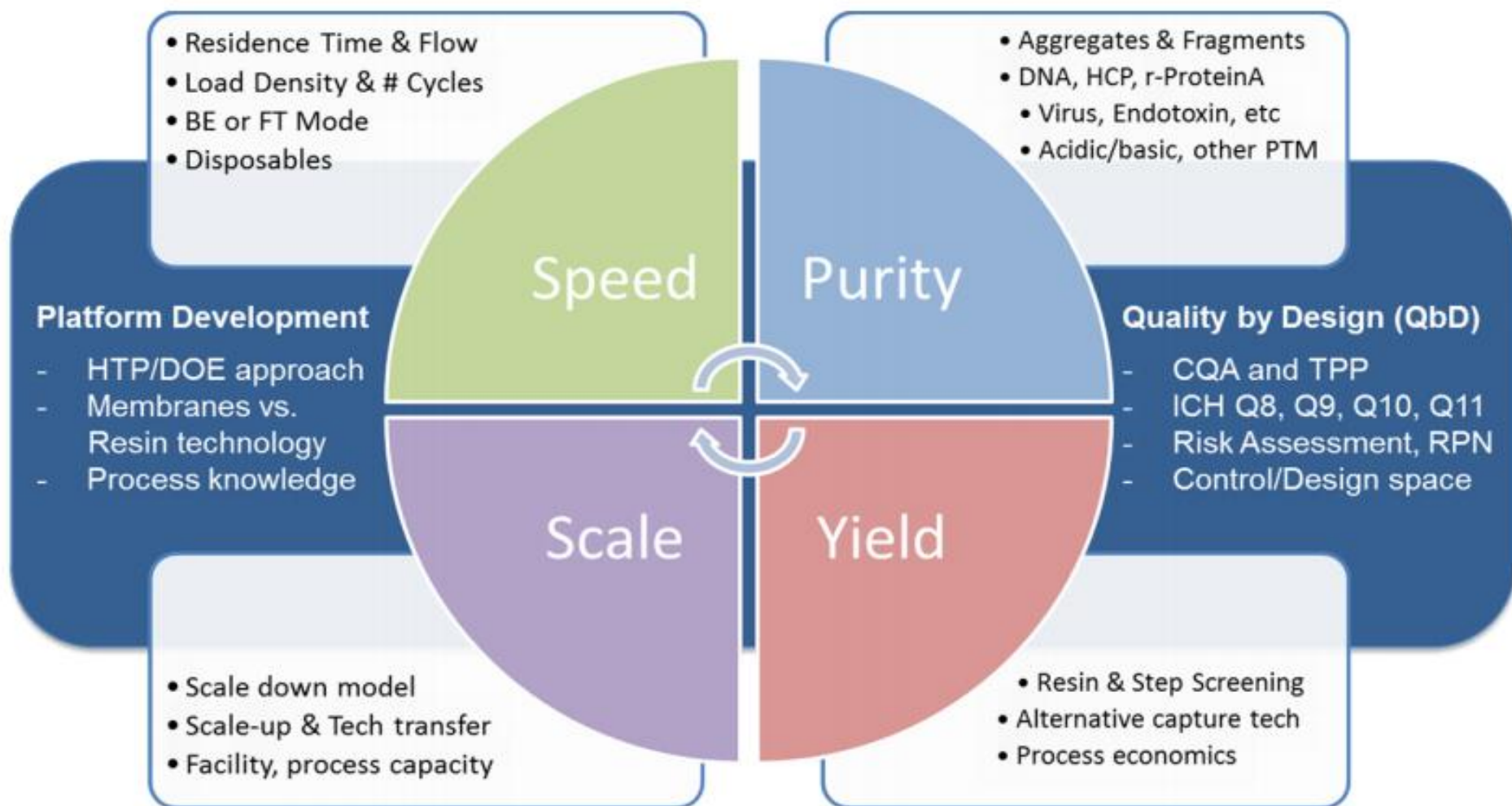
- Custom Resin Development
- CaptureSelect Affinity Ligand (and resin) Development

Pre-Packed Columns & Analytics

- HPLC tools
- Leakage ELISA
- RoboColumns
- 1 & 5ml evaluation columns
- Biotin conjugated ligands

Simplifying purification preparation and enabling custom affinity solutions

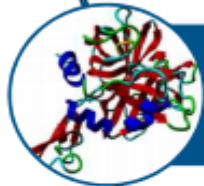
Downstream Process Development



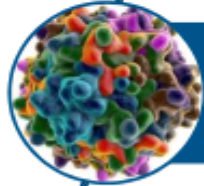
Bioprocess purification products for affinity capture chromatography



Antibody derived therapeutics



Biosimilars, recombinant proteins & plasma proteins



Viral vectors

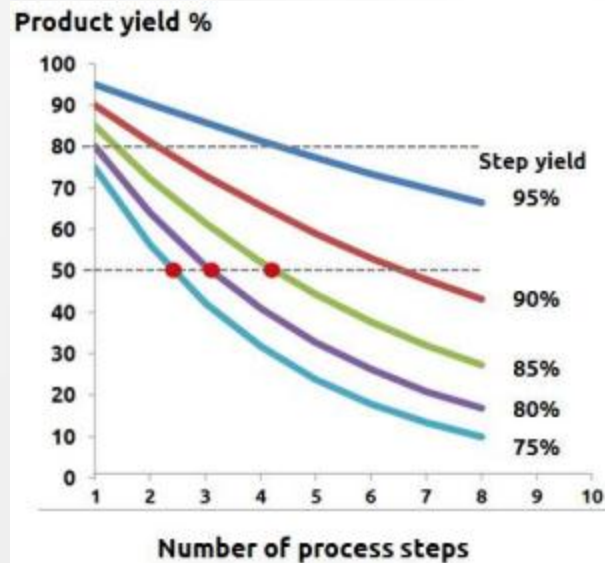
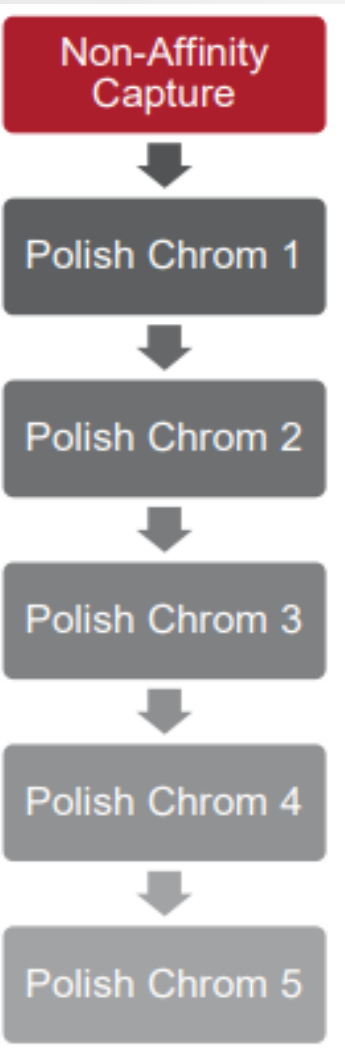


Vaccines

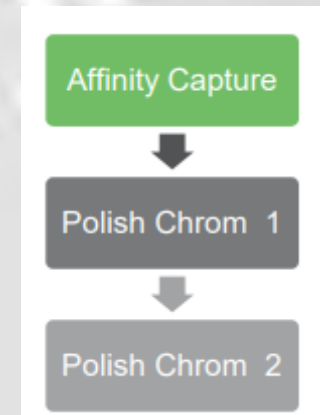
CaptureSelect solution

- High purity in a single capture step;
- Reduction of process steps;
- Mild elution conditions;
- Efficient impurity clearance.

Affinity chromatography – addressing purification challenges



80% reduction
in purification time

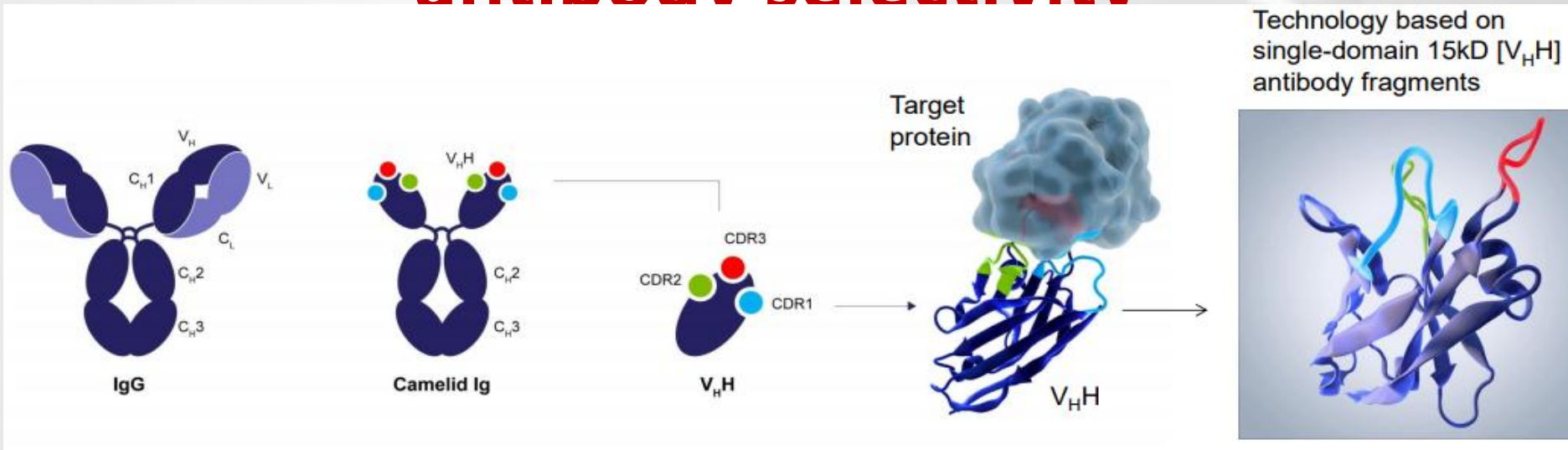


Affinity chromatography process:

- Limit the number of steps in a purification procedure;
- Increase product yield;
- Helps reduce time to market and overall cost of goods;

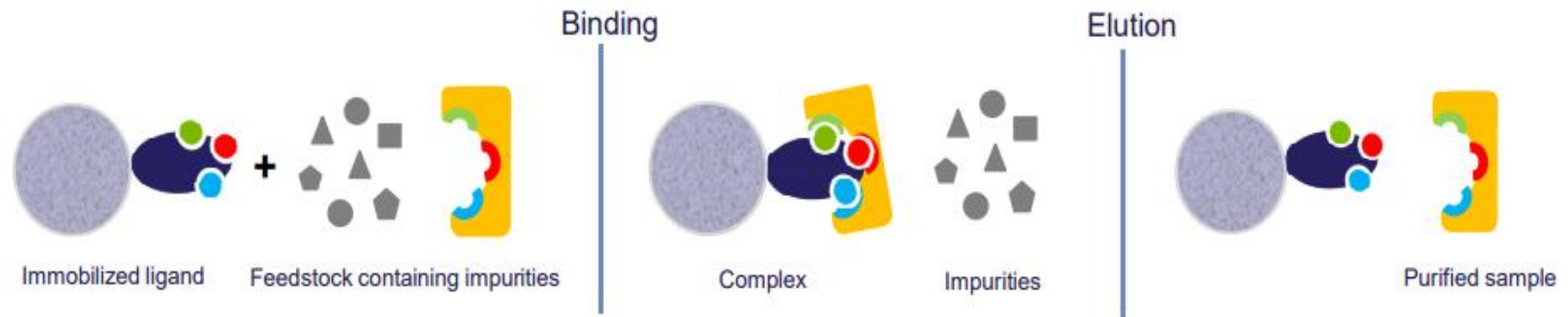
Simplifying biomolecule purification and helping to reduce cost of goods

CaptureSelect ligands : affinity through antibody selectivity

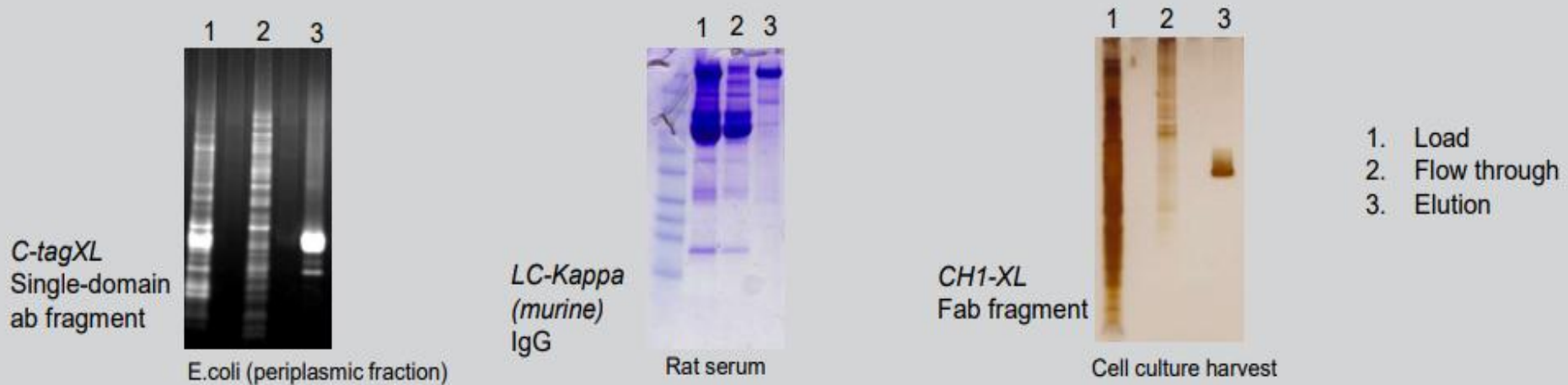


- The three CDR regions of the V_H H ligand provide unique, tunable specificity;
- Unique screening technology for target specificity, mild elution & stability (resin design features);
- Animal origin free production process (*Saccharomyces cerevisiae*);
- Technology used in commercial purification processes.

One-step selectivity for challenging purification needs

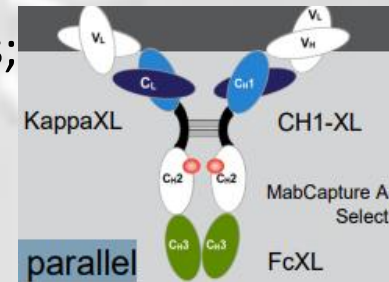


high target purity in a single capture step, independent of feed stock



Antibody and antibody fragment purification

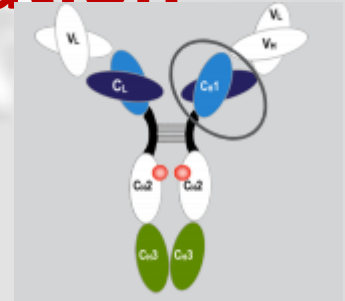
- Helping you develop the next generation of antibody therapeutics;
- Unique target specificity and mild elution protocols;
- Defined antibody region specificity eliminates the need to test multiple resins in parallel



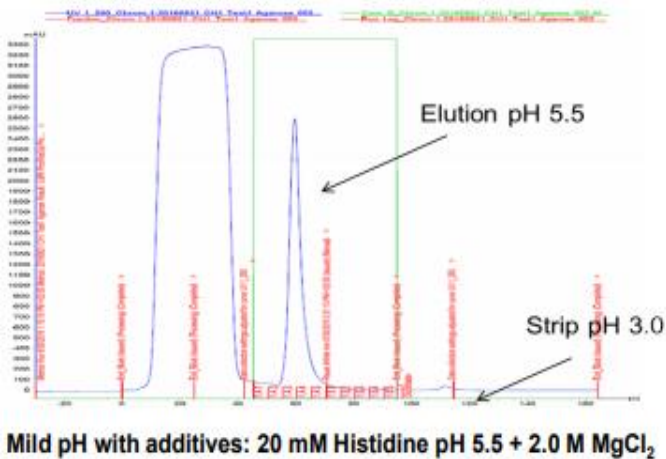
Product	Target applications	Uniqueness
CaptureSelect KappaXL	<ul style="list-style-type: none"> • Human IgG • Fab fragments containing a kappa light chain 	Mild elution for fragments and antibodies
CaptureSelect FcXL	<ul style="list-style-type: none"> • Human IgG • Fc-fusion proteins 	CH3 binding domain, mild elution
CaptureSelect CH1-XL	<ul style="list-style-type: none"> • Human IgG antibodies • Fab fragments 	Improved version of IgG-CH1, including increased binding capacity and improved stability
POROS MabCapture A Select	<ul style="list-style-type: none"> • Human IgG 	Cost-effective, high-performance Protein A resin produced from in-house sourced protein A

CaptureSelect CH1XL affinity matrix – A platform for Fab fragment purification

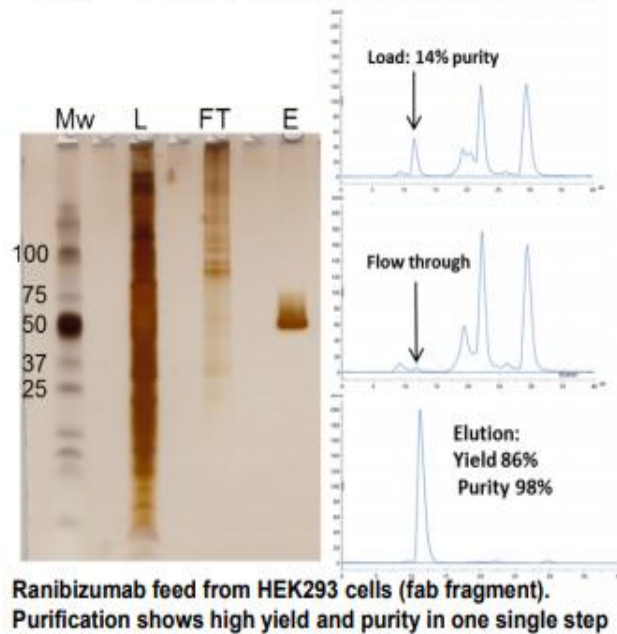
- Binds to the constant heavy domain (CH1) of all human IgG subclasses;
- No co-purification of free light chains (only correct assembled Fabs);
- High Dynamic Binding Capacity*; 19 mg/ml for polyclonal Fab;
- Efficient elution at mild pH;



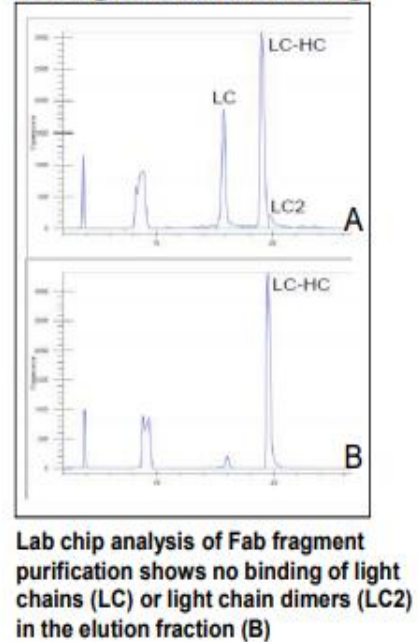
Efficient elution at mild pH



Single step Fab fragment purification



No light chain binding



Affinity solutions for biosimilars, recombinant proteins & plasma proteins

Improving downstream processing in the development of non – antibody biosimilars and biobetters

- Significant increase in purification yields
- Decrease the number of purification steps



Product	Target applications	Uniqueness
CaptureSelect FSH	Human follicle stimulating hormone	Binds only intact FSH
CaptureSelect HSA	Human serum albumin, albumin fusion proteins	Mild elution conditions help to protect fusion partner
CaptureSelect hCG	Binds to the alpha chain of HCG (human chorionic gonadotropin)	Shows binding to all human gonadotropins
CaptureSelect tPA	Human tissue plasminogen activator	Mild elution conditions
CaptureSelect TSH	Human Thyroid-Stimulating Hormone	Selective for TSH with no cross-binding to FSH, LH and HCG
CaptureSelect hGH	Human Growth Hormone	Selective for Human Growth Hormone

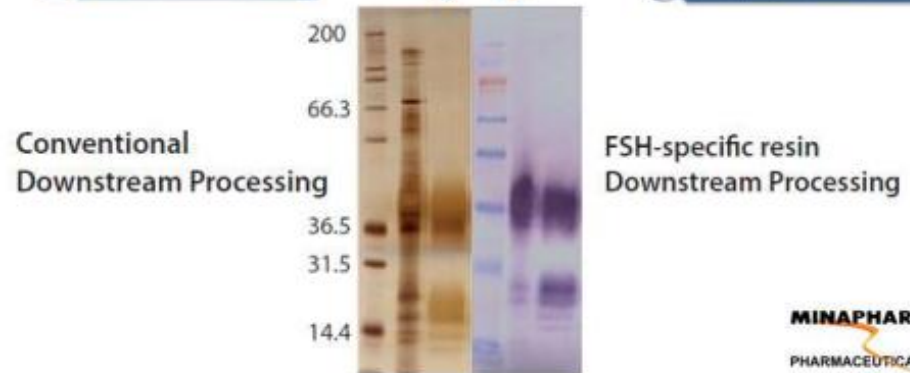
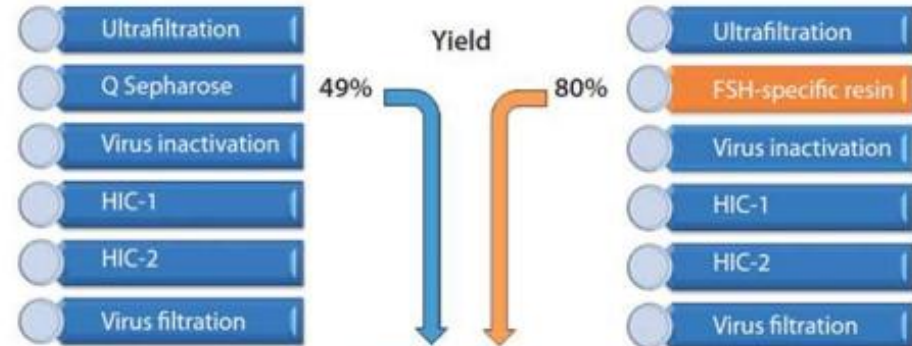
CaptureSelect FSH affinity matrix – selectivity towards conformational epitope



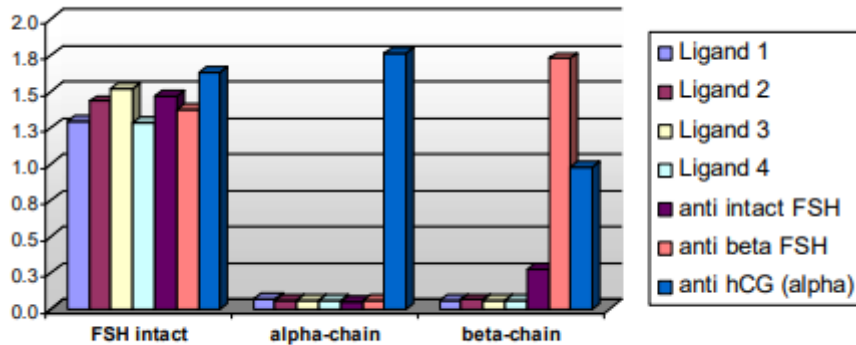
Follicle Stimulating Hormone (FSH)

- Red: Alpha-subunit
- Green: Beta-subunit

Comparing yield and purity in conventional and FSH-specific resin downstream processing



- Highly selective for recombinant intact FSH, with no crossbinding to free alpha or beta-subunits
- Mild elution, retaining biological activity

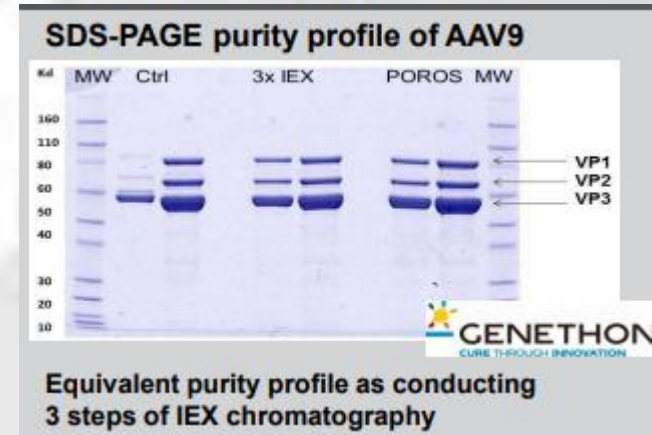


Ligand screening for intact FSH

Affinity solutions for viral vectors

Enabling large scale development of gene therapy vectors

- High specificity: enabling platform purification for AAV purification;
- High capacity: confirmed through multiple customer evaluations;
- Product is engineered on proven high performing POROS base bead;
- Excellent scalability: >80% vector recovery on 200L scale.



Thermo Scientific™ resin

Binding Capacity (vg/mL)

Serotype Affinity

POROS CaptureSelect AAV8

>10¹³

AAV8

POROS CaptureSelect AAV9

>10¹⁴

AAV9

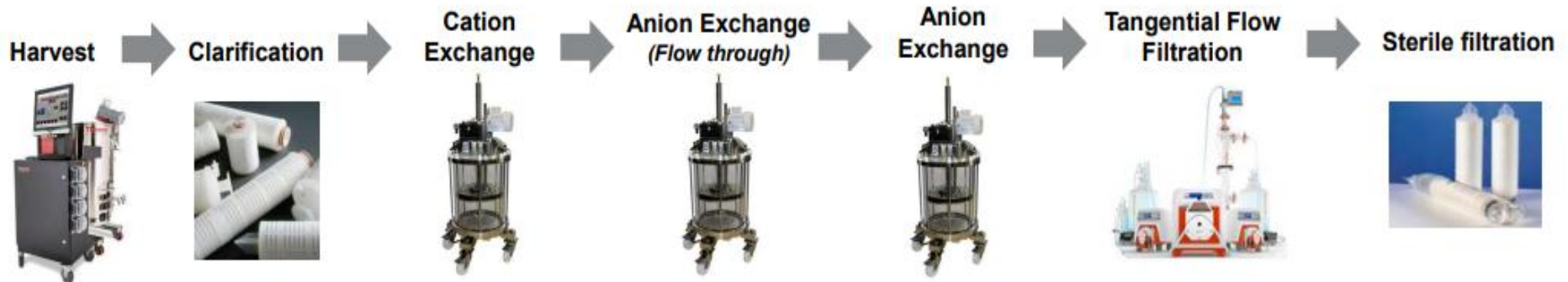
POROS CaptureSelect AAVX

>10¹⁴

* viral genomes per milliliter (vg)/mL, binding capacity will vary based on serotype, feed stream, additives, and mutations to parent serotypes

AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, recombinant and chimeric vectors

Enabling a Paradigm Shift in Viral Vector Purification – a customer case stud



CaptureSelect Paradigm

- Affinity capture and fewer chromatography steps;
- Process simplified;
- Lower cost & complexity;
- Speed to market;
- Fewer steps = higher yield;

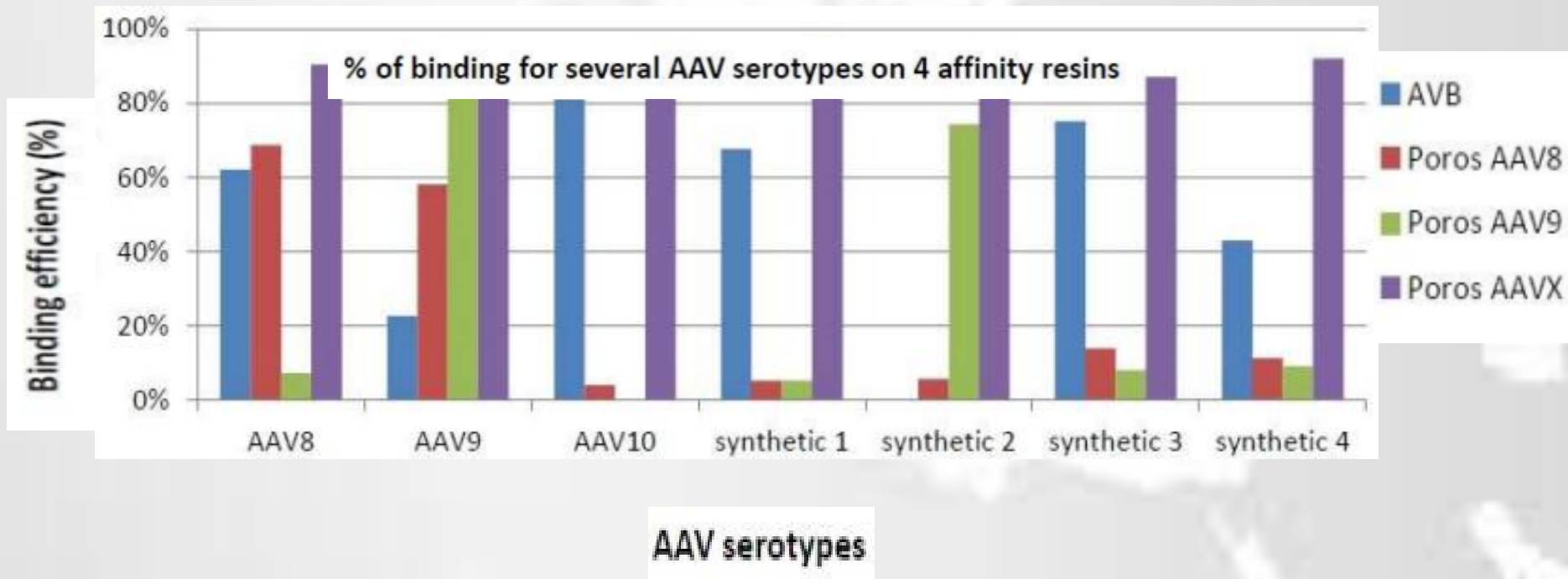
Customer testimonial: “Process yield improvement from 20% to 60% & cost reduction by a factor of 6”



AAV affinity chromatography resins: comparative study

Experimental design

- Comparison of Thermo Scientific POROS AAV resins with a competitor resin;
- 3 native AAV vectors and 4 synthetic vectors were tested in static binding mode.



- **POROS AAV resins show high affinity and capacity ;**
- **POROS AAVX displays broad selectivity to natural and synthetic capsids.**

Affinity solutions for protein based vaccines: C-tagXL

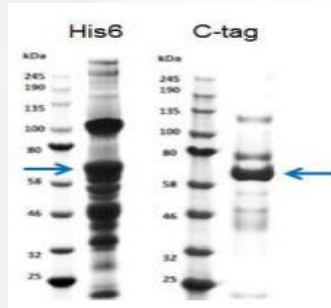
C-tag: C-terminal 4 amino acid **E-P-E-A** tag. Small **inert** tag, limiting effect on protein functionality

Enabling process scale production of protein-based vaccines

- Obtaining sufficient quantities of high quality and pure protein;
- Mild elution, protecting the protein of interest;
- Enabling high target purity and yield from complex mixtures in a “one-step” process.

Product	Target applications	Uniqueness
CaptureSelect C-tagXL	Binds the short C-tag sequence E-P-E-A	Allows for a one-step purification of C-tagged proteins

Improved yield and purity compared to His6- tag purification



Data Obtained from: Jin, J., et al. Accelerating the clinical development of protein-based vaccines for malaria by efficient purification using a four amino acid C-terminal 'C-tag'. *Int. J. Parasitol.* (2017), <http://dx.doi.org/10.1016/j.ijpara.2016.12.001>

Pipeline of CaptureSelect affinity resins

Product Stage	Therapeutic Proteins	Viruses	Antibody Types
Bioprocess Products	Follicle Stimulating Hormone (FSH), Human Serum Albumin (HSA), human Chorionic Gonadotropin (hCG), Tissue plasminogen activator (tPA), C-tagXL, Human Growth Hormone (hGH), Thyroid-Stimulating Hormone (TSH)	Adeno-Associated Virus 8 & 9 (AAV8 & AAV9) , AAV multi serotype (AAVX)	KappaXL FcXL, IgG-CH1, CH1-XL
Stage 5: RUO Products	Antithrombin III, Fibrinogen (Fib), Transferrin, Apolipoprotein H (ApoH), C1-inhibitor (C1-Inh), Granulocyte-macrophage colony-stimulating factor (GM-CSF)	Adenovirus (Adv5)	IgA (Fc), IgA-CH1, IgM, IgE Bovine IgA
Stage 4: Lead Development	Prothrombin, Deoxyribonuclease (DNase)	Influenza (HA)	
Stage 3: Lead Selection	Insulin Universal affinity tag (Uni-Tag), Factor X	Lentivirus (VSV-G)	Rabbit, Mouse & Rat IgG
Stage 2: Lead Screening	Interferon α and β (IFN α/β), Interleukin 2 (hIL2), Factor V, FXI, FXII, FXIII, and FH	Baculovirus, Exosomes	scFv
Stage 1: Library Construction	Erythropoietin (EPO)		IgY

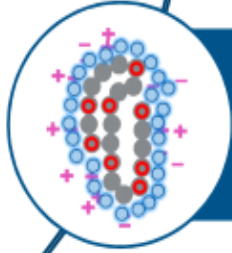
Bioprocess purification products for capture and polishing chromatography



Cation exchange



Anion exchange



Hydrophobic interaction /
Reversed Phase

POROS high performance resins

- High capacity;
- Superior resolution;
- High salt tolerance;
- Higher flowrates / linear pressure flow curves;
- Efficient clearance of HCP, DNA, virus.

POROS- Perfusion Chromatography® media

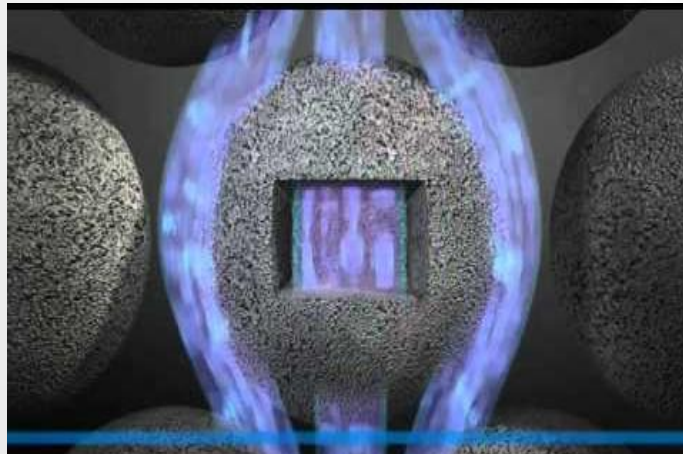
- **POROS MabCapture A:** Increase throughput without increasing the size of your process column. The highest performance Protein A affinity media for large-scale purification of monoclonal antibodies from cell culture, POROS® MabCapture™ A offers unmatched capacity vs. flow rate performance with the physical and chemical stability of a rigid polymeric support.
- **POROS 50 micron HS:** A strong cation exchange media based on a sulfopropyl functionality, POROS® 50 micron HS is designed for the purification of more basic proteins and biomolecules. It is especially well-suited for the separation of process impurities and aggregates and is an excellent choice for both capture and polishing applications.
- **POROS 50 micron HQ:** A strong anion exchange media, POROS® 50 micron HQ uses a unique quaternized polyethyleneimine functional group to separate molecules with selectivity unlike any other commercially available strong anion exchange products..
- **POROS 50 micron PI:** A weak anion exchange media, POROS® 50 micron PI utilizes the same polyethyleneimine functional group as POROS® 50 micron HQ, but with a unique selectivity that allows elution via pH change or increasing ionic strength. The large pore structure of this 50 micron product makes it ideal for purification of large biomolecules, such as plasmids and viral particles.
- **POROS® 50 micron D:** Another weak anion exchanger based on a polyhydroxylated polymer functionalized with dimethyl amino alkyl groups, POROS® 50 micron D is similar to DEAE. If DEAE is a standard step in your process, then POROS® D is a great way to take your resolution and throughput to the next level.
- **POROS® 50 micron Heparin:** A popular affinity ligand media for the purification of serum growth factors, coagulation factors, and lipoproteins, POROS® 50 micron Heparin can be operated at much higher flow rates than conventional soft gel supports..
- **POROS® 50 micron MC:** A metal affinity ligand based on an imidodiacetate group, POROS® 50 micron MC uses metal chelate chromatography to purify histidine-rich proteins and histidine-tagged proteins from complex feed streams. With POROS® 50 micron MC, you can easily scale up metal chelate chromatography from laboratory scale to high-throughput processing.
- **POROS® 50 micron R1 and R2 Reverse:** These products offer fast, high-resolution separation of proteins at process scale. The large pore structure and lower phase ratio of these two products allows recovery of intact proteins or large biomolecules such as plasmids or viral vectors to provide high-resolution, reversed-phase separations without loss of material and with rapid linear velocities.

POROS XS Strong Cation Exchange Resin

POROS® XS Strong Cation Exchange Resin (POROS® XS resin) is a 50 µm, rigid, polymeric cation-exchange chromatography resin that can be used for the chromatography of biomolecules including monoclonal antibodies, recombinant proteins, and peptides.

Description:

- All sorbents POROS has high permittivity;
- High resolution supports better product yield and improved impurity clearance;
- The polymer matrix has a high chemo stability cruel severe regeneration regimes;
- Dynamic binding capacity for proteins, greater than 100 mg/mL at 5% breakthrough
- Robust salt tolerance that maintains dynamic binding capacity up to 150 mM NaCl
- (15 mS/cm)



Support matrix	Cross-linked poly(styrene-divinylbenzene)
Surface functionality	Sulfopropyl (-CH ₂ CH ₂ CH ₂ SO ₃ ⁻
Dynamic binding capacity	≥100 mg/mL 5% breakthrough of Polyclonal Human IgG in 20 mM MES, 40 mM NaCl, pH 5.0 at 300 cm/hr in 0.46 cmD × 20 cmL column
Shipping solvent	20% ethanol
Ionic capacity	88 to 120 µmol/mL
Average particle size	50 µm

POROS Cation exchange resins

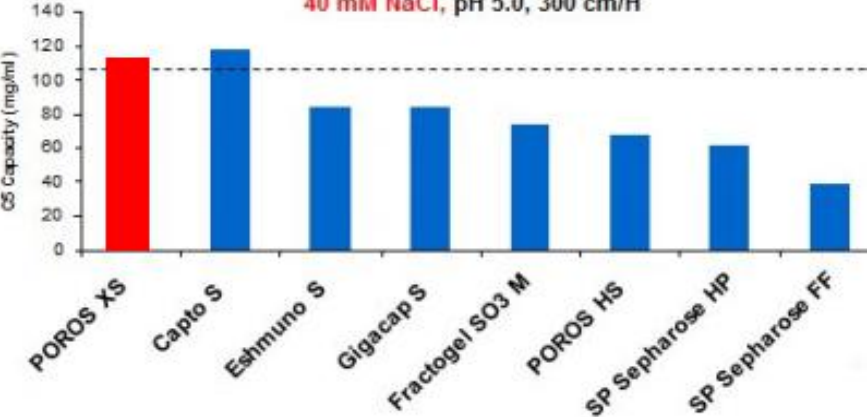
POROS Resin	Type of CEX Resin	Surface Chemistry	IgG Binding Capacity (mg/mL)	Ionic capacity (μmol/ml)	CEX Applications
HS	Strong	Sulphopropyl	75	75	<p>Bind/Elute: Polish of many biomolecules (Mabs, VLP/viruses, fusion proteins, high pl rProteins)</p> <p>Flow Through: Polish for Mabs by binding impurities under normal B/E conditions: impurity removal (aggregates, HCP, DNA, viruses)</p>
XS	Strong	Sulphopropyl	120	107	

- POROS HS and XS both provide excellent product and process related impurity clearance
- POROS XS is optimized to drive higher IgG dynamic binding capacity under higher conductivity conditions
- Ionic capacity of POROS XS is 40-50% higher than for POROS HS

POROS XS Chromatography Resin

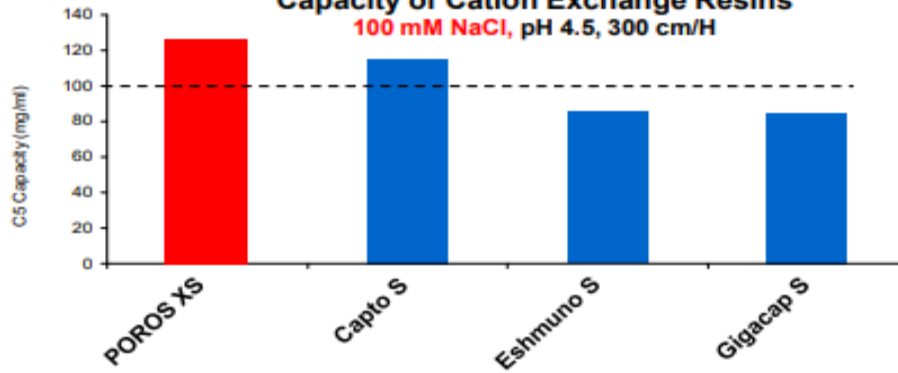
Capacity of Cation Exchange Resins

40 mM NaCl, pH 5.0, 300 cm/H



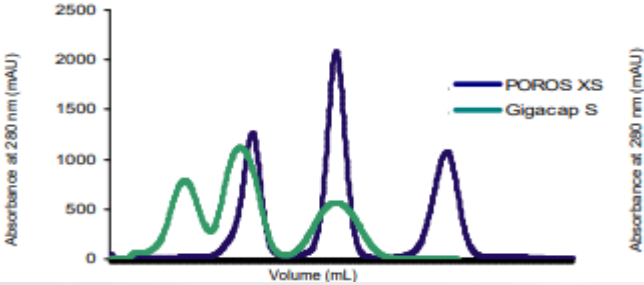
Capacity of Cation Exchange Resins

100 mM NaCl, pH 4.5, 300 cm/H

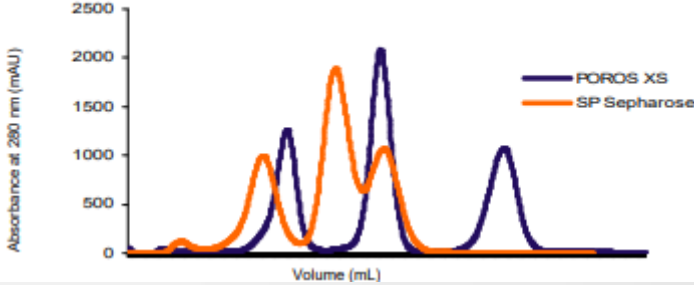


POROS XS - Combining superior capacity, salt tolerance & resolution

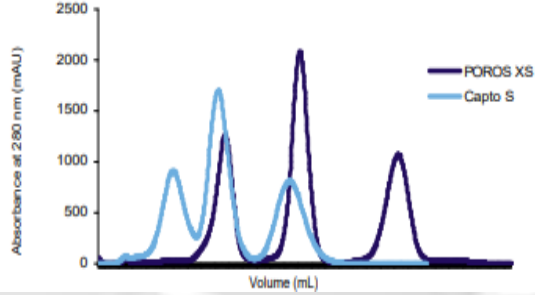
POROS XS vs. GigaCap S



POROS XS vs. SP Sepharose FF



POROS XS vs. Capto S



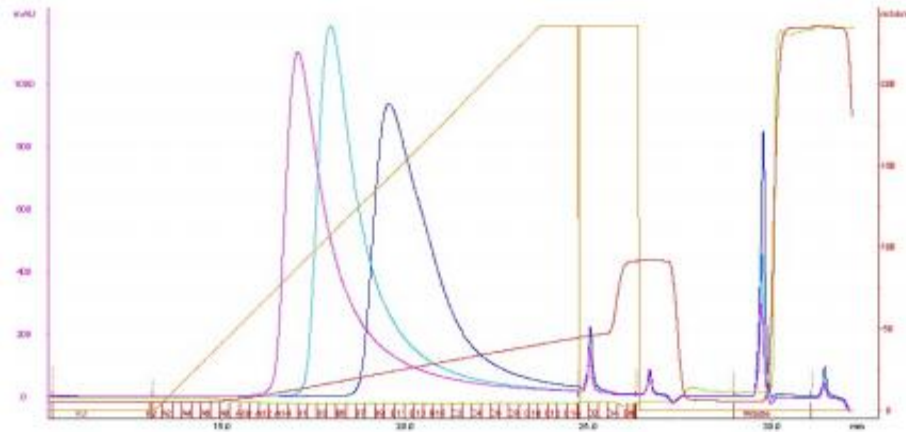
Improved separation with a high resolution resin – POROS XS

Experiment: IgG separation performed on POROS XS vs Soft gel Cation exchanger under the same 3 pH conditions.

Run 1 – pH 5.5

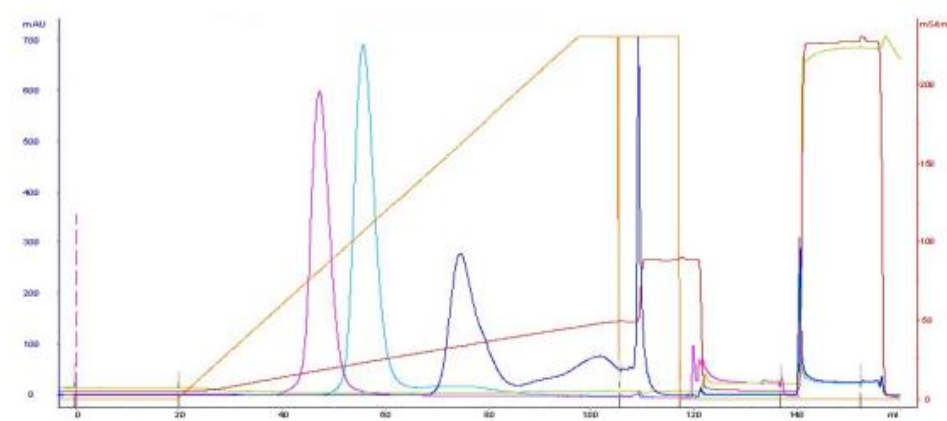
Run 2 – pH 5.0

Run 3 – pH 4.5



Soft gel resin

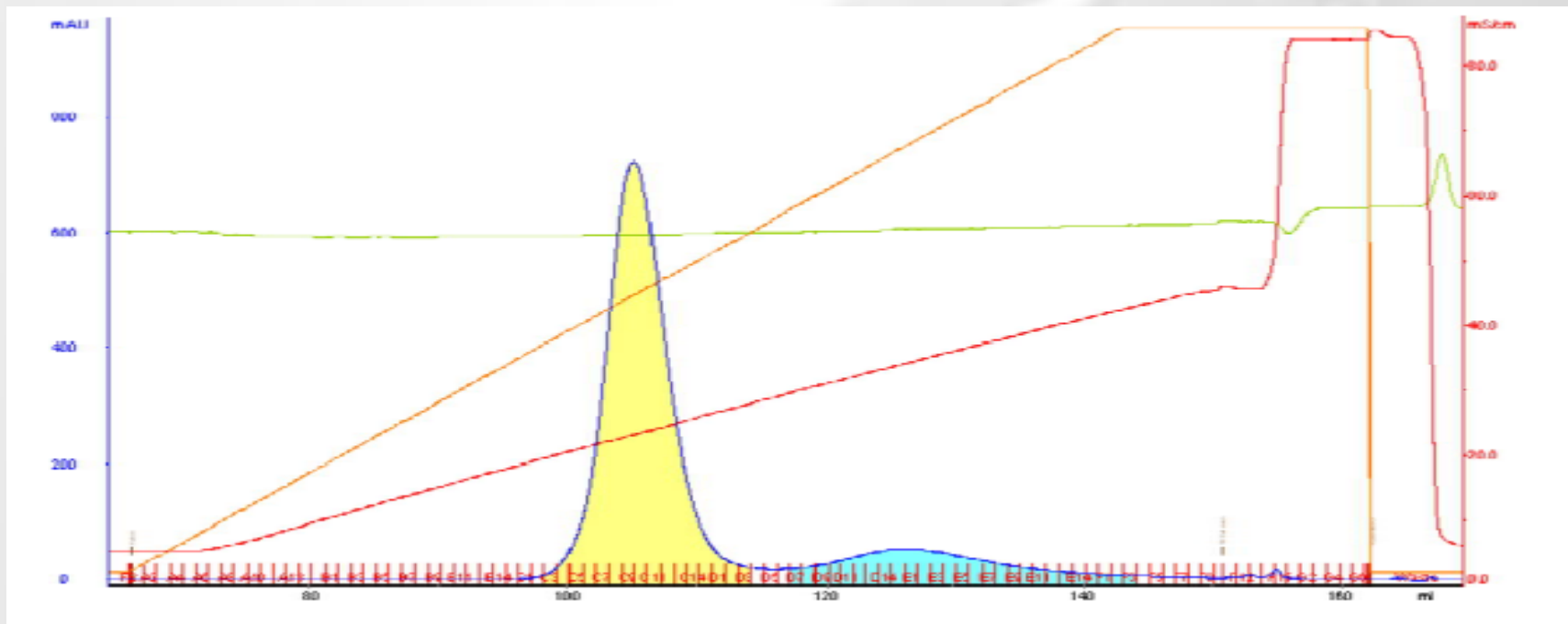
- Tailing elution peaks
- No good separation between monomeric and aggregated IgG



POROS XS

- Tight elution peaks
- At pH 5 and 4.5 aggregate gets separated from the monomer

Optimization on POROS XS



Optimization on POROS XS

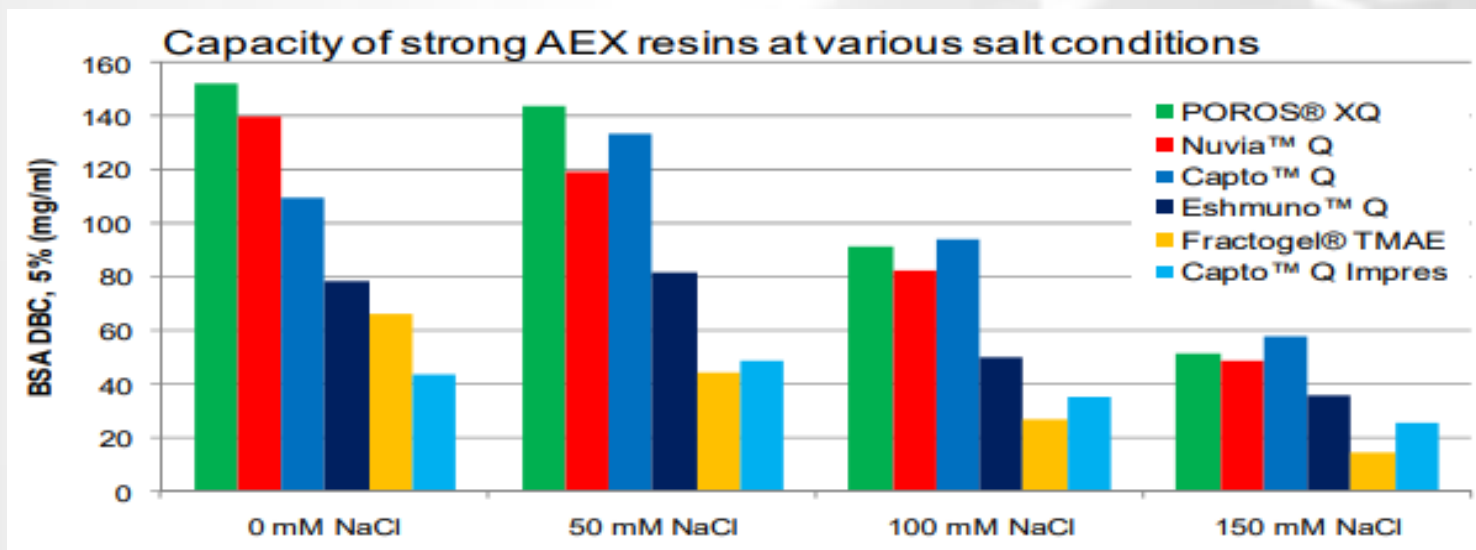
- Optimization of baseline separation;
- Gradient can be optimized to step elution;
- Improved product yield due to efficient separation of monomer and aggregate;

POROS Anion Exchange Resins

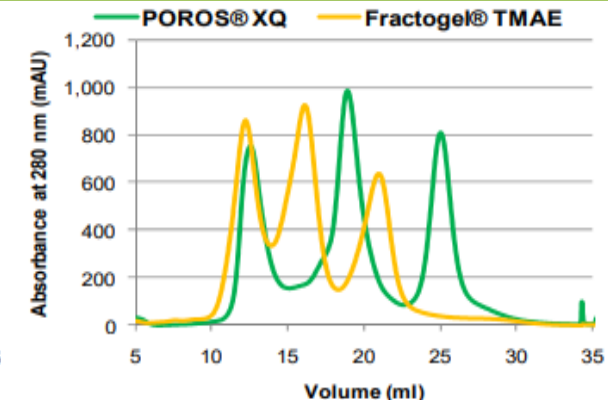
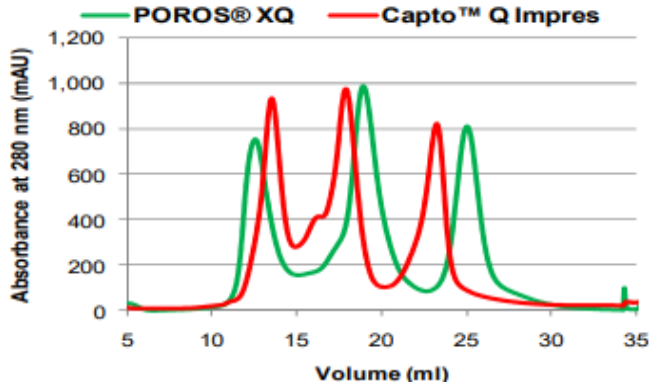
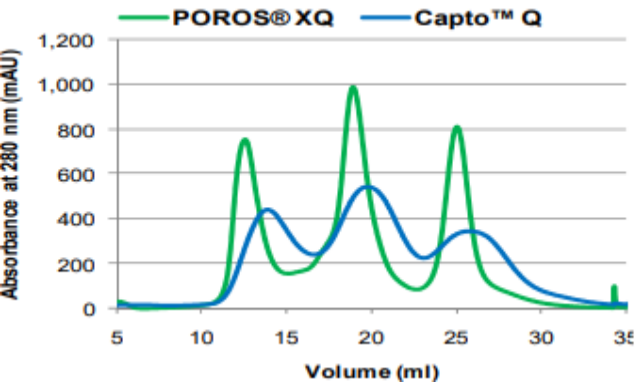
A full range of weak and strong anion exchange resins with unique surface chemistries, that provide unique selectivity

POROS Resin	Type of AEX Resin	Surface Chemistry	BSA Binding Capacity (mg/mL)	AEX Applications
D	Weak	Dimethylaminopropyl	90	Bind/Elute: Protein, virus, plasmid DNA purification Flow Through: Trace impurity removal by binding impurities (DNA, viruses, HCP, aggregates, endotoxin)
PI	Weak	Polyethyleneimine (mixed amine)	80	
HQ	Strong	60% Quaternized polyethyleneimine (mixed amine)	75	
XQ	Strong	Fully quaternized amine	>140	

POROS XQ Strong AEX Chromatography Resin



POROS XQ - Combining superior capacity, salt tolerance & resolution



POROS XQ Case study:

Mab polish in a 2-step process

Goal: Optimize a two-step purification process by designing a scalable AEX flow through polish step for acidic/neutral antibodies ($pI < 8$)

- Maximize impurity clearance (HCP, DNA, HMW, viruses and leached Protein A)
- Maximize product yield

Process Details:

- 7 Mabs tested
- Loading Capacity: 100-300mg of protein/ml of resin
- Study format:
 - HTP Screening: 96 well plates, 40 μ l resin volume in 270 μ l working volume
 - Development: 1.1cmD x 5.3cmL, 5ml columns
 - Pilot: 14cmD x 18.5cmL, 2.9L

Research Article

Pharmaceutical
BIOPROCESSING

Pharm. Bioprocess. (2015) 3(8), 477-487

Development of an acidic/neutral antibody flow-through polishing step using salt-tolerant anion exchange chromatography

POROS XQ Case study:

Mab polish in a 2-step process

POROS XQ:

- Eliminated the need for load dilution and diafiltration steps
- Reduced DNA and Protein A leachate levels to below quantification limit

Table 3. Summary of XQ purification process performance.

Antibody	Equilibration buffer	Process loading (mg/ml)	Yield (%)	HMW (%)	HCP (ng/mg)	DNA (pg/mg)	ProA (ng/mg)
mAb-A	20 mM Tris, pH 8.5 3.21 mS/cm	150	93	2.14	20	BQ	0.2
mAb-FL	50 mM Tris, pH 7.5 9.18 mS/cm	314	98	0.66	11	BQ	0.4
mAb-FY	50 mM Tris, pH7.5 10.10 mS/cm	114	91	1.31	26	BQ	BQ
mAb-R	20 mM NaPi, pH 6.8 7.50 mS/cm	100	89	1.36	7	ND	ND
mAb-T	20 mM NaPi, pH 7.2 6.00 mS/cm	100	92	0.58	15	BQ	ND
mAb-T (Pilot)	20 mM NaPi, pH 6.8 7.10 mS/cm	100	93	0.80	7	BQ	0.1
mAb-P	50 mM Tris, pH7.5 9.02 mS/cm	300	97	1.13	26	BQ	ND
mAb-C	50 mM Tris, pH7.5 10.10 mS/cm	100	90	0.54	4	BQ	BQ

BQ: Below quantification limit; HCP: Host cell proteins; HMW: High molecular weight species; ND: Not determined.

**Up to 8 Fold
HMW
Clearance**

**Up to 9 Fold
HCP Clearance**

Effective clearance while maintaining high product yield in flow-through mode

Data from Kang, Yun (Kenneth), et al (Eli Lilly) "Development of an acidic/neutral antibody flow-through polishing step using salt tolerant anion exchange chromatography", *Pharmaceutical Bioprocess* (2015), V8 (8), pages 477-487, DOI 10.4155/pbp.15.28

Viral Clearance on POROS XQ resin with increasing conductivity

POROS XQ			
Load Conductivity	Load Capacity (IgG/ml of resin)	XmuLV Log10 Reduction	MMV Log10 Reduction
5 mS/cm	500	$>4.31 \pm 0.12$	$>5.10 \pm 0.09$
10 mS/cm	500	$> 4.39 \pm 0.14$	1.61 ± 0.23
15 mS/cm	500	3.46 ± 0.29	0.19 ± 0.28

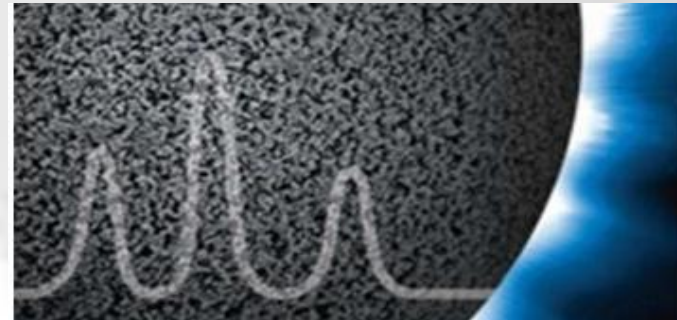
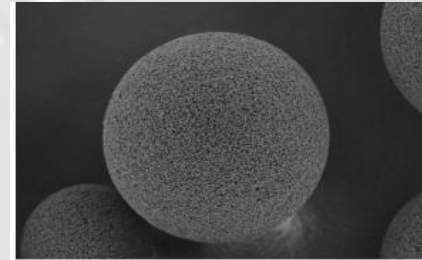
Viral clearance with higher salt concentrations allows for increased flexibility when designing a purification scheme

- Minimizes need for load dilution and diafiltration steps
- Allows for more efficient and cost effective processes

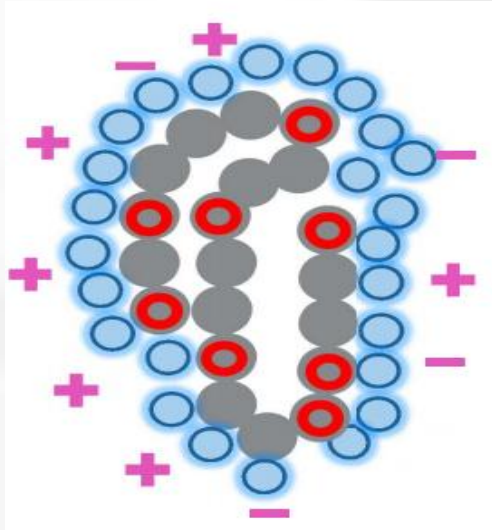
POROS XQ resin provides excellent viral clearance for both model viruses under typical FT/Wash conditions

Introducing the POROS HIC Resin Family

- **A family of differentiated Thermo Scientific™ POROS™ HIC resins:**
 - POROS Ethyl Resin
 - POROS Benzyl Resin
 - POROS Benzyl Ultra Resin
- **Unique products engineered with novel hydrophobic ligands and bead to provide highest resolution for impurity removal**
- **Resin features include:**
 - Superior resolution capability
 - High capacity for a range of molecules
 - Use of lower salt concentrations & weaker lyotropic salts
 - Flow rate independent performance
 - Robust stability



Hydrophobic Interaction Chromatography – “Salting Out” Model



Example Protein

- Ionic charges
- Hydrophobic charges
- Hydrogen bonding
- Van Der Waals interaction

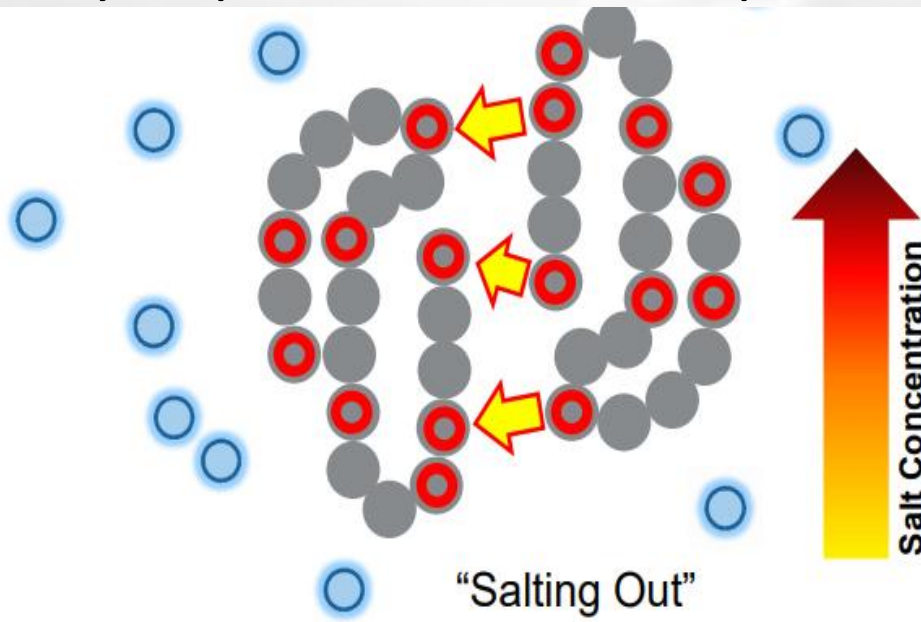
In an Aqueous solution...

- Water surrounds the molecule in an ordered manner to favor ionic interactions and hydrogen bonding
- Hydrophobic moieties are sequestered in the protein core (red spheres)

Hydrophobic Interaction Chromatography – Salting out

As lyotropic salt concentration increases

- Water molecules are displaced
- Ionic charges are masked
- Hydrophobic areas are exposed



- Hydrophobic molecules are attracted to other hydrophobic molecules (or resin surfaces)
- At precise salt concentrations, proteins will precipitate out of solution

POROS HIC resins – A range of hydrophobicity

POROS Benzyl Ultra

Key Application:

Flow-through Mode in Lower Salt such as Aggregate Removal

POROS Benzyl

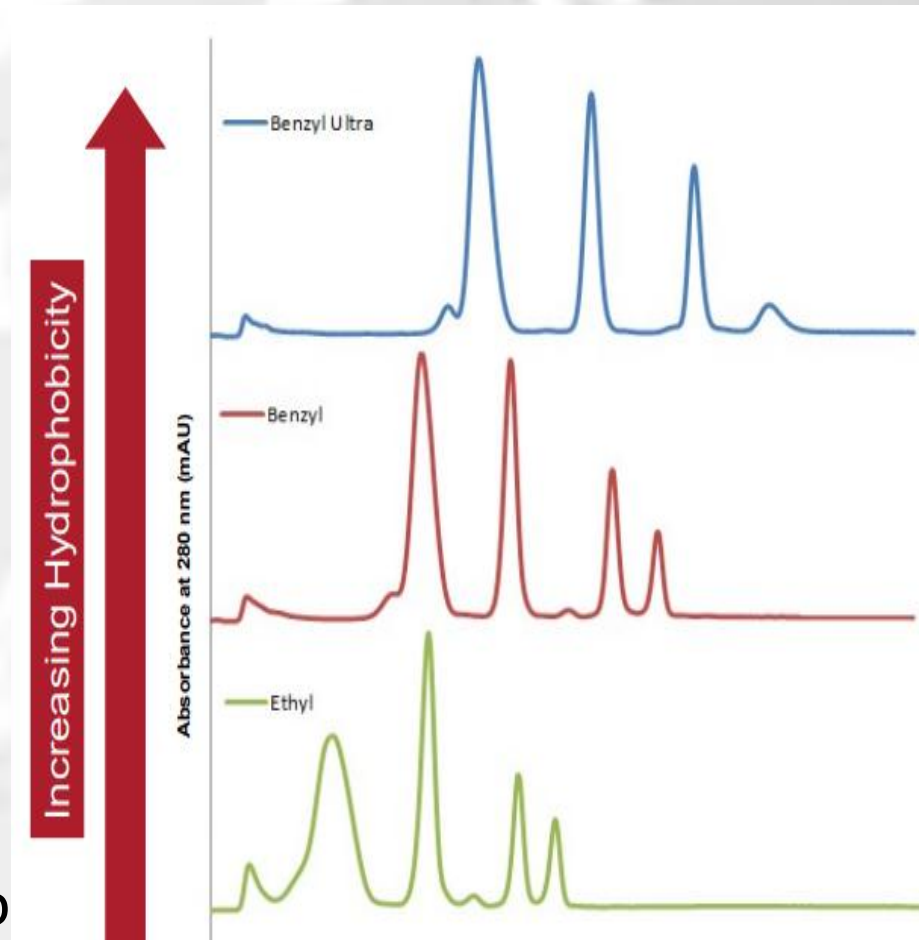
Key Application:

Bind/Elute Mode or Flow-through Mode depending on molecule

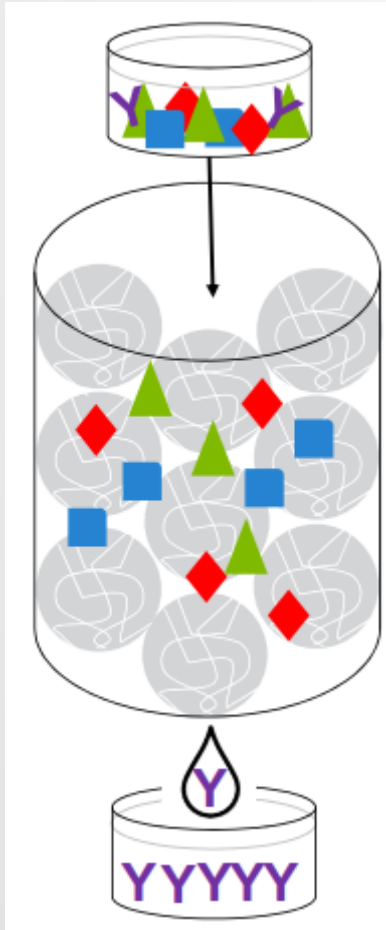
POROS Ethyl

Key Application:

Bind/Elute Mode of Moderately to Considerably Hydrophobic Molecules



What is Flow Through Chromatography?

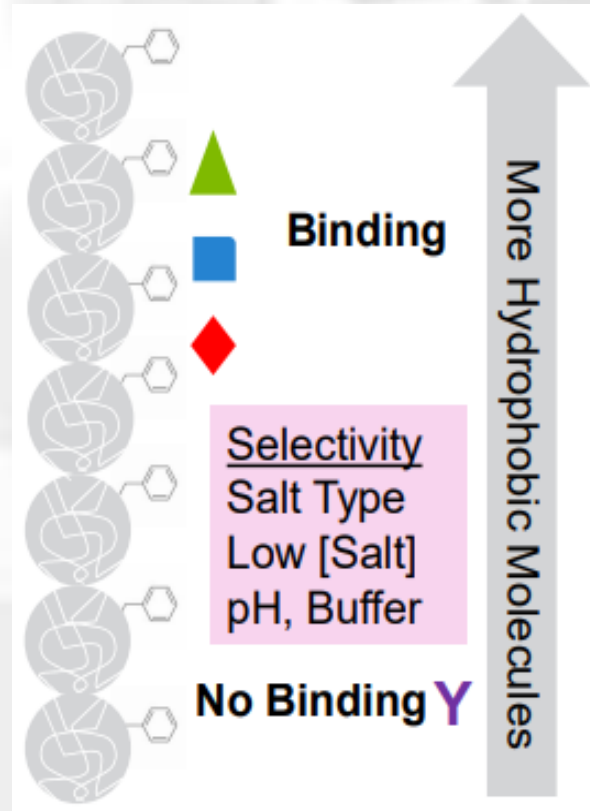


**Load
mAb + Impurities**

**Hydrophobic
Impurities and
Aggregates
Retained**

**mAb Product
Flow Through**

POROS Benzyl Ultra



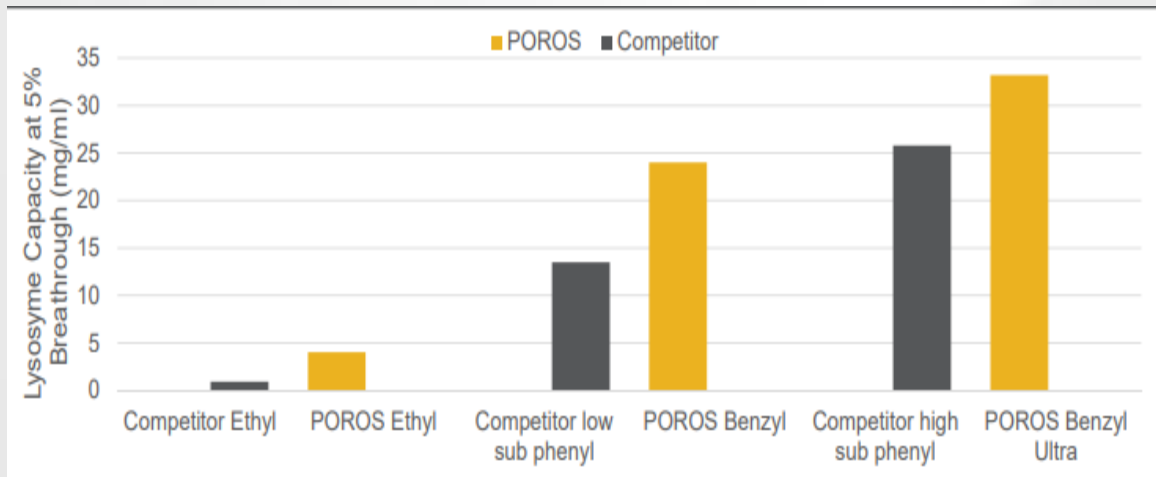
Binding

Selectivity
Salt Type
Low [Salt]
pH, Buffer

No Binding Y

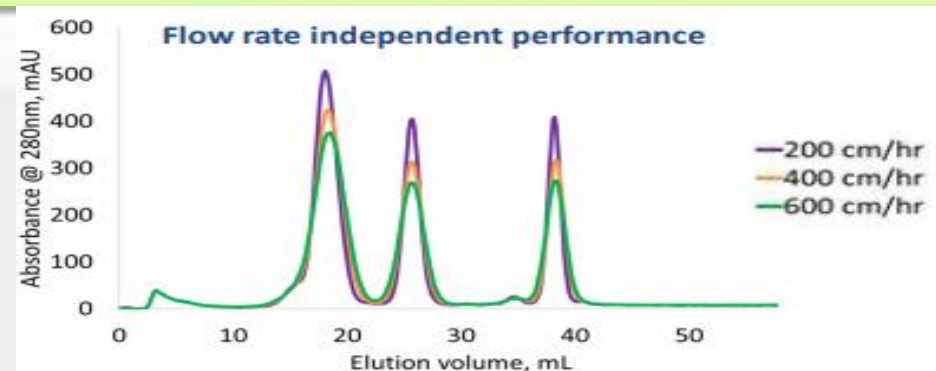
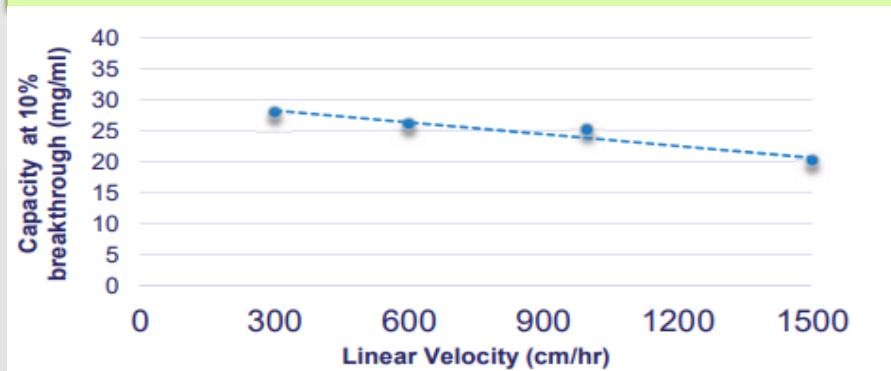
- mAb aggregates
- Hydrophobic HCPs
- ResDNA, virus, other contaminants
- mAb monomer

POROS HIC resins – strong performance

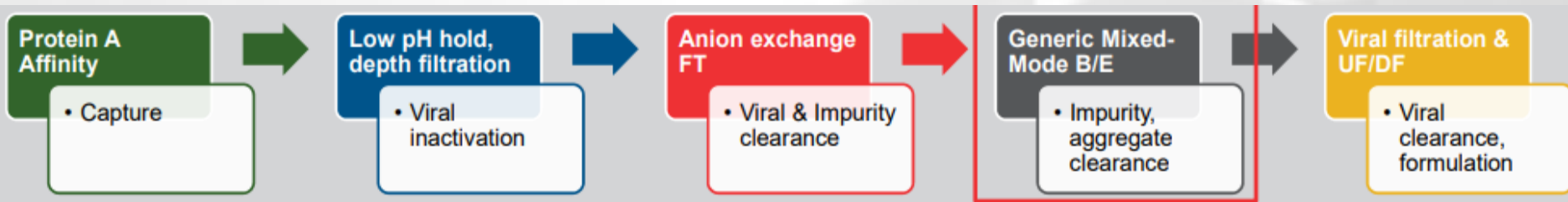


- Superior resolution
- User of lower salt concentrations
- Robust stability

POROS HIC resins - Superior capacity & resolution independent of flow rate

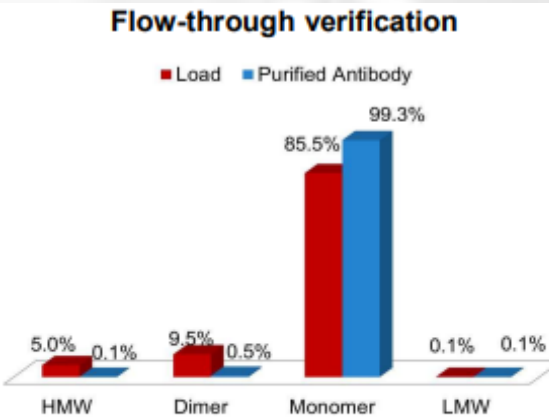
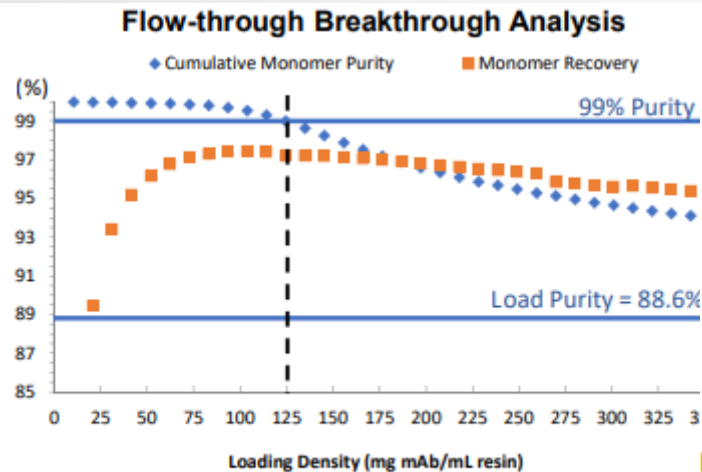


POROS Benzyl Ultra Case study: Optimizing a Mab purification polishing step in flow through mode



Goal: optimize the purification process of a monoclonal antibody feed containing high levels of aggregate (12%)

Focus: replacing generic Mixed-Mode in bind elute mode with a POROS HIC resin in flow through mode



Effective reduction of dimer and HMW with high recovery

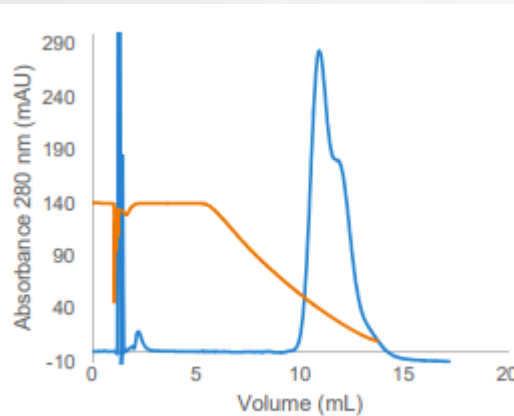
mAb-A Process	Mixed-Mode BE	HIC FT
Load Density (g/L resin)	25	80
Monomer Purity FT (%)	99	>99
Mon. Recovery (%)	90	98
HCP (ppm)	<LLOQ	<LLOQ
Residence time (min)	6.0	1.2

A successful Mab polish step for high efficient aggregate removal and near complete monomer recovery

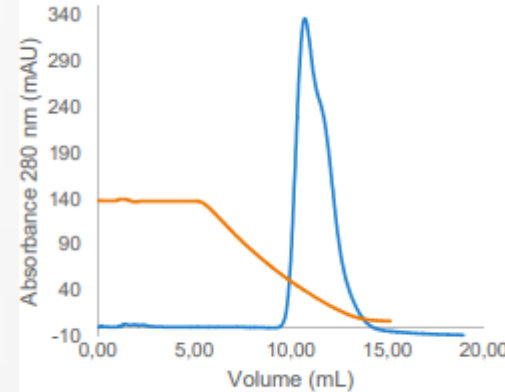
125 g/L resin loading at 1% breakthrough of aggregates in low conductivity solutions, 97% monomer recovery

Comparative case study: ADC Purification by HIC in bind elute mode

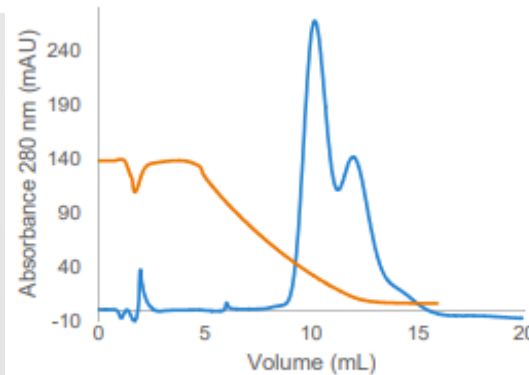
Goal: Resolve individual Antibody Drug Conjugates by Drug to Antibody Ratio (DAR)



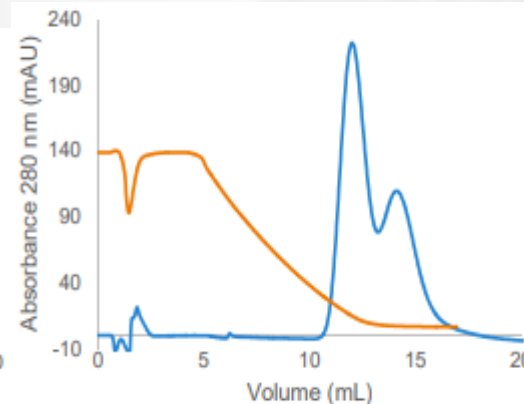
Competitor resin 1



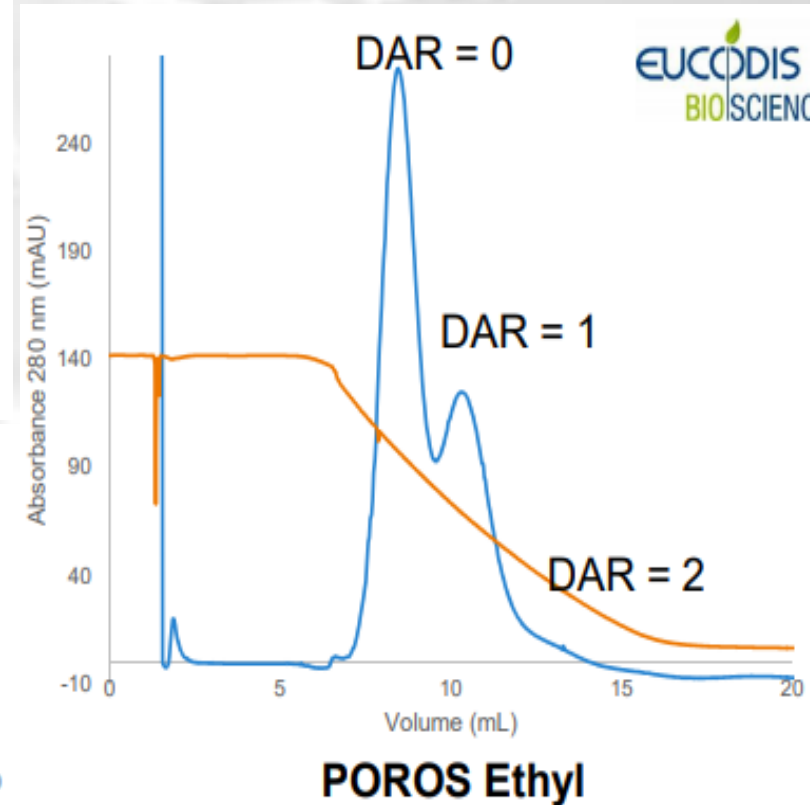
Competitor resin 2



POROS Benzyl



POROS Benzyl Ultra

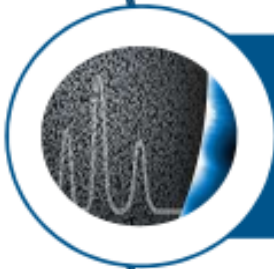


Matched molecule and surface hydrophobicity leads to highest resolution

Custom production solutions & supporting products



Custom affinity resin development



Custom POROS resin development



Supporting products

CaptureSelect™ resin development: set-up and timings

WP1
10 wks

Library Construction

1 to 10 targets

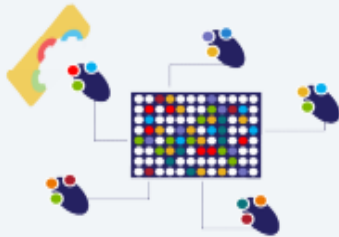


Or
Existing libraries from
collection

WP2
10 wks

Lead Screening

200 → 24 → 6



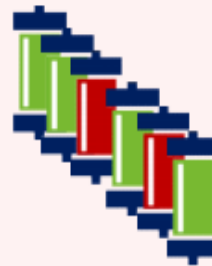
Ligand Screen

Specificity
Binding Kinetics
Mild elution
Stability

WP3
12 wks

Prototype Resin Testing

6 → 2



Ligand Scalability

Production / Purification

Resin Screen

Capacity, Purity
Elution profiles
Ligand Stability
Resin samples to customer

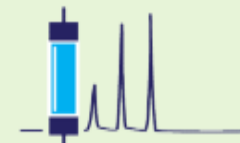
WP4
6 wks

Lead Selection

2 → 1 (RUO)

Resin optimization

- Δ backbone
- Δ ligand density



RESEARCH

RUO resin

- multiple liter scale
- process development

Analytics

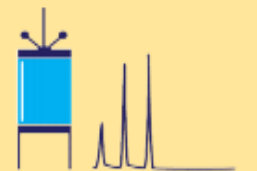
- HPLC columns
- Biotin conjugates

WP5
~ 8 months

Lead Development

cGMP use

Resin suitable for
cGMP use



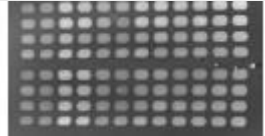
BIOPROCESS

cGMP development

- Process validation
- 3x 5L lots
- Leakage ELISA
- RSF

CaptureSelect Lead screening

- Unique array-based 96 single clone screening set-up:



Array



Kinetic data: on-off rates

CaptureSelect Biotin conjugates

- CaptureSelect Biotin conjugates available against a wide range of antibody and protein targets

• Antibody Targets

- Biotin anti IgG-Fc (human)
- Biotin anti-IgG-Fc (multi species)
- Biotin anti-IgG-CH1
- Biotin anti-IgA (human)
- Biotin anti-IgM
- Biotin anti-IgE
- Biotin anti-LC-kappa (human)
- Biotin anti-LC-lambda (human)
- Biotin anti-LC-kappa (murine)
- Biotin anti-LC-lambda (mouse)
- Biotn anti-LC-lambda (rat)
- Biotin anti-IgG3 and anti-IgG4
- Biotin anti-IgG-Fc (rabbit)
- Biotin anti-Free LC-kappa (human)
- Biotin Human Fab-kappa kinetics
- Biotin Human Fab-lambda kinetics
- Biotin Human IgG-Fc PK

• Non-Antibody Targets

- Biotin anti-FVII
- Biotin anti-FVIII
- Biotin anti-FIX
- Biotin anti-FSH
- Biotin anti-G-CSF
- Biotin anti-hGH
- Biotin anti-Insulin
- Biotin anti-EPO
- Biotin anti-AAV8
- Biotin anti-AAV9
- Biotin anti-AAVX
- Biotin anti-Fibrinogen
- Biotin anti-Transferrin
- Biotin anti-AAT
- Biotin anti-C1-Inhibitor
- Biotin anti-ApoA1
- Biotin anti-C-tag
- Biotin anti-Gonadotropin
- Biotin anti-HSA
- Biotin anti- α 1-Acid Glycoprotein
- Biotin anti-Haptoglobin
- Biotin anti-C3
- Biotin anti-vWF
- Biotin anti- α 2 Macroglobulin
- Biotin anti-Antithrombin-III
- Biotin anti-Prothrombin



Custom solutions – POROS resin development

WP1 (6 wks)

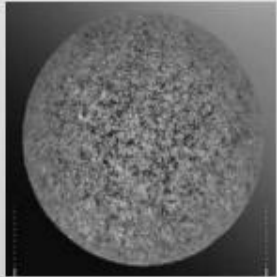
WP2 (8-10 wks)

WP3 (6 months)

Prototype resin development

Process definition

Commercialization



- Customer information
- Experimental design
- Proposal

- Process optimization
- Scale-up

- Process validation
- Stability studies
- Regulatory support package

Affinity or other surface chemistry resins can be developed specific to your downstream processing needs

Supporting bioprocess development and manufacturing



Leakage ELISA's

- Product specific ELISA's to measure any ligand potentially leaching from the column

Process Development columns

- POROS and CaptureSelect RoboColumns® for highthroughput resin screening and optimization
- CaptureSelect MiniChrom columns and POROS GoPure™ columns, designed for benchscale screening and process development

HPLC columns

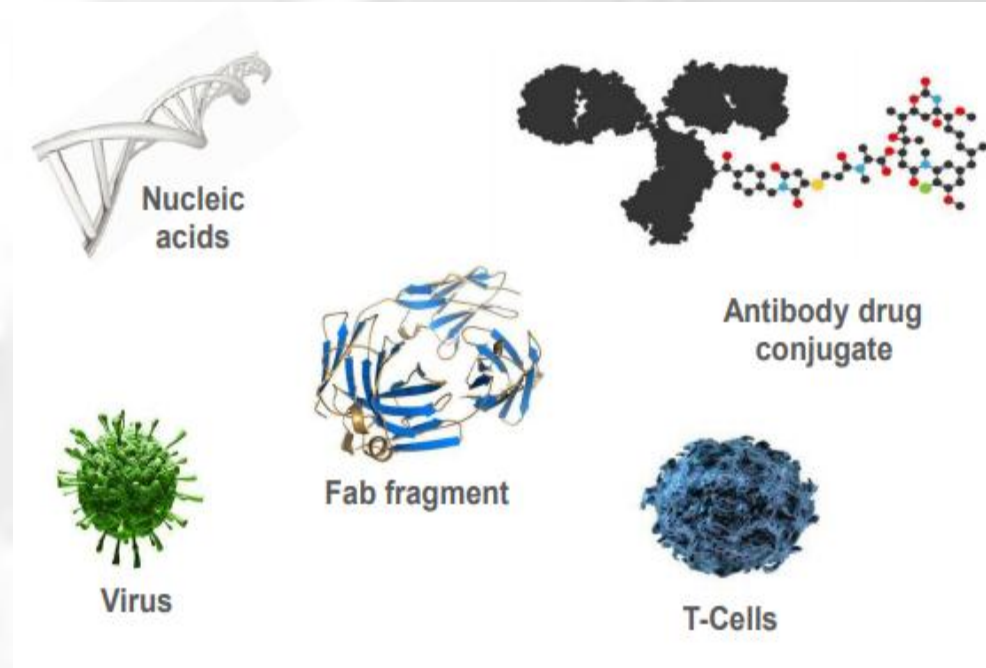
- Prepacked POROS & CaptureSelect columns for rapid quantitation of your target molecules
- Applications include titer determination and small scale purification

Conjugated ligands

- Biotinylated ligands for use in a range of analytical assays
- Applications include Capture ELISA, Western blot, MSIA, Gyrolab®-based immunoassays, and labelfree detection platforms

Purifying next generation biotherapeutics

- Superior performance and resolution
- Unique affinity portfolio
- State of the art manufacturing capabilities
- Strong partner in quality and supply
- Expert field applications and service



High performing chromatography solutions to meet today's purification challenges

Analytical and Preparative chromatography columns (GoPure™) POROS

Table 1. Analytical chromatography columns .

Diameter, mm	Length, mm	Volume, мл
2,1; 4,6; 10,0	30; 50; 100	0,1; 0,8; 1,7; 7,9

The columns are supplied in plastic and metal Embodiment

Table 2. Preparative chromatography columns .

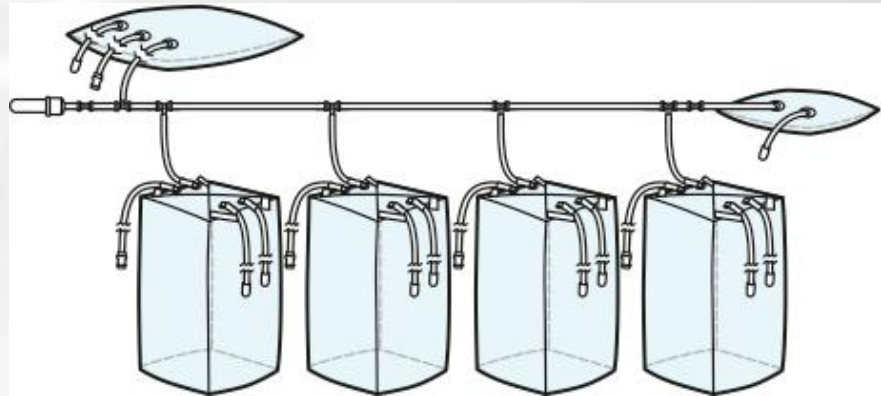
Diameter, cm	Length, cm	Volumeмл
1,2; 8,0; 20,0	5; 10; 15; 20	5-22; 250-1000; 1600-6300

Standard NPT threaded connection



Thermo Fisher bioprocess analytics

Single-use systems widely used in the production of biological products, including for the production of animal cell cultures. Single-use containers, filters systems, exchanger, reservoir, aseptic sampling system, mixing systems and bioreactors for primary processing are the most frequently used types of equipment in the manufacture of preparations for preclinical and clinical trials.



Single-use systems are designed for single use. All elements in contact with the product are made of high-quality polymer materials

Industrial equipment



Preparative chromatography



Bioreactors

Fermenters



Filtration systems

Reservoir



Mixers systems

