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

- 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.
Growing Diversity of Biological Molecules in Development

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

- Superior resolution
- High capacity
- High throughput

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

Increased salt tolerance

Superior resolution

BSA DBC 5% (mg/ml)

Absorbance at 280 nm (AU)

Volume (ml)
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-pores on 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

### Bioprocess Non-Affinity Resins
- **ANION EXCHANGE**
  - XQ
  - HQ50
  - D50
  - PI50
- **CATION EXCHANGE**
  - XS
  - HS50
- **HYDROPHOBIC INTERACTION**
  - Ethyl
  - Benzyl
  - Benzyl Ultra
- **REVERSED PHASE**
  - OLIGO R3

### 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

### Resins for bioprocessing at any scale
- Affinity solutions from screening to final manufacturing
- Simplifying purification preparation and enabling custom affinity solutions
Downstream Process Development

- Residence Time & Flow
- Load Density & # Cycles
- BE or FT Mode
- Disposables

- Aggregates & Fragments
- DNA, HCP, r-ProteinA
- Virus, Endotoxin, etc
- Acidic/basic, other PTM

Platform Development
- HTP/DOE approach
- Membranes vs. Resin technology
- Process knowledge

Quality by Design (QbD)
- CQA and TPP
- ICH Q8, Q9, Q10, Q11
- Risk Assessment, RPN
- Control/Design space

- Scale down model
- Scale-up & Tech transfer
- Facility, process capacity

- Resin & Step Screening
- Alternative capture tech
- Process economics
Bioprocess purification products for affinity capture chromatography

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

- Antibody derived therapeutics
- Biosimilars, recombinant proteins & plasma proteins
- Viral vectors
- Vaccines
Affinity chromatography – addressing purification challenges

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

80% reduction in purification time
CaptureSelect ligands: affinity through antibody selectivity

- The three CDR regions of the VH 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

<table>
<thead>
<tr>
<th>Product</th>
<th>Target applications</th>
<th>Uniqueness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaptureSelect KappaXL</td>
<td>• Human IgG</td>
<td>Mild elution for fragments and antibodies</td>
</tr>
<tr>
<td></td>
<td>• Fab fragments containing a kappa light chain</td>
<td></td>
</tr>
<tr>
<td>CaptureSelect FcXL</td>
<td>• Human IgG</td>
<td>CH3 binding domain, mild elution</td>
</tr>
<tr>
<td></td>
<td>• Fc-fusion proteins</td>
<td></td>
</tr>
<tr>
<td>CaptureSelect CH1-XL</td>
<td>• Human IgG antibodies</td>
<td>Improved version of IgG-CH1, including increased binding capacity and improved stability</td>
</tr>
<tr>
<td></td>
<td>• Fab fragments</td>
<td></td>
</tr>
<tr>
<td>POROS MabCapture A Select</td>
<td>• Human IgG</td>
<td>Cost-effective, high-performance Protein A resin produced from in-house sourced protein A</td>
</tr>
</tbody>
</table>
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

Lab chip analysis of Fab fragment purification shows no binding of light chains (LC) or light chain dimers (LC2) in the elution fraction (B)
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

<table>
<thead>
<tr>
<th>Product</th>
<th>Target applications</th>
<th>Uniqueness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaptureSelect FSH</td>
<td>Human follicle stimulating hormone</td>
<td>Binds only intact FSH</td>
</tr>
<tr>
<td>CaptureSelect HSA</td>
<td>Human serum albumin, albumin fusion proteins</td>
<td>Mild elution conditions help to protect fusion partner</td>
</tr>
<tr>
<td>CaptureSelect hCG</td>
<td>Binds to the alpha chain of HCG (human chorionic gonadotropin)</td>
<td>Shows binding to all human gonadotropins</td>
</tr>
<tr>
<td>CaptureSelect tPA</td>
<td>Human tissue plasminogen activator</td>
<td>Mild elution conditions</td>
</tr>
<tr>
<td>CaptureSelect TSH</td>
<td>Human Thyroid-Stimulating Hormone</td>
<td>Selective for TSH with no cross-binding to FSH, LH and HCG</td>
</tr>
<tr>
<td>CaptureSelect hGH</td>
<td>Human Growth Hormone</td>
<td>Selective for Human Growth Hormone</td>
</tr>
</tbody>
</table>
CaptureSelect FSH affinity matrix – selectivity towards conformational epitope

Follicle Stimulating Hormone (FSH)
- Red: Alpha-subunit
- Green: Beta-subunit

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

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

<table>
<thead>
<tr>
<th>Thermo Scientific™ resin</th>
<th>Binding Capacity (vg/mL)</th>
<th>Serotype Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>POROS CaptureSelect AAV8</td>
<td>$&gt;10^{13}$</td>
<td>AAV8</td>
</tr>
<tr>
<td>POROS CaptureSelect AAV9</td>
<td>$&gt;10^{14}$</td>
<td>AAV9</td>
</tr>
<tr>
<td>POROS CaptureSelect AAVX</td>
<td>$&gt;10^{14}$</td>
<td>AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, recombinant and chimeric vectors</td>
</tr>
</tbody>
</table>

*Viral genomes per milliliter (vg)/mL, binding capacity will vary based on serotype, feed stream, additives, and mutations to parent serotypes
Enabling a Paradigm Shift in Viral Vector Purification – a customer case study

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.

<table>
<thead>
<tr>
<th>Product</th>
<th>Target applications</th>
<th>Uniqueness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaptureSelect C-tagXL</td>
<td>Binds the short C-tag sequence E-P-E-A</td>
<td>Allows for a one-step purification of C-tagged proteins</td>
</tr>
</tbody>
</table>

Improved yield and purity compared to His6-tag purification

## Pipeline of CaptureSelect affinity resins

<table>
<thead>
<tr>
<th>Product Stage</th>
<th>Therapeutic Proteins</th>
<th>Viruses</th>
<th>Antibody Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioprocess Products</td>
<td>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)</td>
<td>Adeno-Associated Virus 8 &amp; 9 (AAV8 &amp; AAV9), AAV multi serotype (AAVX)</td>
<td>KappaXL FcXL, IgG-CH1, CH1-XL</td>
</tr>
<tr>
<td>Stage 5: RUO Products</td>
<td>Antithrombin III, Fibrinogen (Fib), Transferrin, Apolipoprotein H (ApoH), C1-inhibitor (C1-Inh), Granulocyte-macrophage colony-stimulating factor (GM-CSF)</td>
<td>Adenovirus (Adv5)</td>
<td>IgA (Fc), IgA-CH1, IgM, IgE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bovine IgA</td>
</tr>
<tr>
<td>Stage 4: Lead Development</td>
<td>Prothrombin, Deoxyribonuclease (DNAse)</td>
<td>Influenza (HA)</td>
<td></td>
</tr>
<tr>
<td>Stage 3: Lead Selection</td>
<td>Insulin Universal affinity tag (Uni-Tag), Factor X</td>
<td>Lentivirus (VSV-G)</td>
<td>Rabbit, Mouse &amp; Rat IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 2: Lead Screening</td>
<td>Interferon α and β (IFNα/β), Interleukin 2 (hIL2), Factor V, FXI, FXII, FXIII, and FH</td>
<td>Baculovirus, Exosomes</td>
<td>scFv</td>
</tr>
<tr>
<td>Stage 1: Library Construction</td>
<td>Erythropoietin (EPO)</td>
<td></td>
<td>IgY</td>
</tr>
</tbody>
</table>
Bioprocess purification products for capture and polishing chromatography

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® MabCaptureTM 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 a 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)

<table>
<thead>
<tr>
<th>Support matrix</th>
<th>Cross-linked poly(styrene-divinylbenzene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface functionality</td>
<td>Sulfopropyl (-CH2CH2CH2SO3–)</td>
</tr>
<tr>
<td>Dynamic binding capacity</td>
<td>≥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</td>
</tr>
<tr>
<td>Shipping solvent</td>
<td>20% ethanol</td>
</tr>
<tr>
<td>Ionic capacity</td>
<td>88 to 120 µmol/mL</td>
</tr>
<tr>
<td>Average particle size</td>
<td>50 µm</td>
</tr>
</tbody>
</table>
# POROS Cation exchange resins

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

- 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

POROS XS - Combining superior capacity, salt tolerance & resolution
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 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

<table>
<thead>
<tr>
<th>POROS Resin</th>
<th>Type of AEX Resin</th>
<th>Surface Chemistry</th>
<th>BSA Binding Capacity (mg/mL)</th>
<th>AEX Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>Weak</td>
<td>Dimethylaminopropyl</td>
<td>90</td>
<td>Bind/Elute: Protein, virus, plasmid DNA purification</td>
</tr>
<tr>
<td>PI</td>
<td>Weak</td>
<td>Polyethyleneimine (mixed amine)</td>
<td>80</td>
<td>Flow Through: Trace impurity removal by binding impurities (DNA, viruses, HCP, aggregates, endotoxin)</td>
</tr>
<tr>
<td>HQ</td>
<td>Strong</td>
<td>60% Quaternized polyethyleneimine (mixed amine)</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>XQ</td>
<td>Strong</td>
<td>Fully quaternized amine</td>
<td>&gt;140</td>
<td></td>
</tr>
</tbody>
</table>
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, 40µl resin volume in 270µl working volume
  - Development: 1.1cmD x 5.3cmL, 5ml columns
  - Pilot: 14cmD x 18.5cmL, 2.9L
**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>
<thead>
<tr>
<th>Antibody</th>
<th>Equilibration buffer</th>
<th>Process loading (mg/ml)</th>
<th>Yield (%)</th>
<th>HMW (%)</th>
<th>HCP (ng/mg)</th>
<th>DNA (pg/mg)</th>
<th>ProA (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb-A</td>
<td>20 mM Tris, pH 8.5 3.21 mS/cm</td>
<td>150</td>
<td>93</td>
<td>2.14</td>
<td>20</td>
<td>BQ</td>
<td>0.2</td>
</tr>
<tr>
<td>mAb-FL</td>
<td>50 mM Tris, pH 7.5 9.18 mS/cm</td>
<td>314</td>
<td>98</td>
<td>0.66</td>
<td>11</td>
<td>BQ</td>
<td>0.4</td>
</tr>
<tr>
<td>mAb-FY</td>
<td>50 mM Tris, pH 7.5 10.10 mS/cm</td>
<td>114</td>
<td>91</td>
<td>1.31</td>
<td>26</td>
<td>BQ</td>
<td>BQ</td>
</tr>
<tr>
<td>mAb-R</td>
<td>20 mM NaPi, pH 6.8 7.50 mS/cm</td>
<td>100</td>
<td>89</td>
<td>1.36</td>
<td>7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>mAb-T</td>
<td>20 mM NaPi, pH 7.2 6.00 mS/cm</td>
<td>100</td>
<td>92</td>
<td>0.58</td>
<td>15</td>
<td>BQ</td>
<td>ND</td>
</tr>
<tr>
<td>mAb-T (Pilot)</td>
<td>20 mM NaPi, pH 6.8 7.10 mS/cm</td>
<td>100</td>
<td>93</td>
<td>0.80</td>
<td>7</td>
<td>BQ</td>
<td>0.1</td>
</tr>
<tr>
<td>mAb-P</td>
<td>50 mM Tris, pH 7.5 9.02 mS/cm</td>
<td>300</td>
<td>97</td>
<td>1.13</td>
<td>26</td>
<td>BQ</td>
<td>ND</td>
</tr>
<tr>
<td>mAb-C</td>
<td>50 mM Tris, pH 7.5 10.10 mS/cm</td>
<td>100</td>
<td>90</td>
<td>0.54</td>
<td>4</td>
<td>BQ</td>
<td>BQ</td>
</tr>
</tbody>
</table>

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

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Up to 8 Fold HMW Clearance
Up to 9 Fold HCP Clearance
Effective impurity 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

<table>
<thead>
<tr>
<th>POROS XQ</th>
<th>Load Conductivity (mS/cm)</th>
<th>Load Capacity (IgG/ml of resin)</th>
<th>XmuLV Log10 Reduction</th>
<th>MMV Log10 Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>500</td>
<td>&gt;4.31 ± 0.12</td>
<td>&gt;5.10 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>500</td>
<td>&gt; 4.39 ± 0.14</td>
<td>1.61 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>500</td>
<td>3.46 ± 0.29</td>
<td>0.19 ± 0.28</td>
</tr>
</tbody>
</table>

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 favors 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
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 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

---

### Flow-through Breakthrough Analysis

- Cumulative Monomer Purity
- Monomer Recovery

![Graph showing flow-through analysis](image)

- Load Density (g/L resin): 88.6%
- Load Purity: 99%
- Effective reduction of dimer and HMW

**125 g/L resin loading at 1% breakthrough of aggregates and 97% monomer recovery**

### Flow-through verification

- Load: 99.3%
- Purified Antibody: 99.3%

### Table: mAb-A Process Comparison

<table>
<thead>
<tr>
<th></th>
<th>Mixed-Mode BE</th>
<th>HIC FT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load Density (g/L resin)</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>Monomer Purity FT (%)</td>
<td>99</td>
<td>&gt;99</td>
</tr>
<tr>
<td>Mon. Recovery (%)</td>
<td>90</td>
<td>98</td>
</tr>
<tr>
<td>HCP (ppm)</td>
<td>&lt;LLOQ</td>
<td>&lt;LLOQ</td>
</tr>
<tr>
<td>Residence time (min)</td>
<td>6.0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

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

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

WP2
10 wks
Lead Screening
200 → 24 → 6

WP3
12 wks
Prototype Resin Testing
6 → 2

WP4
6 wks
Lead Selection
2 → 1 (RUO)

WP5
~ 8 months
Lead Development
cGMP use

Or
Existing libraries from collection

Ligand Screen
Specificity
Binding Kinetics
Mild elution
Stability

Ligand Scalability
Production / Purification
Resin Screen
Capacity, Purity
Elution profiles
Ligand Stability
Resin samples to customer

Resin optimization
• Δ backbone
• Δ ligand density

RUO resin
• multiple liter scale
• process development

Analytics
• HPLC columns
• Biotin conjugates

Resin suitable for cGMP use

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
Elution screening: 35 buffers on 96 lead ligands

Selection of ligand candidates based on:
- Specificity √
- Binding affinity √
- Elution efficiency √
- Stability √
- Ligand sequence √
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)
- Biotin 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-α1-Acid Glycoprotein
- Biotin anti-Haptoglobin
- Biotin anti-C3
- Biotin anti-vWF
- Biotin anti-α2-Macroglobulin
- Biotin anti-Antithrombin-III
- Biotin anti-Prothrombin
Custom solutions – POROS resin development

Affinity or other surface chemistry resins can be developed specific to your downstream processing needs.
### 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 (GoPureTM) POROS

Table 1. Analytical chromatography columns .

<table>
<thead>
<tr>
<th>Diameter, mm</th>
<th>Length, mm</th>
<th>Volume, мл</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,1; 4,6; 10,0</td>
<td>30; 50; 100</td>
<td>0,1; 0,8; 1,7; 7,9</td>
</tr>
</tbody>
</table>

The columns are supplied in plastic and metal

Embodiment

Table 2. Preparative chromatography columns .

<table>
<thead>
<tr>
<th>Diameter, см</th>
<th>Length, см</th>
<th>Volume, мл</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2; 8,0; 20,0</td>
<td>5; 10; 15; 20</td>
<td>5-22; 250-1000; 1600-6300</td>
</tr>
</tbody>
</table>

Standard NPT threaded connection
Single-use systems are designed for single use. All elements in contact with the product are made of high-quality polymer materials.
Industrial equipment

- Bioreactors
- Fermenters
- Reservoir
- Mixers systems
- Filtration systems
- Preparative chromatography