BioToolomics

Bioprocess Chromatography Specialists

BioToolomics
Single-Use Disposable Chromatography Solutions

Company Background -1

- **Date of Operation**: Since February 2006
- **Location**: Consett, County Durham, United Kingdom
- **Expertise**: Over 50 years combined experience in design, development and manufacturing of process chromatography resins and columns within our management team
- **Staff level**: 10 - 15 people (demand depending)
- **Company Status:** Privately held, cash positive

Company Background - 2

- **Quality**: Certified to ISO 9001:2015 quality management system since 2011 (by LRQA)
- **Certification**: Regulatory support files and leachable / extractable data for the manufacturing of biologics (protein medicine or regenerative medicine), food and beverage etc.
- **UK-Made**: All our products made in our UK facility

Company Background - 3

- **Resin Capability**:
  - Up to 5,000 litres of agarose-based chromatography resins p.a. in our purpose-built facility
  - Further facility expansion, e.g. installation of 500L – 1000L reactors, is under plan
- **Column Capability**:
  - Scalable columns from 5 mm to over 450 mm
  - Class 7 clean room for packing GMP grade columns

Customers

BioToolomics mainly serves biopharma and life science companies world wide

Agarose Technology Platform

	HighRes	High Flow	Large Beads	Other
Bead size (μm)	20 - 50	50 -150	150 - 350	<20 or other size range
Porosity	Controlled to suit different applications, varies from 10 nm to over 300 nm			
Cross-linking	Standard: highly cross-linked			Lower or Higher than standard
Non-specific binding	Very low non-specific binding			
pH stability	Typical pH 3 to 12 (long term) and pH 1 to 14 (short term)			
Chemical stability	Stable to most commonly used aqueous buffers: 6 M guanidine, 8 M urea, 1 M NaOH			
Temperature	Typical 4°C – 30°C			>121°C possible
Clean-in-place	Application dependent			

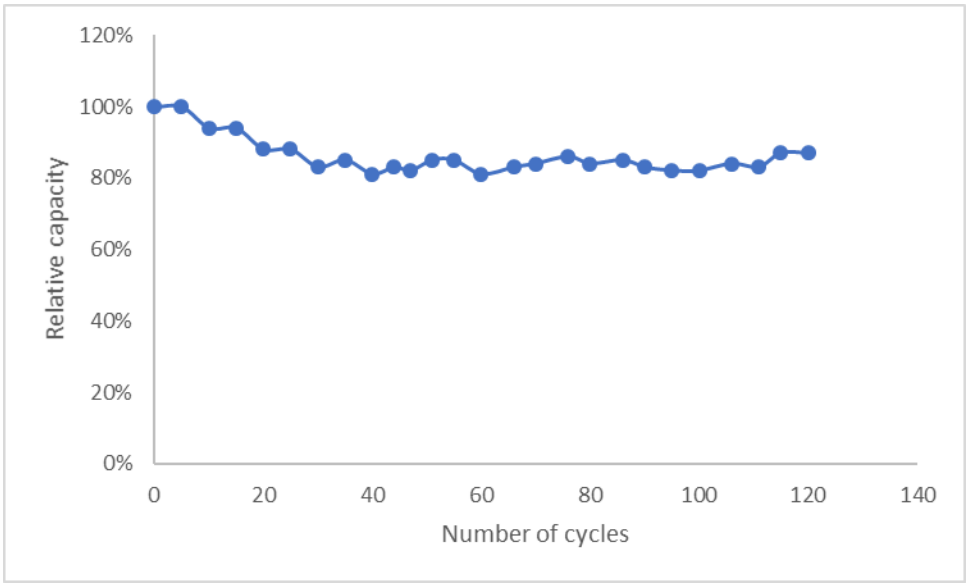
Process Chromatography Resins

- **Affinity chromatography**: Protein-A, Alkaline-stable Protein-A, Protein-G, IMAC, GST, Heparin, pre-activated resins
- **Ion-Exchange**: Broad range (35 micron, 90 micron, 200 micron, dextran grafted, high binding capacity, high resolution (Q, DEAE, SP, CM)
- **HIC**: SepFast Butyl, Phenyl, Octyl
- **Mixed-Mode**: Mainly for negative chromatography applications
- **Gel filtration**: High selectivity and high resolution (equivalent to Superdex 75, Superdex 200, Superose 6)
- **Magnetic** chromatography resins

Affinity Resins

- Protein A
  - SepFast
  - SepFast Endure
    - (i.e. caustic stable)
  - SepFast Large Beads
  - SepFast Large Beads Endure
  - SepFast HighRes
- Protein G SepFast
- IMAC SepFast
  - (Ni, Co, Zn)
- Glutathione SepFast
- Heparin
  - SepFast,
  - SepFast HighRes

Example: Alkaline-stable Protein-A SepFast



Alkaline stability tested at 0.5 M NaOH: the resin was cleaned with 0.5 M NaOH for 15 mins after each binding / elution cycle with hIgG

No loss of binding capacity after soaking in 0.1 M NaOH for 1 week (data not shown)

MabSelect SuRe

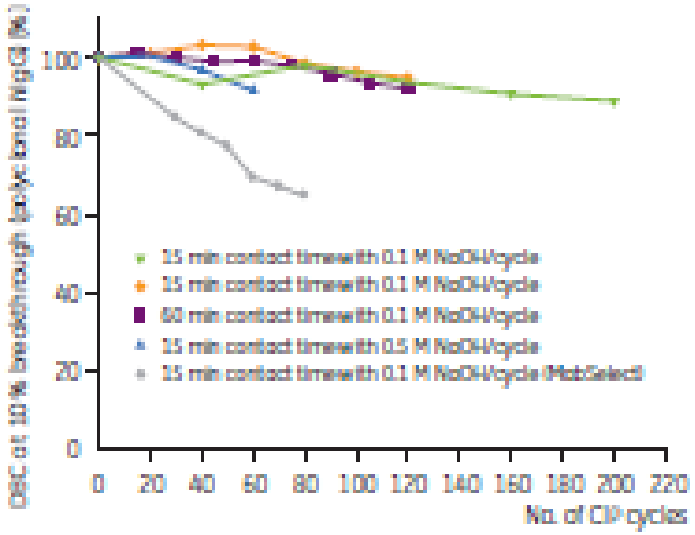


Fig 2. Dynamic binding capacity of MabSelect SuRe and MabSelect for polyclonal human IgG after CIP with 0.1–0.5 M NaOH for up to 200 cycles.

MabSelect SuRe:
Alkaline stability data
Only tested to 60 cycles at 0.5 M NaOH (the blue line)
*The data sheet file of MabSelect SuRe published by GE Healthcare

Pre-activated Resins for Protein Immobilisation

- Aldehyde-activated SepFast
- Amine-activated SepFast
- Carboxyl-activated SepFast
- CNBr-activated SepFast
- Epoxy-activated SepFast
- Irreversible Thiol-coupling SepFast
- NHS-activated SepFast

Standard IEX Resins

Q, DEAE, SP, CM in different variants:

- SepFast HighRes (20-50 µm)
- SepFast 6HF (50-150 µm)
- SepFast 6HF Plus (i.e. dextran grafted)
- SepFast Large Beads (150-350 µm)
- SepFast Large Beads Plus

Other IEX Resins
(particle size 20 – 50 μm)

- **SepFast Supor Q (DEAE, S, CM)**: high binding capacity to viruses
- **SepFast Macro Q and S**: particularly designed for high resolution purification of very large proteins such as pegylated antibodies etc
- **SepFast HighRes Ultra**: High capacity, high resolution, up to 20 bar pressure

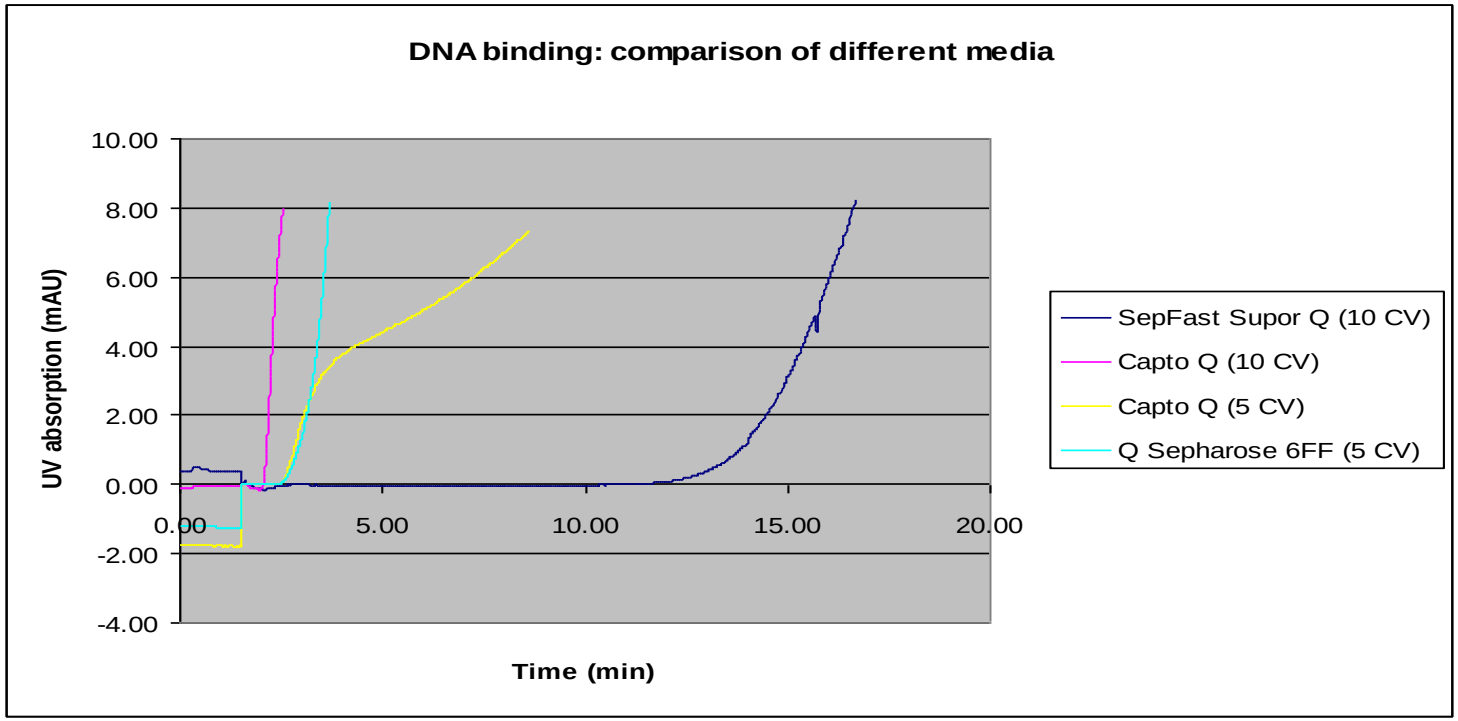
Custom Resins for Insulin, Peptide, Oligonucleotide

- **Capture Step**: Agarose-based IEX resins of small pore size (10-20 nm) to achieve high binding capacity; bead size 20-50 μm or 50-150 μm
- **Intermediate Purification**: Same as above; bead size 20-50 μm
- **Polishing Step**: High pressure (up to 20 bar), high resolution (10-20 μm or 20-30 μm) agarose-based IEX or HIC resins

Example: SepFast Supor Q

- The base matrix possesses easily accessible inter-connected small pores (50 – 100 nm) and large pores (μm level)
- High dynamic binding capacity to both nano-sized structures (such as plasmid, virus or virus-like-particle) and proteins
- The base matrix is made of polysaccharide composites, compatible with most commonly used chemicals in the bioprocessing area
- Highly cross-linked with good rigidity
- In pre-packed column format, ready-to-use and disposable

SepFast Supor Q



Note: 1 ml resin was packed at 1 cm bed height to capture the calf thymus DNA fragment

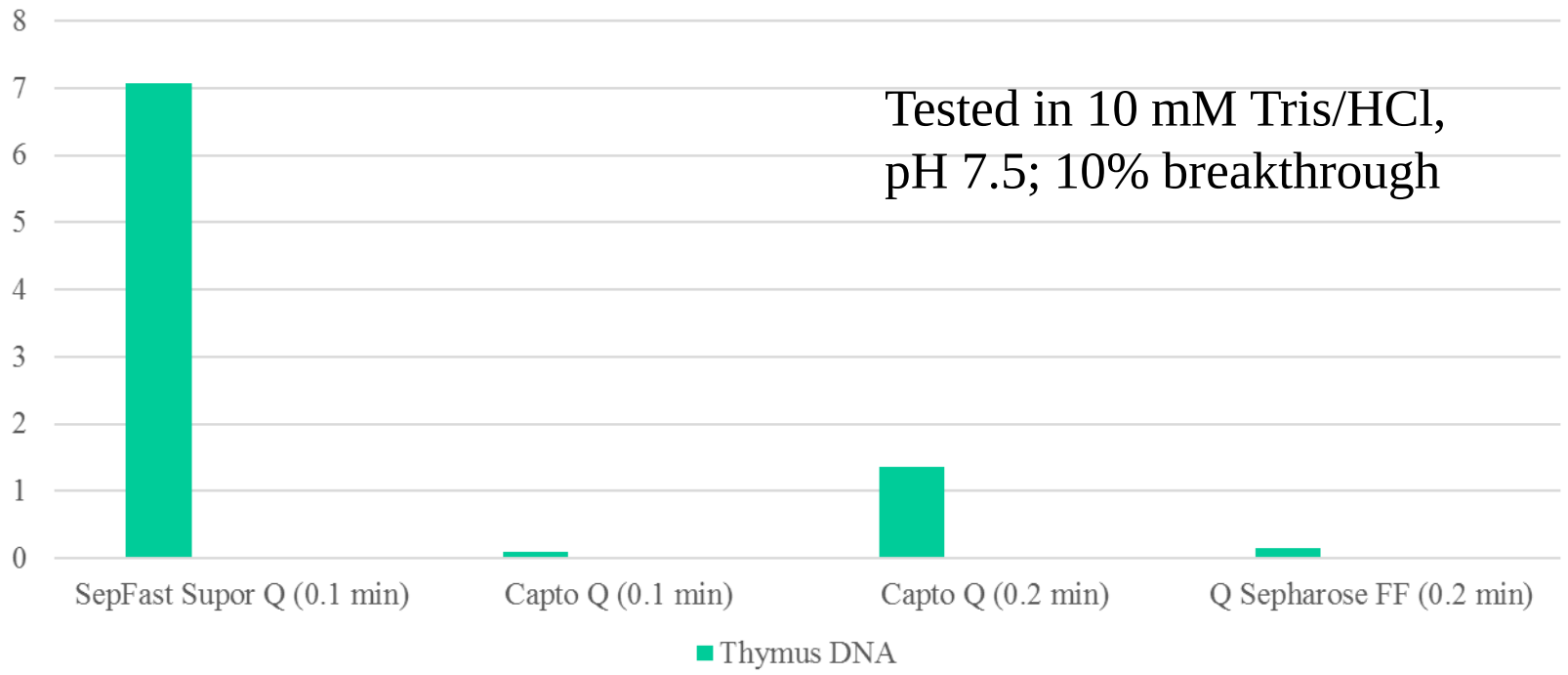
SepFast Supor Q

Comparison of the Dynamic Binding Capacity (DBC)

	SepFast Supor Q (flowrate @10 CV)	Capto Q (flowrate @ 10 CV)	Capto Q (flowrate @ 5 CV)	Q Sepharose FF (flowrate @ 5 CV)
Calf thymus DNA fragment	7075 μg/ml	105 μg/ml	1367 μg/ml	157 μg/ml
Protein (BSA)	133 mg/ml	84 mg/ml	116 mg/ml	1.7 mg/ml

Note: The testing was done at a bed height of 1 cm.

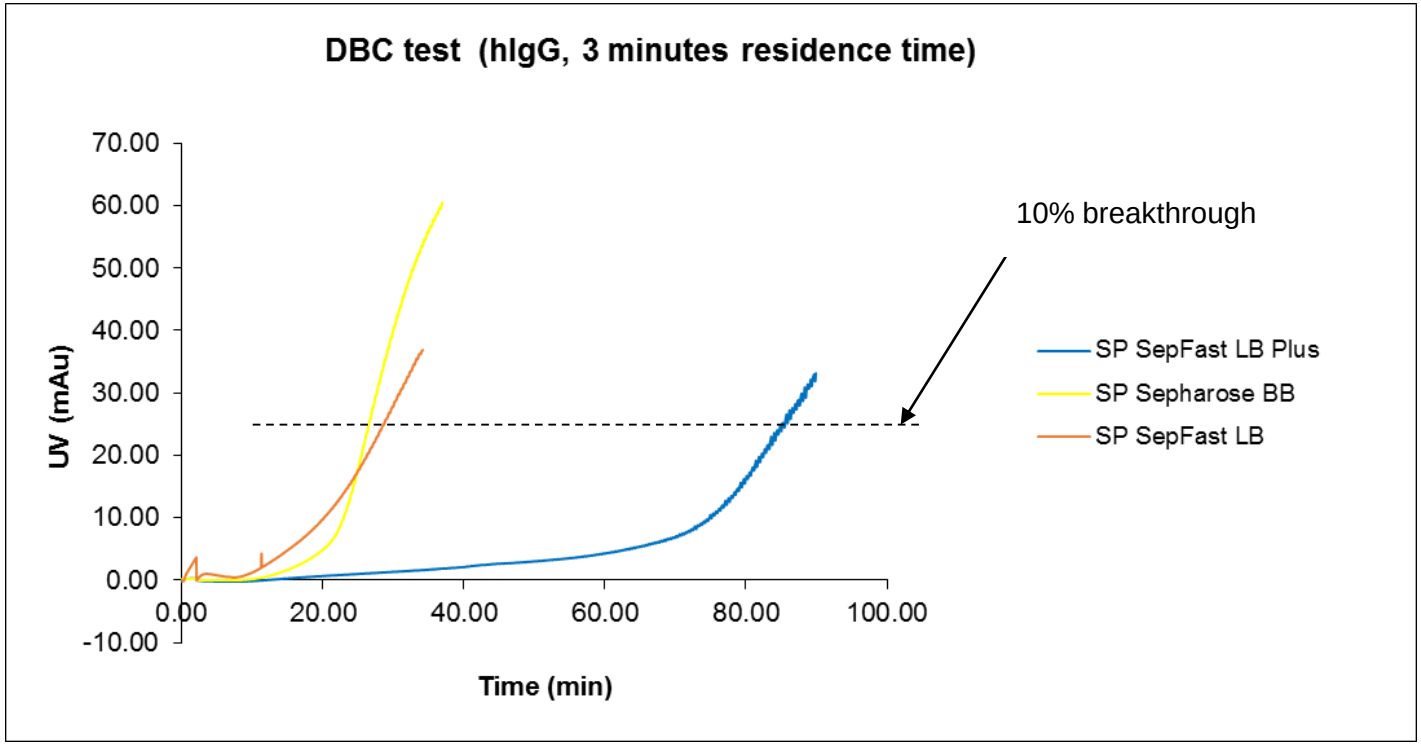
Dynamic binding capacity (mg/ml) of large DNA molecule



Applications for SepFast Supor Q

- Flow through polishing of Mabs to remove trace amount of impurities (such as host cell proteins, DNA, virus, endotoxin)
- Purification of nano-sized complexes (e.g. plasmids, viruses, VLPs) in the capture or inter-mediate step
- Purification of recombinant proteins at very short residence times

Example: SP SepFast Large Beads Plus



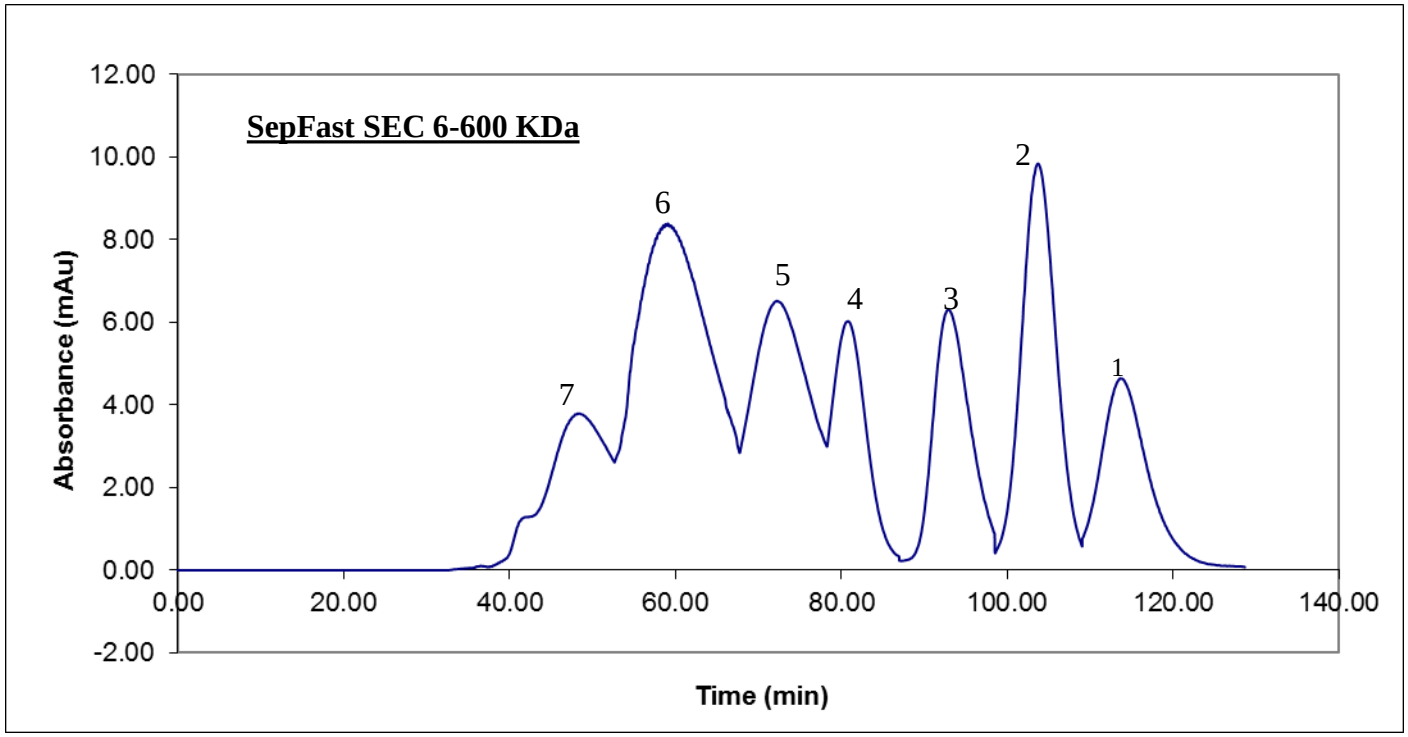
Good to purify large molecules or viruses from viscous or cell-containing broth

Gel Filtration (SEC)

A broad choices for various applications:

- SepFast SEC 3 – 70 KDa (equiv. to Superdex 75 pg)
- SepFast SEC 6 – 600 KDa (equiv. to Superdex 200 pg)
- SepFast SEC 6 – 5000 KDa, (equiv. to Superose 6 pg)
- SepFast CL-2B (2% agarose beads)
- SepFast 4B, SepFast CL-4B (4% agarose beads)
- SepFast 6B, SepFast CL-6B (6% agarose beads)
- Desalting columns
- Gel filtration columns

Example: Separation Power of Gel Filtration Resin



Separation of test substances on a 16 x 600 mm gel filtration column. Flowrate: 1 ml/min (30 cm/hr); sample loading 0.5 ml; mobile phase: PBS (phosphate buffered saline); model proteins 0: vitamin B-12 (1200); 1: aprotinin (M_r 6500); 2: cytochrome c (M_r 12300); 3: β-lactoglobulin (M_r 35000); 4: BSA (M_r 67000); 5: γ-globulin IgG (M_r 158000; 6: apoferritin (M_r 440000); 7: thyroglobulin (M_r 669000)

Hydrophobic Interaction (HIC) Resins

- Butyl SepFast 4HF
- Butyl SepFast 6HF
- Octyl SepFast 4HF
- Octyl SepFast 6HF
- Phenyl SepFast 6HF
- Phenyl SepFast 6HF (high sub)
- Phenyl SepFast HighRes
- Phenyl SepFast Large Beads

Mixed-Mode Resin Technology Platform

BioToolomics has an established technology platform to make novel mixed-mode chromatography resins for various applications:

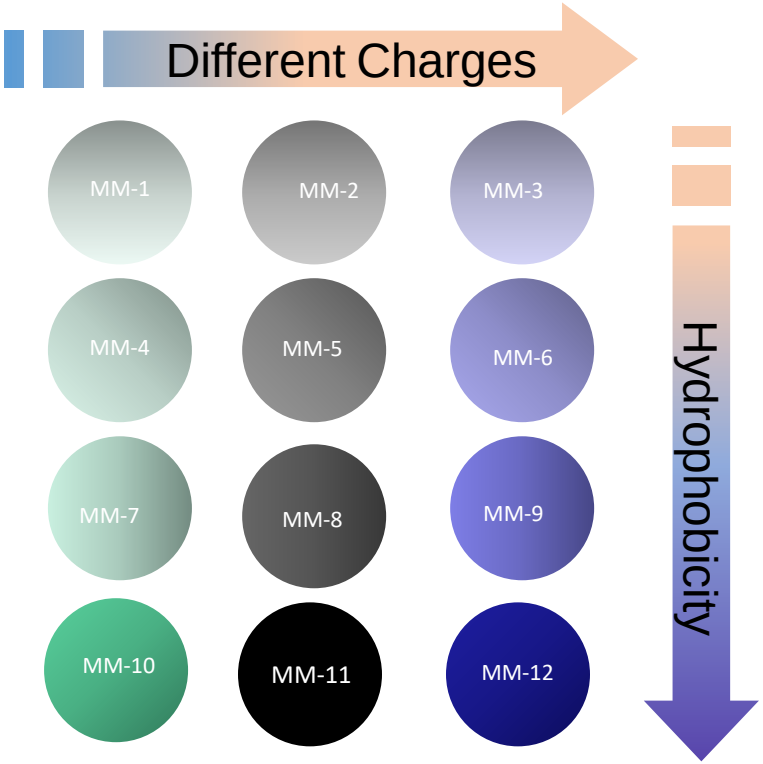
- Large mixed-mode resin library to screen
- Unique bead formulation technology
- Selective removal of host cell proteins

Mixed-mode chromatography library

BioToolomics mixed-mode ligands cover all the possible combinations of the following functional moieties:

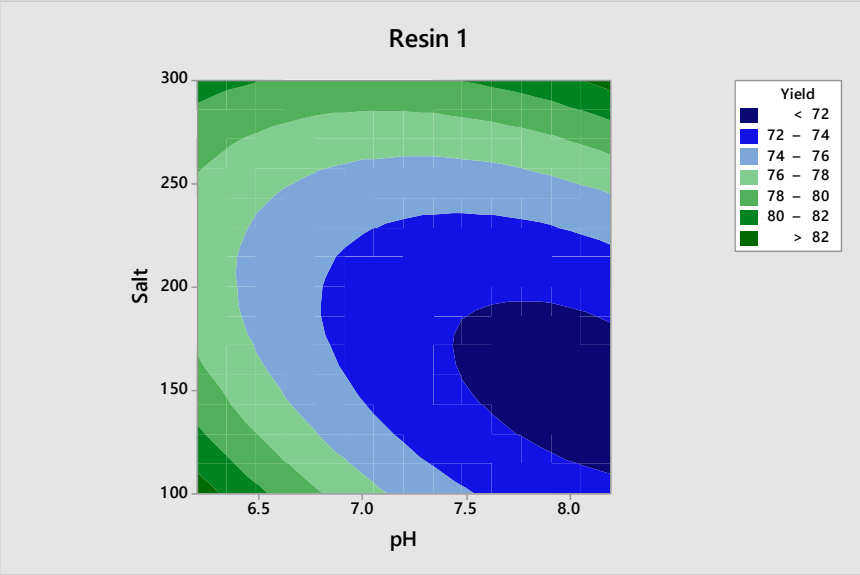
- **Strong anion exchange group**
- **Weak anion exchange group**
- **Strong cation exchange group**
- **Weak cation exchange group**
- **Strong hydrophobic group**
- **Weak hydrophobic group**
- **Hydrogen bond**
- **π-π interaction**

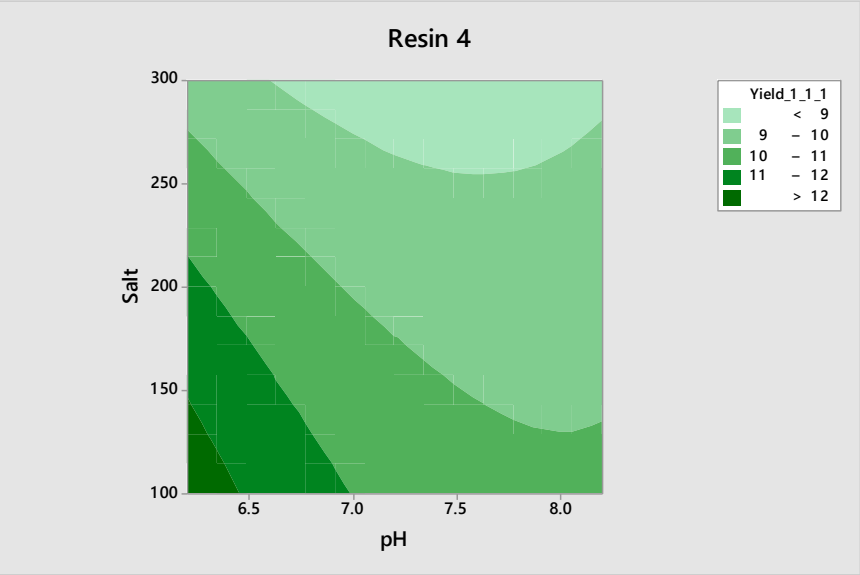
Screening Mixed-Mode Resin Library



- **Primary screening**: 96-well high-throughput format
- **Verification**: RoboColumn format
- **Process Development**: 10 ml packed column

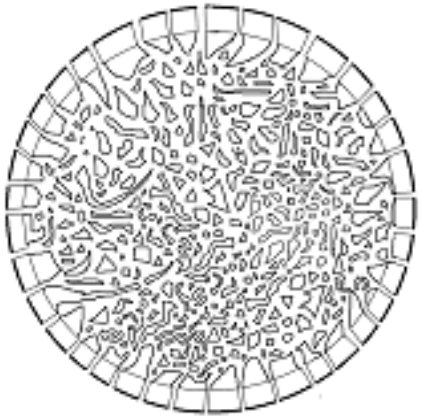
Example data of HTS – mAb yield





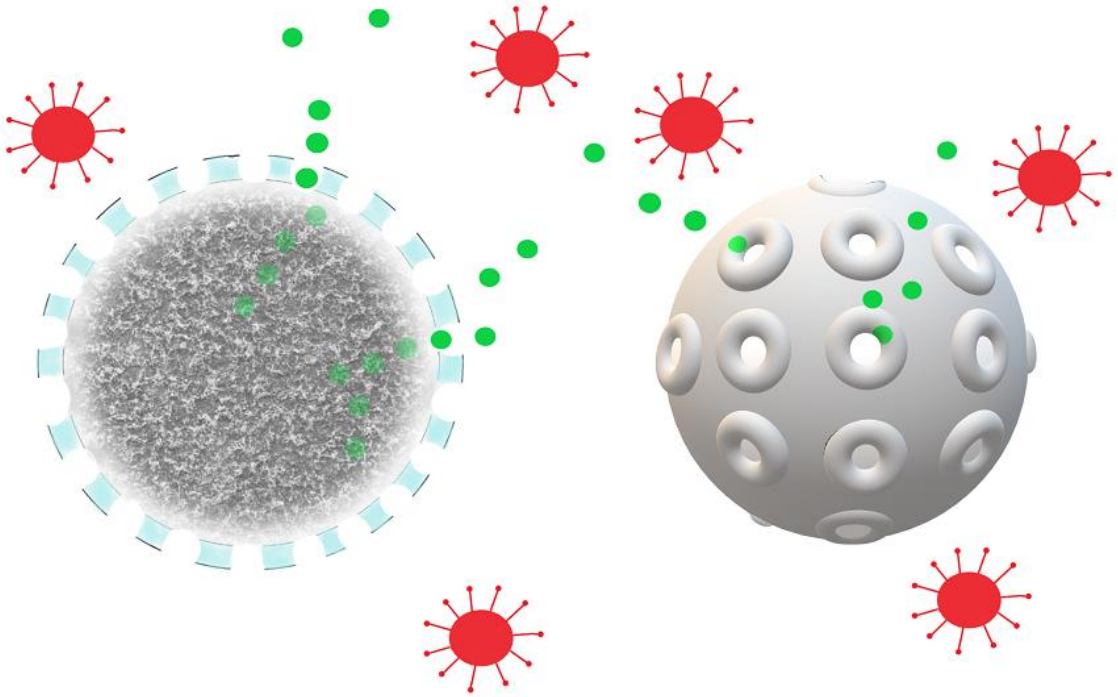
Shelled Bead Technology

Conventional
Porous bead

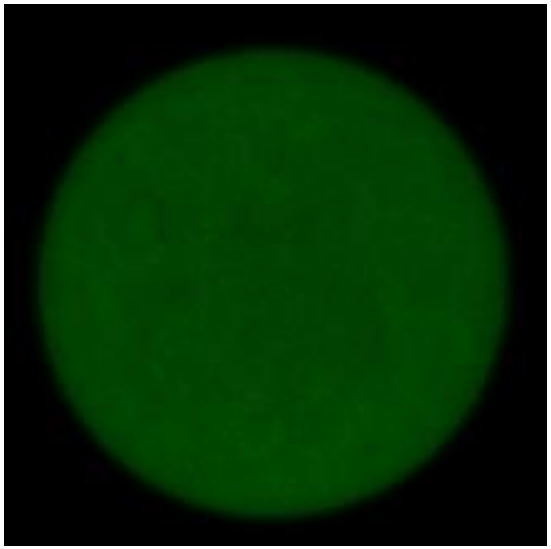


Shelled bead

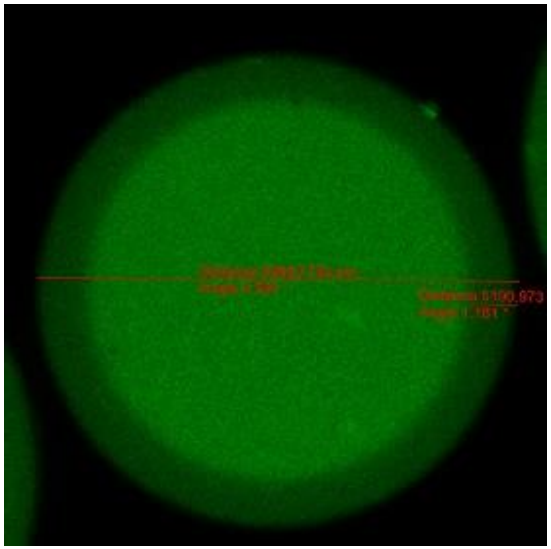
Shelled bead: 3D illustration



Confocal microscopy visualisation

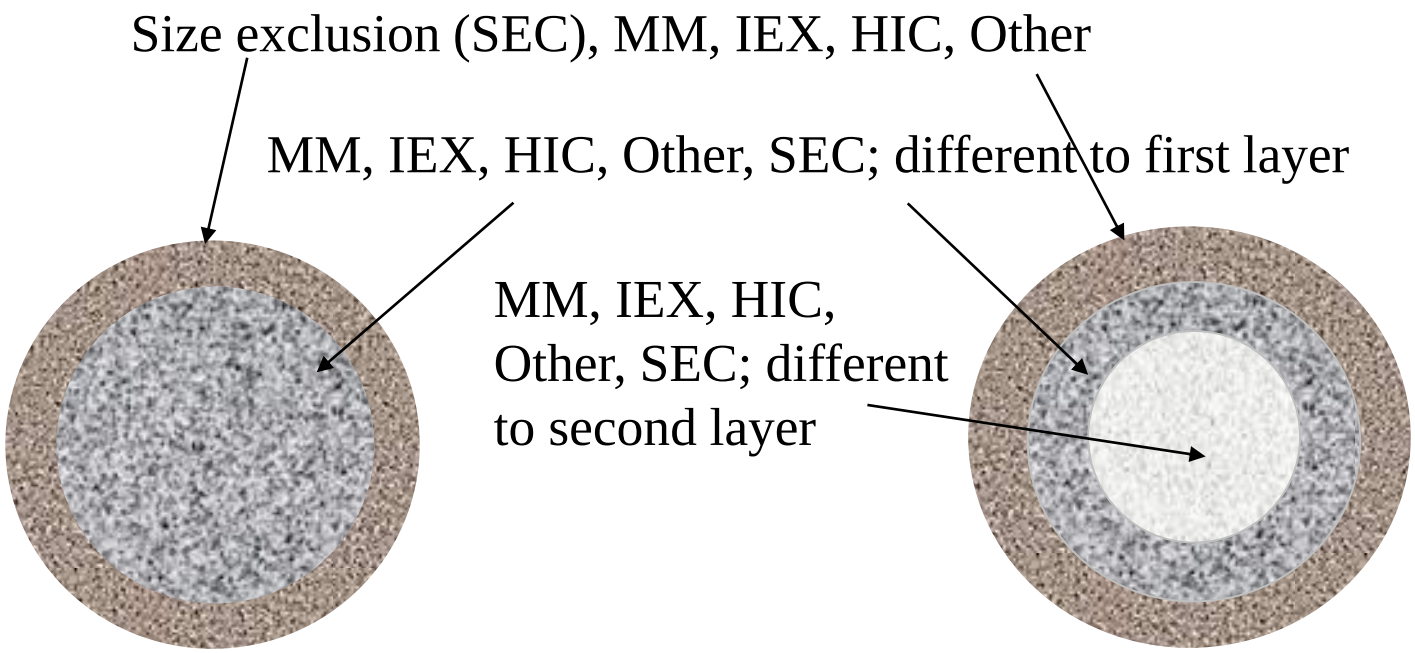


Conventional bead



Shelled bead (1 layer)

Possible Configurations of Shelled Beads



Bead with one layer

Bead with two layers*

Note: More than two layers can be made

Non-Capture Antibody Purification (NCAP) Technology

- Based on our Mixed-mode Resin Technology and Shelled Bead Technology, BioToolomics has developed novel chromatography resins tailored for the purification of monoclonal antibodies (mAb) in flow-through mode.
- A mAb could be purified without using Protein A resins.
- A mAb purification process based on BioToolomics NCAP resins could be more efficient and cost-effective than conventional Protein-A based processes.

MabPolish™

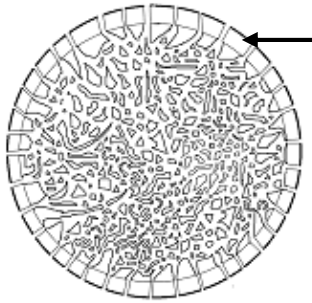
MabPolish Type I and Type II are a group of special mixed mode resins showing high binding capacity to host cell proteins (HCP) with little binding to mAbs.

	Application Guide
MabPolish™ Type I	Anion mixed-mode resin with very mild hydrophobicity. It can remove high level of HCPs at pH 4 to 5 and salt concentration up to 0.15 M.
MabPolish™ Type II	Anion mixed-mode resin with hydrophobicity stronger than Type I. It can remove more hydrophobic species at pH 4 to 5 and salt concentration up to 0.15 M.

MabPolish™ DUO
SepFast™ DUO

- MabPolish DUO and SepFast DUO are a class of novel resins with inert shell having size-exclusion effect that blocks molecules based on their molecular weights. It is a very gentle method with little loss of product.

Conventional
Porous bead



MabPolish DUO (mixed-mode ligand)
SepFast DUO (ion-exchange ligand)

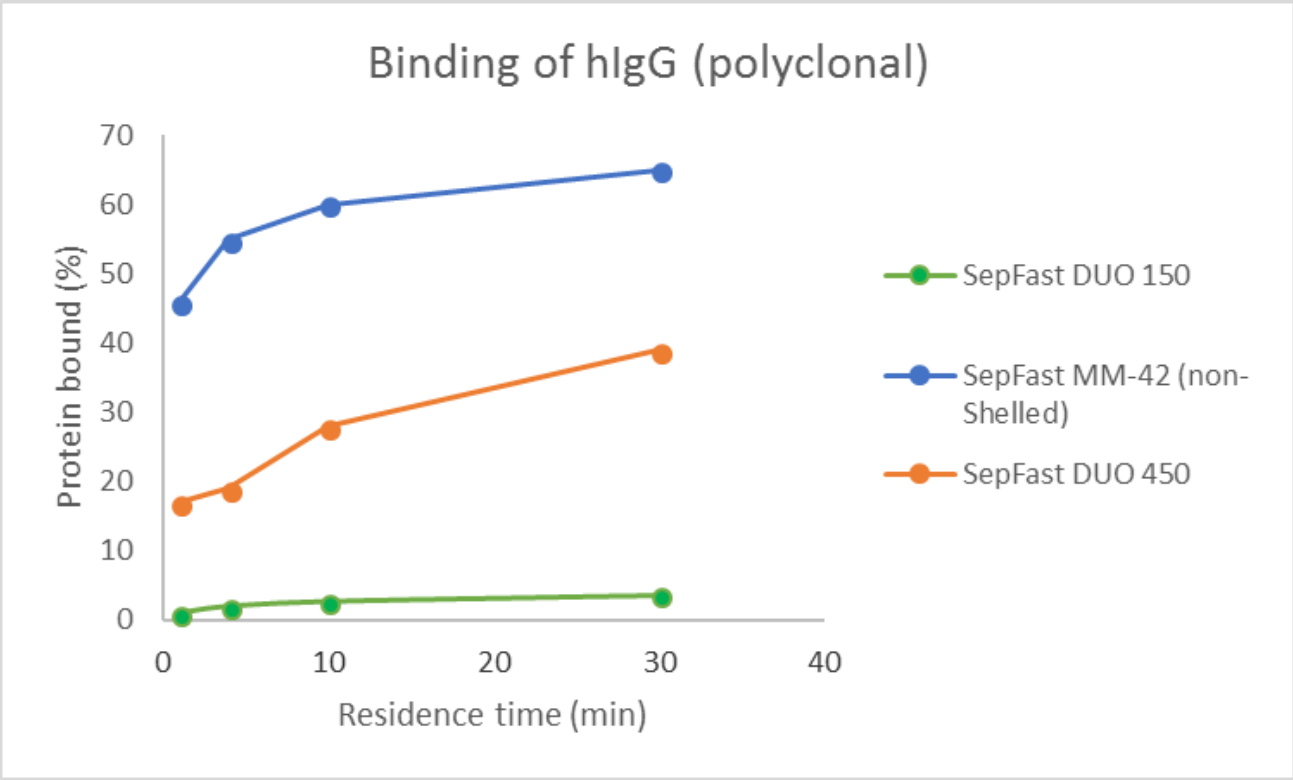
MabPolish DUO: Application Guide

	Key Features	Application Guide
MabPolish™ DUO 150A **MabPolish™ DUO 400A**	Shelled anion mixed-mode resin. The shell of 150A blocks molecules of 150 KDa. The shell of 400A blocks molecules around 400 KDa.	Flow-through mode to capture impurities smaller than IgGs at a wide range of pH and conductivity. MabPolish DUO 400 range can be run at faster flow velocity to remove high molecular weight species with low loss of target antibodies.
MabPolish™ DUO 150C **MabPolish™ DUO 400C**	Shelled cation mixed-mode resin. The shell of 150C blocks molecules of 150 KDa. The shell of 400C blocks molecules around 400 KDa.	

SepFast DUO: Application Guide

	Key Features	Application Guide
SepFast™ DUO 150 Q **SepFast™ DUO 400 Q**	Shelled strong anion-exchange resin. The shell of 150 Q blocks molecules of 150 KDa. The shell of 400 Q blocks molecules around 400 KDa.	Flow-through mode to capture impurities smaller than IgGs based on charges. SepFast DUO 400 range can be run at faster flow velocity to remove high molecular weight species with low loss of target antibodies.
SepFast™ DUO 150 S **SepFast™ DUO 400 S**	Shelled strong cation-exchange resin. The shell of 150 S blocks molecules of 150 KDa. The shell of 400 S blocks molecules around 400 KDa.	

Comparison of Shelled and Non-Shelled Mixed-Mode Resin



SepFast MM-42 is a non-shelled mixed-mode resin; SepFast DUO 450 has a SEC outlayer of mol. Cut-off of 450 KDa; SepFast DUO 150 has a SEC outlayer of mol. Cutoff of 150 KDa

Case Study: Conventional Platform Process for Antibody Purification

Cell Culture

↓

Clarification

↓

Protein-A affinity capturing

↓

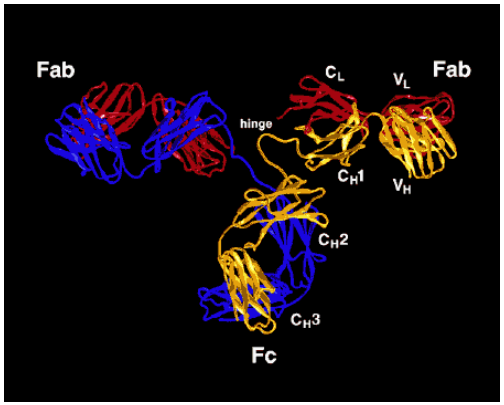
Virus inactivation

↓

CIEX

↓

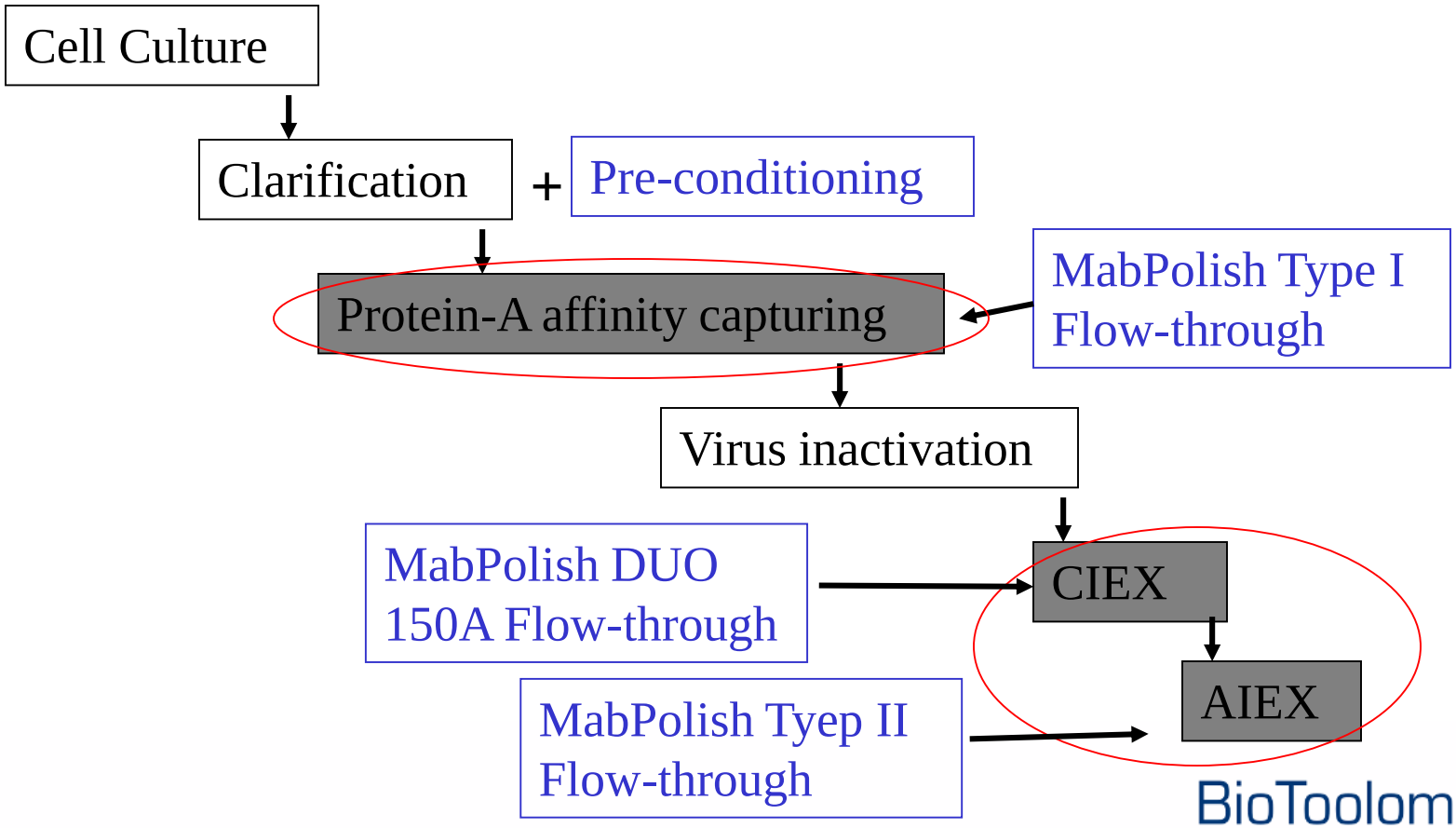
AIEX



BioToolomics

Single-Use Disposable Chromatography Solutions

Case Study: A Full Flow-Through mAb Purification Process (No Buffer Adjustment)



Result: Removal of Host Cell Proteins (HCP)

Flow-through step	HCP level by ELISA (U/mg mAb)	Yield of mAb (%)
Starting feedstock	112276	n/a
1. MabPolish Type I	28336	93
2. MabPolish DUO 150A	6868	84
3. MabPolish Type II	1779	89

Key benefits to UCB

Non Capture Antibody Purification (NCAP)

Collaboration with BioToolomics Ltd.

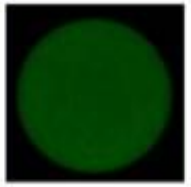
SIMPLE
- Reducing process complexity
- Minimising use of buffers

FAST
- Flow-through mode ('capture-free')
- Continuous and fully integrated steps

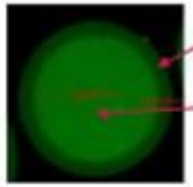
COST
- Target 20% cost saving
- Avoid Protein A (expensive)
- Consider environmental impact as a key driver

HTPD
- Extensive use of HTP systems for resin screening and parameter ranging

NOVEL RESIN DESIGN
- Dynamic resin development to match the biophysical properties of impurities



Confocal image of standard porous agarose bead.



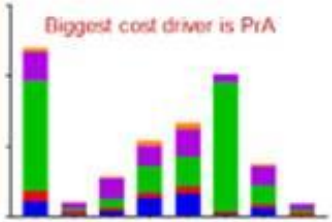
Shelled agarose bead. Improved selectivity; enables more efficient, new purification processes.

SEC out-layer preventing Antibody from getting inside the bead

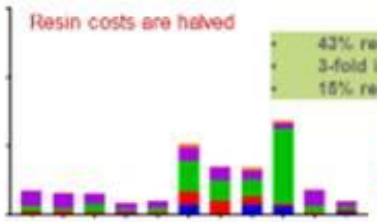
Mixed-mode, HIC or IEX ligand in the core to remove impurities smaller than antibody in flow-through mode

Independent of titre!!

PrA bind/elute process



Optimised NCAP Process 1



- 43% reduction in process costs
- 3-fold increase in throughput
- 18% reduction in water usage

BioToolomics

Single-Use Disposable Chromatography Solutions

High Capacity, High Throughput, Ready-to-Use, Disposable

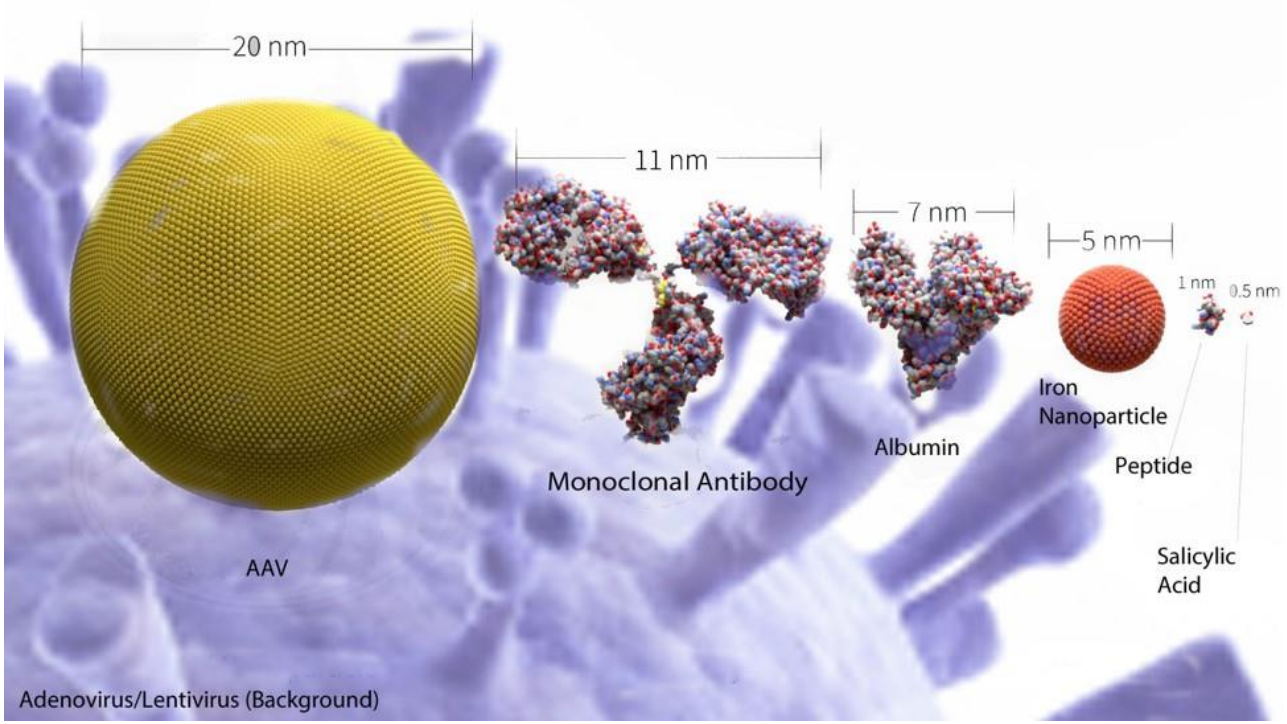
Accelerate Your Purification Process

www.biotoolomics.com

Viral Vectors for Gene Therapy:

- Gene therapy as a viable medical method has built up the momentum to rapidly grow in the coming years.
- Demand of high quality therapeutic viral vectors will be very high.
- Current chromatography resins in the market have poor performance.

Sizes of Viruses



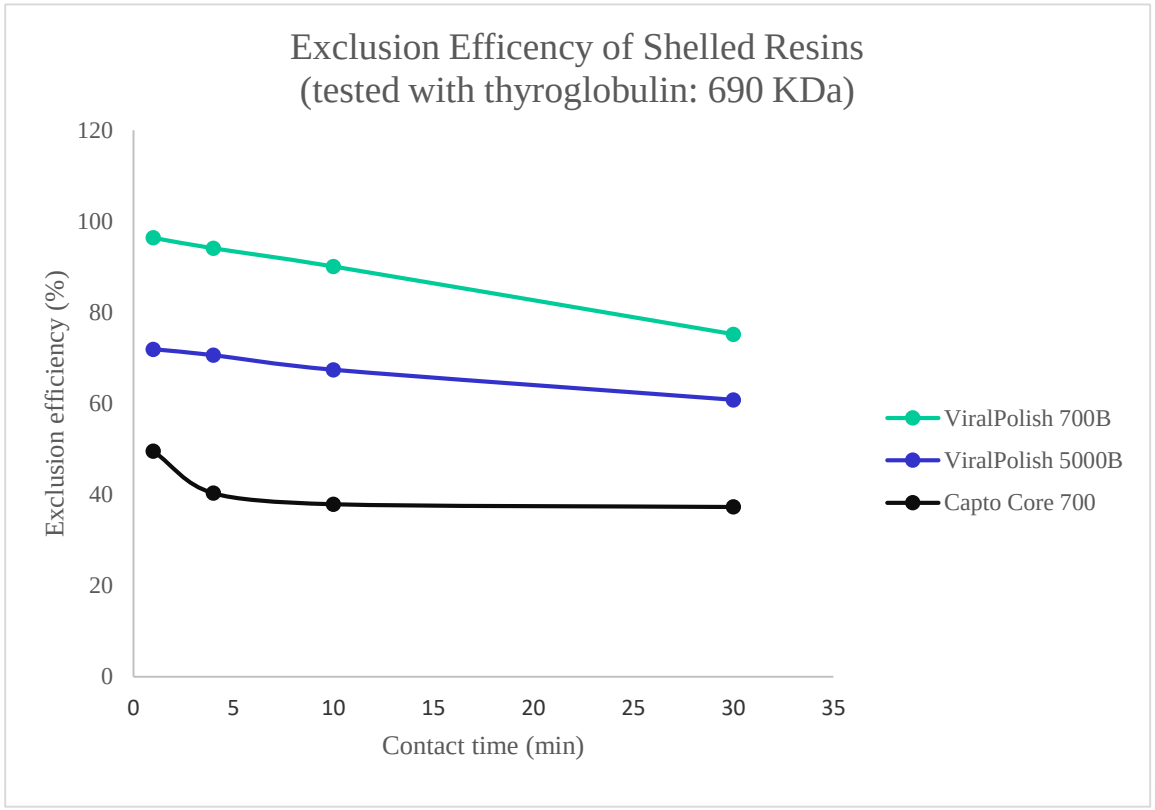
Novel Chromatography Resins (Based on Shelled Bead Technology) for Viral Purifications

- **ViralPolish 400A, 400B, 400C**
- **ViralPolish 700A, 700B, 700C**
- **ViralPolish 5000A, 5000B, 5000C**
- **SepFast DUO 400 Q, 400 S**
- **SepFast DUO 700 Q, 700 S**
- **SepFast DUO 5000-Q, 5000 S**

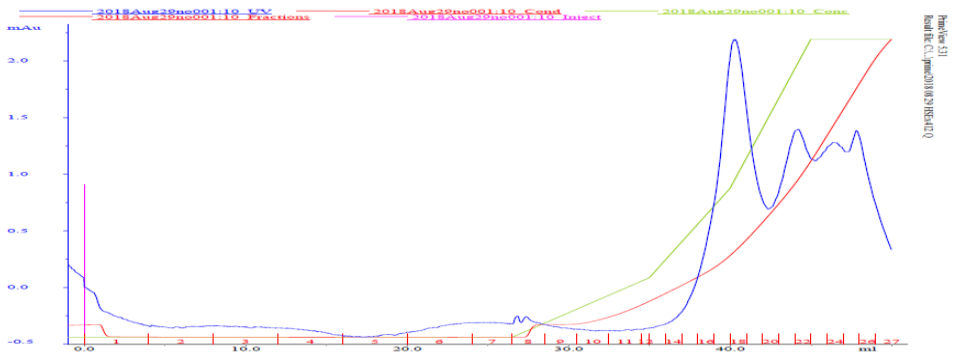
Shelled Mixed-Mode Resin	Application Guide	Key Features
ViralPolish™ 5000A **ViralPolish™ 5000B** **ViralPolish™ 5000C**	Good for lentivirus, adeno virus, VLPs and other viruses of large sizes AAV may be purified if very high molecular-weight impurity (e.g. truncated AAV or empty capsid) need be removed.	The inert out shell has molecular cut-off of 5000 KDa, 700 KDa and 400 KDa, respectively, to block viral particles of different sizes. Mixed-mode ligand is immobilised inside to capture impurities. “A” has mild hydrophobicity; “B” has strong hydrophobicity, “C” has even stronger hydrophobicity than “B”. “A” type resins can be easily re-generated and re-used. “B” & “C” types of resins have higher loading capacity but is difficult to re-generate. They are more suitable for single-use.
ViralPolish™ 700A **ViralPolish™ 700B** **ViralPolish™ 700C**	Good for AAVs, other viruses and VLPs of similar sizes, plasmids etc	
ViralPolish™ 400A **ViralPolish™ 400B** **ViralPolish™ 400C**	Good for certain vaccine antigens, large proteins etc	

Shelled IEX Resin	Application Guide	Key Features
SepFast™ DUO 5000 Q **SepFast™ DUO 5000 S**	Good for lentivirus, adeno virus etc. AAV may be purified if very high molecular-weight impurity (e.g. truncated AAV or empty capsid) need be removed.	The inert out shell has molecular cut-off of 5000 KDa, 700 KDa and 400 KDa, respectively, to block viral particles of different sizes. Strong ion-exchange ligand is immobilised inside to capture impurities. Q: strong anion-exchanger S: strong cation-exchanger
SepFast™ DUO 700 Q **SepFast™ DUO 700 S**	Good for AAVs, other viruses and VLPs of similar sizes, plasmids	
SepFast™ DUO 400 Q **SepFast™ DUO 400 S**	Good for certain vaccine antigens, large proteins etc	

Shelled Resin: Effectiveness in Blocking 700KDa Molecules



Two-step Purification of Adeno Viruses



One-step process: Q resin in bind-elute mode

Two-step process: ViralPolish 700 Q flow-through mode then Q resin bind-elute mode

BioToolomics
Single-Use Disposable Chromatography Solutions

Magnetic Resins

- A broad range of magnetic affinity resins including Protein A, Protein G, Ni / Co / Zn etc
- Pre-activated magnetic resins (Amine, carboxyl, CNBr, NHS, epoxy, thiol-link etc) for making custom affinity resins
- Magnetic SepFast Supor Q (DEAE, S, CM) for high-throughput screening of phage libraries

Custom Resins

- Parameters:
  - particle size,
  - pore size,
  - cross-linking,
  - coupling chemistry,
  - ligands,
  - magnetic particles,
  - very dense particles
  - others

- BioToolomics develops and produces custom-made agarose-based chromatography resins

Examples of Literatures Using BioToolomics Resins

- Purification of bacteriophage M13 by anion exchange chromatography, Razieh Monjezia et al., Journal of Chromatography B, 878 (2010) 1855–1859
- Purification of long helical capsid of newcastle disease virus from *Escherichia coli* using anion exchange chromatography, Chee Fai Yap et al., Biotechnology Progress, Volume 29, Issue 2, March/April 2013, Pages 564–567
- Preclinical assessment of viral vectored and protein vaccines targeting the Duffy-binding protein region II of *Plasmodium vivax*, Simone C. de Cassan et al., Front. Immunol., 08 July 2015
- Purification of rabbit polyclonal immunoglobulin G with ammonium sulphate precipitation and mixed-mode chromatography, Mariam et al., Sep. Purif. Technol., 144 (2015), pp. 133-138
- Identification of an allosteric binding site for RORγt inhibition, Marcel Scheepstra et al., *Nature Communications* 6, article number: 8833 (2015)
- Perlman syndrome nuclease DIS3L2 controls cytoplasmic non-coding RNAs and provides surveillance pathway for maturing snRNAs, Anna Łabno et al., *Nucleic Acids Research*, Volume 44, Issue 21, 1 December 2016, Pages 10437–10453
- A short splicing isoform of HBS1L links the cytoplasmic exosome and SKI complexes in humans, Katarzyna Kalisiak et al., *Nucleic Acids Research*, Volume 45, Issue 4, 28 February 2017, Pages 2068–2080

Chromatography Columns

- BioToolomics offers scalable disposable or non disposable columns particularly suitable for sample preparation, process development and up-scale cGMP production applications.

- Column inner diameter includes 6.2 mm, 7 mm, 11 mm, 16 mm, 26 mm, 50 mm, 80 mm, 100 mm, 129 mm and 258 mm.
- Larger diameter columns can also be made.

Key Features and Benefits

- Scalable column design for bioprocessing applications
- Low cost
- Excellent flow distribution mechanism with minimum dead volumes
- Robust and long life time
- Disposable
- Materials used are compatible with common buffers and approved for targeted use

Empty Columns

Column Volume (ml)	Column Diameter (mm)	Operational Pressure	
0.33, 0.67, 1	6.2	6 bar	
5, 10	11	3 bar	
20, 30, 40	16	3 bar	

Column Configuration -1

- 6.2 mm i.d. SepFast column for robust process development use;
- 1 ml and 5 ml HiSep columns for routine sample preparation
- Compatible to most FPLC systems including AKTA
- Compression factor taken into account
- Column material: mainly polypropylene, compatible to most buffers

SepFast column

HiSep column

Column Configuration - 2

- 11 mm i.d. and 16 mm I.d. columns
- Standard sizes: 5 ml, 10 ml, 20 ml, 30 ml and 40 ml
- Compatible to AKTA systems
- Compression factor taken into account
- Column body: Acrylic

Column Packing Service

- We have extensive experience and expertise in packing various types of chromatography resins: from soft gels to rigid particles (such as ceramics or glass particles), from small particles to large particles, from spherical particle to irregular particles, or particles of higher settling densities.
- The packing procedure for each column is fully documented under our ISO 9001:2015 quality management system.
- GMP-grade columns packed in our class 7 clean room.

Packing Service: Medium to High Throughput Screening / Purification Applications

Product Type	Packed Volume		
96 well filter plate	1µl to 500µl		
Spin column	20µl to 500µl		
Gravity column	1 ml to >10 ml		
1 ml, 5 ml column	1 ml / 5 ml		

BioToolomics
Single-Use Disposable Chromatography Solutions

Packing Service: Process Development / Validation / Small-Scale Purification

Column Diameter	Packed Volume (/cm of bed height)	Operational Pressure	
7 mm	0.38 ml	Standard: 5 bar Optional: 20 bar	
11 mm	0.95 ml		
16 mm	2 ml		
26 mm	5.3 ml		

Each column comes with the results certificate for the following tests: HETP, Peak Asymmetry and Flow Properties (if required)

Packing Service: cGMP Scaling-up & Production Applications

Column Diameter	Packed Volume (/cm of bed height)	Operational Pressure	
50 mm	20 ml	3 bar	 
80 mm	50 ml		
100 mm	78 ml		
129 mm	130 ml		
258 mm	522 ml		

Column diameter >258 mm available on request

The wetted materials of each column are of the USP class VI and/or FDA CFR 177 grades. Each column is packed and sanitised in our clean room (class 7) and will come with the results certificate for the following tests: ***HETP, Peak Asymmetry, Endotoxin Level and Bioburden Level.***

BioToolomics
Single-Use Disposable Chromatography Solutions

Summary / Conclusion

- High flexibility to meet customer needs
- Strong technical support with fast response
- Regulatory support
- Very cost-effective
- On-time delivery / fast production