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Accelerating antibody drug development with subdomain-specific affinity ligands

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INTRODUCTION

With the development of novel bio-therapeutic antibody formats, such as trifunctional and bi-specific monoclonal antibodies or antibody fragments, new purification challenges in the downstream process of these molecules arise.

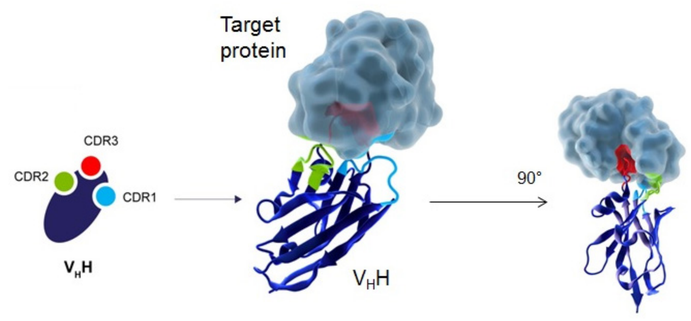
Thermo Scientific™ CaptureSelect™ antibody subdomain-specific affinity products and analytical tools are developed for the discovery and manufacturing of therapeutic antibodies and antibody fragments. The affinity resins provide high target purity in a single step, independent of feedstock.

CAPTURESELECT TECHNOLOGY – UNIQUE AFFINITY PURIFICATION SOLUTION

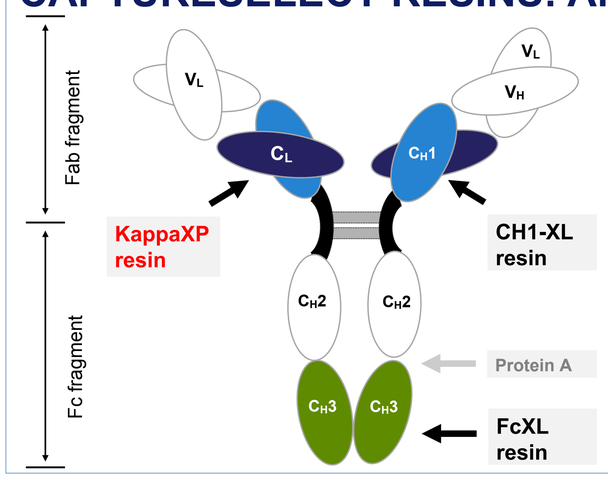
- Affinity through antibody selectivity: technology based on Camelid single domain [V_HH] antibody fragments
- Unique V_HH screening technology to determine final resin properties such as target specificity, mild elution & ligand stability
- Animal origin free production process (*Saccharomyces cerevisiae)*
- Technology used in commercial purification processes

Fig.1 CaptureSelect ligands are V_HH fragments (single domain antibody fragments – sdAb), the smallest antigen binding molecule.

The small size of V_HH fragments (15kD) allows binding at difficult to reach epitopes. Overall, V_HH fragments offer high specificity, affinity and stability.



CAPTURESELECT RESINS: ANTIBODY BINDING REGIONS



A unique set of CaptureSelect affinity ligands has been developed (fig 2.), directed against a variety of antibody subdomains, providing tools for researchers and manufacturers to help facilitate purification of a broad range of antibody formats.

Fig.2 CaptureSelect Antibody Selectivity
Binding regions of CaptureSelect resins for affinity purification of antibodies and antibody fragments.

PURIFICATION OF ANTIBODY THERAPEUTICS

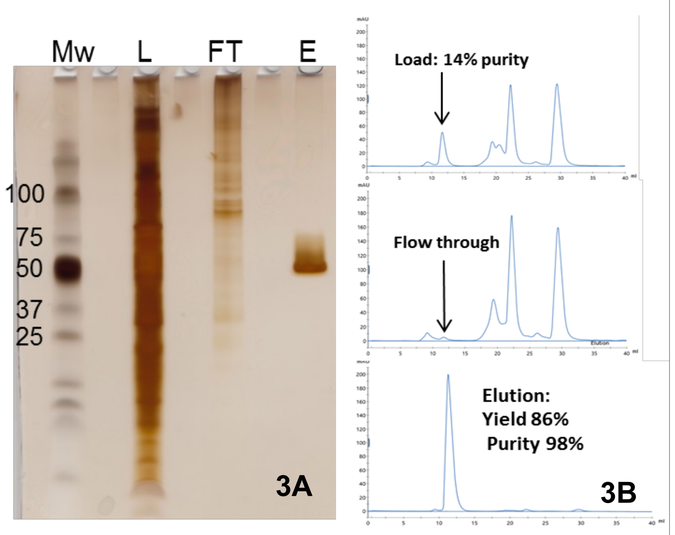
CaptureSelect CH1-XL affinity matrix
Fab fragment purification platform

- CH1 binding domain ligand
- No co-purification of free light chains (only correct assembled Fabs)
- Efficient elution at milder pH (4 – 4,5)

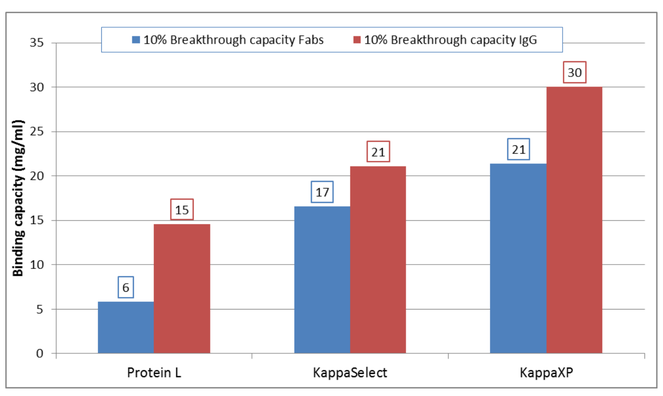
Fig. 3 Ranibuzimab feed from HEK293 cells. Analysis of the fractions after purification with CaptureSelect CH1-XL resin shows high yield and purity in a single step.

3A: SDS-PAGE silver staining of the load (L), flow through (FT) and elution (E) fractions, showing no presence of light chains in the elution pool.

3B: Gel filtration analysis showing 98% purity of the Fab fragment in the elution fraction with a yield of 86%



CaptureSelect KappaXP affinity matrix
Next generation Kappa light chain binder: improved DBC, efficient elution



- 100% Kappa subtype coverage for all IgG's containing a Kappa light chain
- High dynamic binding capacity (up to 45 g/L measured with monoclonal IgG)
- Efficient elution at milder (pH 5-6) with additives

Fig.4 KappaXP 10% breakthrough analysis; comparison with alternative kappa light chain affinity resins. Capacities measured with Polyclonal IgG and Polyclonal Fab at 6,1 min residence time on 1 mL columns. Bound protein eluted using 20 mM Citric Acid pH 3.5.

CaptureSelect FcXL affinity matrix
Scalable solution for poorly protein A binding IgGs and Fc-fusion proteins

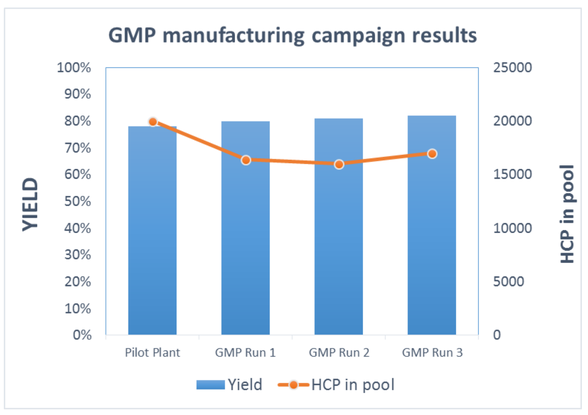


Fig.5 The FcXL affinity matrix shows high consistency between development, pilot and GMP scales (data kindly provided by Roche)

- CH3 domain binding ligand
- Excellent scalability
- High dynamic binding capacity (25/35 gL)
- Efficient elution at milder (pH 5-6) with additives, making it suitable for Fc fusion proteins

"The implementation of FcXL resulted in a reduction of chromatography steps (from 4 to 3), higher DBC, higher purity and better pool stability" - Roche

ANALYTICAL TOOLS

POROS™ CaptureSelect HPLC columns (fig 6)

POROS CaptureSelect affinity columns combine speed, selectivity, method automation and high precision when monitoring antibody titers and yield during manufacturing.

CaptureSelect Biotin conjugated ligands (fig 7)

CaptureSelect biotinylated ligands can be used to develop a range of analytical assays, including ELISA, Western Blot and assays for label-free detection platforms such as Surface Plasmon Resonance (SPR).

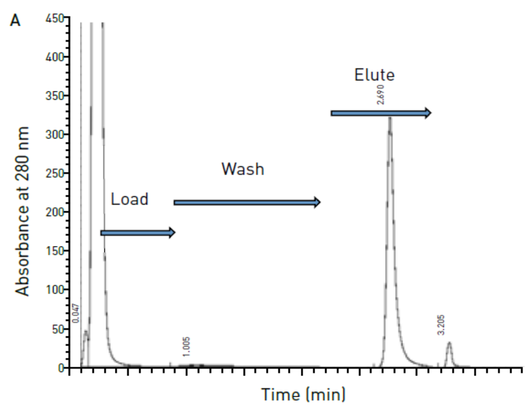


Fig. 6. Chromatogram showing elution of a protein recovered from CHO-conditioned supernatant spiked to a final concentration of 1 mg/mL.

Column: POROS CaptureSelect IgG-Fc.

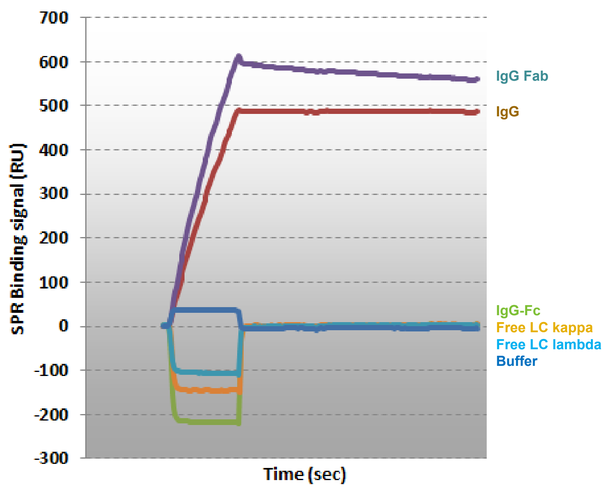


Fig. 7. SPR binding curves showing binding with intact IgG or Fab fragment and no cross binding with IgG-Fc or free light chains.

Conjugate: CaptureSelect Biotin anti-IgG-CH1.

CAPTURESELECT ANTIBODY AFFINITY PRODUCTS

	CaptureSelect Ligand	Species	Biotin conjugated ligand	HPLC POROS	Research resin	cGMP resin + Robocolumn
IgG all subclasses	CH1-XL (CH1)	human		X	X	X
	IgG-CH1	human	X			
	FcXL (CH3)	human + primate		X	X	X
	IgG-Fc	human	X	X		
	IgG-Fc	multi-species	X		X	
	IgG-Fc	rabbit	X		X	
Light chains	KappaXL & XP (CL)	human + primate		X	X	X
	LC-kappa (CL)	human + primate	X	X		
	LC-kappa (CL)	murine	X		X	
	LC-lambda	human + primate	X	X	X	
	LC-lambda	mouse	X		X	
	LC-lambda	rat	X		X	
	LC-lambda	ungulate	X		X	
Isotype specific	IgM	human , mouse, rat	X	X	X (POROS)	contact us
	IgA (Fc)	human	X	X	X	
	IgA (CH1)	human			X	
	IgA	bovine		X	X	
	IgE	human	X		X	
IgG subclass specific	IgG1	human			X	
	IgG3	human	X		X	
	IgG4	human	X		X	

For more information visit:**www.thermofisher.com/Captureselect**

CONCLUSION

CaptureSelect antibody subdomain-specific affinity resins address the purification challenges in therapeutic antibody development by providing unique selectivity, high purity and yields in a one-step purification process.

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