

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-derived single domain [V_HH] antibody fragments
- Unique screening technology for target specificity, mild elution & stability
- Animal origin free production process (*Saccharomyces cerevisiae*)
- Technology used in commercial purification processes

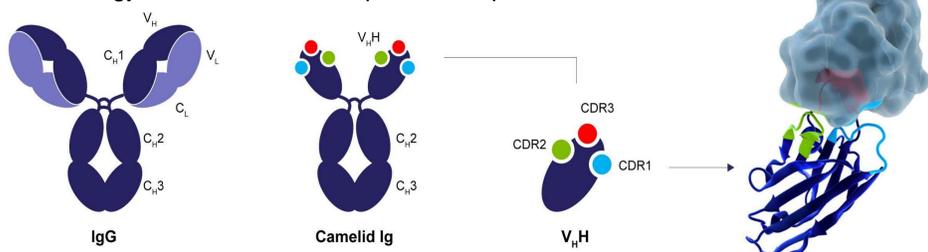


Fig.1 Regular IgG antibody compared to a Camelid heavy-chain antibody. The V_HH antibody fragments offer high specificity, affinity and stability.

ANTIBODY SUBDOMAIN TARGETS

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.

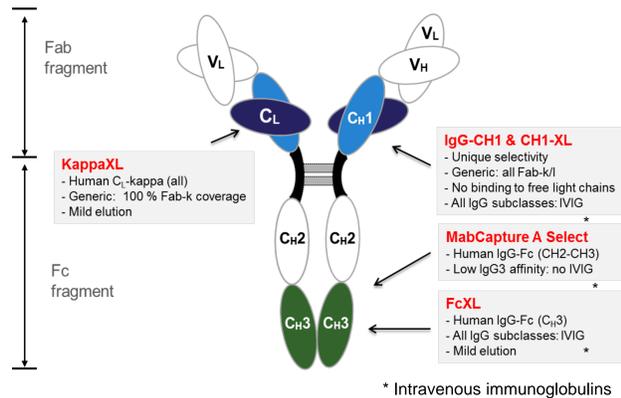


Fig.2 Available subdomain-specific affinity ligands

Product coverage

- Isotype specific: IgG, IgM, IgA, and IgE
- Subclass specific: IgG1, 3 and 4
- Species: human, murine, multi-species
- Fragments: Fab (C_H1 or LC), Fc-fusions (C_H3)

PURIFICATION OF ANTIBODY THERAPEUTICS

CaptureSelect CH1-XL affinity matrix

- ✓ A true platform for Fab fragment purification
- No co-purification of free light chains (only correct assembled Fabs)
- Efficient elution at mild pH

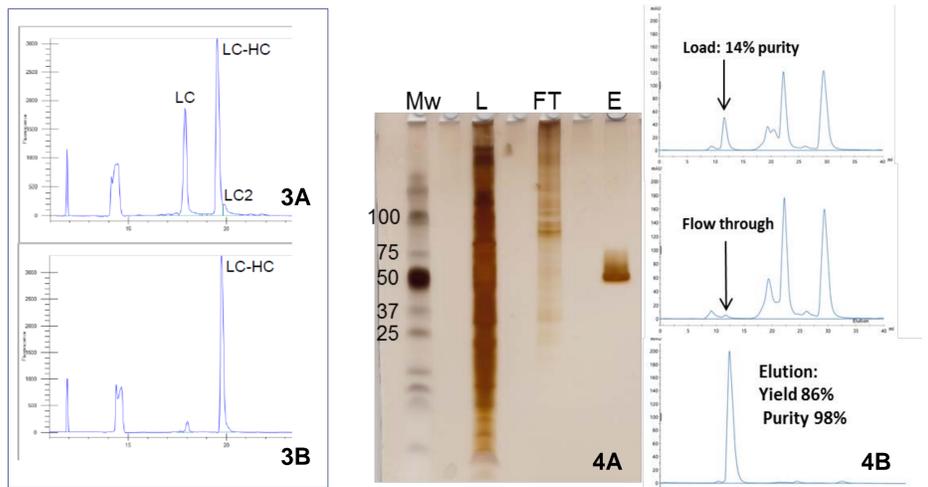


Fig. 3 LabChip® analysis of Fab fragment purification shows no binding of light chains (LC) or light chain dimers (LC2) in the elution fraction (B). 3A: Analysis of feed stock, 3B: Analysis of elution fraction. LabChip® is a registered trademark of PerkinElmer

Fig. 4 Ranibizumab feed from HEK293 cells. Analysis of the fractions after purification with CaptureSelect CH1-XL resin shows high yield and purity in a single step. 4A: 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. 4B: Gel filtration analysis showing 98% purity of the Fab fragment in the elution fraction with a yield of 86%

CaptureSelect FcXL affinity matrix

- ✓ The alternative for more sensitive IgGs and Fc-fusion proteins
- High selectivity for human IgG-Fc (C_H3 domain) of all subclasses
- High dynamic binding capacity
- Efficient elution at milder (pH 5 -6) with additives

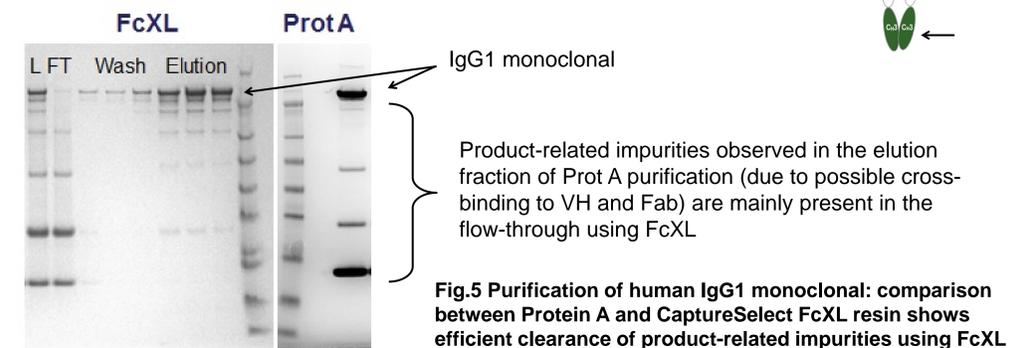


Fig.5 Purification of human IgG1 monoclonal: comparison between Protein A and CaptureSelect FcXL resin shows efficient clearance of product-related impurities using FcXL

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 ligand conjugates (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).

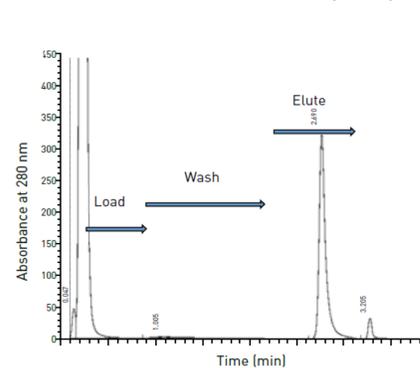


Fig. 6. Chromatogram showing elution of a protein recovered from CHO-conditioned supernatant spiked to a final concentration of 1 mg/mL. Column: POROS CaptureSelect IgG-Fc.

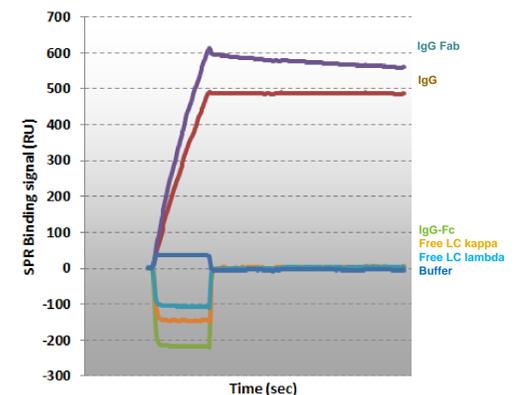


Fig. 7. SPR binding curves showing binding with intact IgG or Fab fragment and no binding with IgG-Fc or free light chains. Conjugate: CaptureSelect Biotin anti-IgG-CH1.

CAPTURESELECT ANTIBODY AFFINITY PRODUCTS

	CaptureSelect Ligand	Species	Biotin conjugate	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			
	IgG-Fc	multi-species	X		X	
Light chains	KappaXL (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	
Isotype specific	IgM	human, mouse, rat	X	X	X (POROS)	contact us
	IgA (Fc)	human	X	X		
	IgA (CH1)	human			X	
	IgE	human	X		X	
IgG subclass specific	IgG1	human			X	
	IgG3	human	X		X	
	IgG4	human	X		X	
	Kinetics & analytics	Fab-kappa kinetics	human + primate	X		
Fab-lambda kinetics		human + primate	X			
IgG-Fc PK (CH2)		human	X			
Free LC-kappa (CL)		human	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.