

# Accelerating antibody analytics

Utilizing subdomain-specific affinity ligands that cover a broad variety of antibody-based platforms



A Thermo Fisher Scientific Brand

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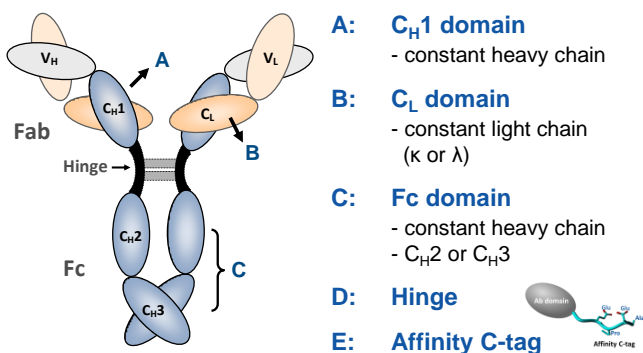
## CaptureSelect™ Affinity Ligands

CaptureSelect™ purification and affinity separation products are developed for the discovery and manufacturing of therapeutic proteins, including antibodies and antibody fragments. These products help enable a reduced time-to-market, increased purity and yield, and an overall lower cost of goods for biopharmaceutical development.

## Antibody Subdomains

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

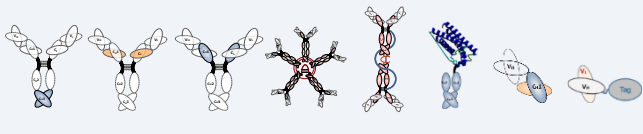
### Anti-subdomain affinity ligands



## Antibody Coverage

Whole antibodies	Ab fragments	Species selectivity
IgG	A-B-C	Fab
IgG-1*	A	Fc
IgG-3*	D	Fc-fusion
IgG-4*	C	(sc)Fv
IgA	A-B-C	V <sub>H</sub>
IgM	B-C	V <sub>L</sub>
IgD	B	
IgE	B	

\* human subclass-specific  
† others = primate + dog, cat, horse, donkey, guinea pig, Syrian hamster



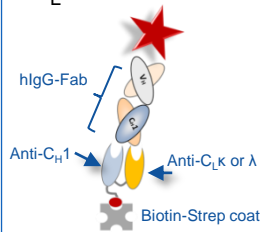
## Application Areas

Discovery Screening	Detection Quantitation	Pharma Analytics	Purification
Affinity ranking	Western	MSIA LC-MS	Small scale
Cell line screening	ELISA	PK studies	Manufacturing
	Label-free		
	HPLC		

## Discovery & Screening

### Affinity ranking

High affinity capture of Fab fragments from human IgG for screening binding kinetics. Robust Fab capture through avidity by anti C<sub>H</sub>1-C<sub>L</sub> κ or λ bi-head.



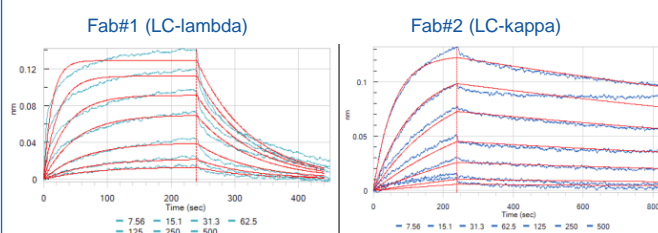
Fab-kappa kinetics biotin conjugate  
Fab-lambda kinetics biotin conjugate

- no significant dissociation of Fab when bivalently captured
- compatible with any streptavidin based biosensor
- Regeneration at low pH (re-use)

### Example

A study was performed by MorphoSys AG in which Octet® SA biosensors were pre-coated with biotin anti C<sub>H</sub>1-C<sub>L</sub>κ and C<sub>H</sub>1-C<sub>L</sub>λ in a 1:1 mixture. The functionalized biosensors were then allowed to capture and analyze 2 different human IgG Mabs (C<sub>L</sub>κ or λ), either as whole antibody or Fab fragment thereof, following kinetic analysis with the target antigen (Octet® QK384 or HTX, forteBIO®). A good correlation was found for the calculated K<sub>D</sub> values between Fab and IgG for each Mab analyzed.

### Real-time binding charts



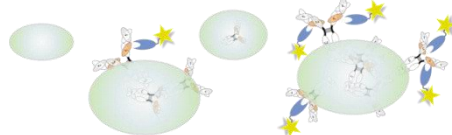
### Binding kinetics

Format	Antibody #1 (LC-lambda)			Antibody #2 (LC-kappa)		
	k <sub>a</sub> (1/Ms)	k <sub>d</sub> (1/s)	K <sub>D</sub> (nM)	k <sub>a</sub> (1/Ms)	k <sub>d</sub> (1/s)	K <sub>D</sub> (nM)
Fab	1.33	1.18	89.3	3.42	4.23	12.4
IgG	1.70	1.09	64.1	3.31	4.31	13.0

### Cell line screening

For isolation of highly productive cells from pools of antibody expressing clones, various anti-subdomain affinity ligands can be used in FACS when labeled to, e.g., Alexa Fluor® 488 dye (Life Technologies™)

e.g., CaptureSelect™ anti-IgG-C<sub>H</sub>1 conjugate (no binding to over-expressed free light chains)



## Detection & Quantitation

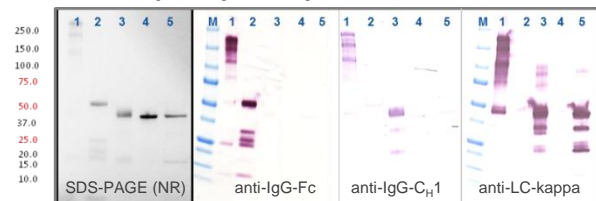
### Western blot

Simple detection of antibodies and antibody fragments in western blot using CaptureSelect™ biotin conjugates in combination with streptavidin-enzyme reagents

Biotin anti-IgG-Fc (C<sub>H</sub>3, human-specific)  
Biotin anti-IgG-C<sub>H</sub>1  
Biotin anti-LC-kappa (human-specific)

### Example

M: MW Marker, 1: hlgG, 2: hlgG-Fc, 3: hlgG-Fab, 4: free LC-lambda, 5: free LC-kappa

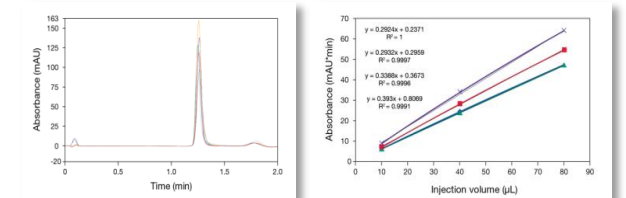


## POROS® CaptureSelect™ HPLC columns

Rapid quantitation of antibody samples in HPLC format.

### Example

POROS® CaptureSelect™ LC-kappa



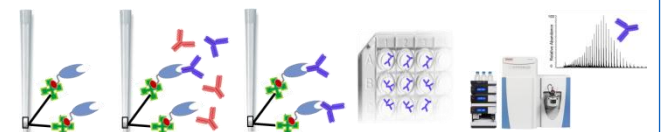
Column: 2.1 x 30 mm, PEEK™ System: Agilent® 1200  
Sample: 50 µL of purified kappa light chain at 0.5 mg/mL  
Flow rate: 2 mL/minute  
Elution: 12 mM HCl, 150 mM NaCl pH 1.9

## Pharma Analytics

### MSIA LC-MS

Mass Spectrometric ImmunoAssays enabled with CaptureSelect™ biotin conjugates following the MSIA D.A.R.T.'S™ streptavidin workflow:

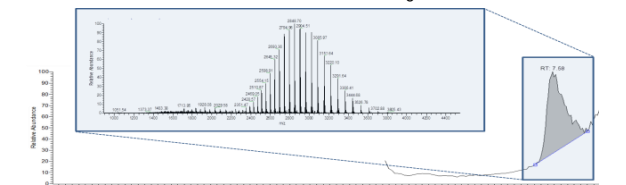
CaptureSelect™ D.A.R.T.'S. Load sample Purify Elute LC-MS analysis



### Example

CaptureSelect™ Biotin anti human-IgG-Fc PK

- Highly specific for human IgG, no binding to, e.g., mouse, rat and cyno IgG
- D.A.R.T.'S. functionalized with 5 µg Biotin PK conjugate
- Sample: 250 µL mouse serum + 250 µL PBS + 0.5 µg Adalimumab (human IgG1)
- Elution with 2% formic acid in 10% MeOH following LC-MS



Intact Mab analysis with LC-MS → human selectivity demonstrated

## Purification

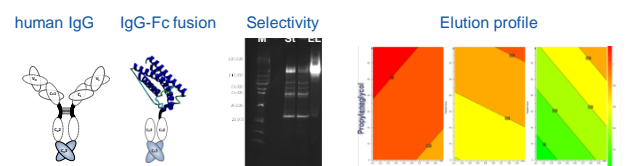
### From R&D to cGMP-scale manufacturing

High one-step purity and yield in combination with mild elution.

### Example

CaptureSelect™ FcXL affinity matrix

- High selectivity for human IgG C<sub>H</sub>3 domain (all 4 subclasses), DBC ~25-30 mg/mL
- Efficient elution at pH 5 and higher
- Ideal for low pH sensitive Mabs and Fc-fusion constructs



## CaptureSelect™ Products

CaptureSelect™ Ligand	Species	Biotin	HPLC POROS®	Purification R&D	Purification cGMP
IgG-Fc (C <sub>H</sub> 3)	Hu + primate	+	+	+	-
FcXL (C <sub>H</sub> 3)	Hu + primate	-	-	+	+
IgG-Fc PK (C <sub>H</sub> 2)	Hu only	+	-	-	-
IgG-Fc	Multi	+	-	+	-
IgG-C <sub>H</sub> 1	Hu +	+	on request	+	+
IgG-1, 3 or 4	Hu +	+	-	+	-
IgA (Fc or C <sub>H</sub> 1)	Hu +	+	+	+	+
IgM	Hu, Mo, Rt	+	+	+	+
LC-kappa / lambda	Hu + primate	+	+	+	+
LC-kappa / lambda	Murine	+	-	-	-
KappaXL	Hu + primate	-	-	+	+
Free LC-kappa	Hu + primate	+	-	-	-
Fab-kappa kinetics	Hu + primate	+	-	-	-
Fab-lambda kinetics	Hu + primate	+	-	-	-
HSA	Hu	-	+	+	+
C-tag	-	+	-	+	-