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CaptureSelect Affinity Ligands for Antibody Detection and Characterization

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Abstract

CaptureSelect affinity ligands are a unique group of heavy chain antibodies that can be designed for almost any purification target, with as narrow or as broad specificity as is required. These ligands can be chemically biotinylated for use in capture assay formats incorporating streptavidin-coated surfaces such as those used in ELISA, SPR and BLI, enabling a broad range of novel selectivities for antibody analytics.

CaptureSelect biotin conjugates demonstrate excellent compatibility with Streptavidin Biosensors and can serve as a valuable tool for detection, quantitation and characterization of multiple antibody subclasses and isotypes using the Octet® platform. Octet® systems can be used to quantify protein concentrations and monitor protein-protein and other biomolecular interaction kinetics (k_a , k_d and K_D) to inform research and development decisions earlier in the process, while their high throughput enables accelerated timelines.

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Introduction

BAC's CaptureSelect affinity ligands provide a unique group of molecules that can be designed for almost any purification target, with as narrow or as broad specificity as is required. The fragments are derived from the unique heavy chain antibodies found in Camelidae. Heavy chain antibodies are devoid of the entire light chain and CH1 domain found in conventional antibodies (Figure 1). Without the light chain, antigens are bound only by the variable domain of the heavy chain (VHH), without a loss in binding affinities compared to conventional antibodies.

The VHH domain is the smallest functional antigen-binding fragment known that shows high affinity and stability. The equivalent fragment from conventional antibodies is the Fv domain, which consists of the heavy chain variable domain and the light chain variable domain held together by a non-covalent bond. If this bond is broken, all antigen binding functionality will be lost. Because the VHH domain exists as a single polypeptide chain, it is an extremely stable and easy to produce antibody fragment that retains the full antigen binding activity of the parent heavy chain antibody.

During the screening process, VHH molecules that meet very specific requirements for specificity, affinity, elution profile and cleaning conditions are selected. In this way VHH affinity ligands can be created to match exactly the processes they are intended for. VHH fragments have been used successfully as affinity ligands in chromatography processes for the purification of many types of protein, including antibodies,^{1-4,6,9} blood factors⁵ and adeno-associated viruses^{7,8,10} among others. For more information on CaptureSelect affinity ligands, please visit www.captureselect.com.

Antibody Purification

Using Protein A, Protein G, and Protein L for antibody purification can be highly effective. However, there are gaps in the functionality that they provide, and when a target protein falls into one of these gaps the process of finding an alternative purification strategy can be extremely frustrating.

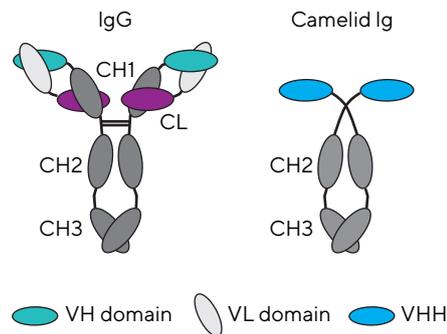


Figure 1: Comparison of conventional IgG structure versus Camelid heavy chain antibody.

The main binding characteristics of Proteins A, G, and L are listed below:

Protein A and G

- Bind to IgG Fc domain (CH2-CH3 interface)
- Bind IgG of many species including bovine and mouse (not human specific)
- Protein A binds human IgG1, 2 and 4 but not IgG3; Protein G binds all sub-types

Protein L

- Binds to VL domain of an antibody light chain
- Binds human VL-kappa 1, 3 and 4 but not to VL-kappa 2 nor to any VL-lambda
- Binds mouse VL-kappa 1

Since more and more antibody-based therapeutics, (e.g., Fab fragments) lack a regular Protein A or G binding site, it becomes a challenge to find a suitable equivalent for primary capture of antibody formats. For this reason, BAC has developed a range of CaptureSelect affinity ligands directed against a unique panel of antibody sub-domains, providing an antibody toolbox for researchers and manufacturers, facilitating affinity purification and detection of virtually any antibody-based format. A schematic representation of an IgG antibody and the specific domains that are targeted by various CaptureSelect affinity ligands is presented in Figure 2.

Besides antibody purification at both a research and bioprocess scale, analytical applications such as online monitoring of antibody expression and/or analyzing product yield and purity during downstream processing, are facilitated by incorporating CaptureSelect affinity ligands onto HPLC-compatible backbones (e.g., POROS).

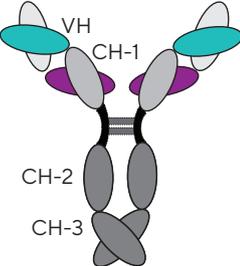
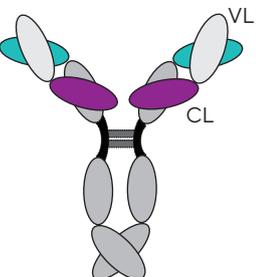
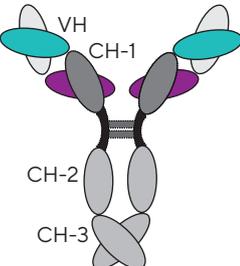
| Antibody Fc domains | CaptureSelect affinity ligands |
|--|---|
|  | <p>Human IgG-Fc</p> <ul style="list-style-type: none"> ▪ Binds all subclasses, human-specific ▪ Human IgG subclass-specific: IgG1, IgG3 and IgG4 ▪ Human isotope specific: IgM, IgA multi-species IgG-Fc |
| Antibody light chains (LC) | CaptureSelect affinity ligands |
|  | <p>Human LC-kappa and LC-lambda</p> <ul style="list-style-type: none"> ▪ Human-specific ▪ Binds all human Ig antibodies (e.g., IgG, IgM, IgA, IgD, IgE) ▪ Binds human Fab fragments |
| Antibody IgG CH1 | CaptureSelect affinity ligands |
|  | <p>IgG-CH1</p> <ul style="list-style-type: none"> ▪ Binds all human IgG subclasses ▪ Covers all human IgG Fab fragments ▪ Binding independent of type of light chain ▪ No binding to free light chains |

Figure 2: Overview of different antibody domains targeted by a variety of CaptureSelect affinity ligands.

To further broaden the range of analytical tools for antibody detection and characterization, a set of CaptureSelect affinity ligands has been chemically biotinylated for use in capture assay formats incorporating streptavidin-coated surfaces (as used in ELISA, SPR and BLI). Octet® Streptavidin (SA) Biosensors demonstrated excellent compatibility with the biotinylated CaptureSelect affinity ligands, enabling a whole range of novel selectivities for antibody analytics on the Octet® platform.

Several CaptureSelect biotin conjugates have been studied in detail using an Octet® system and the results are described.

Binding Selectivity of CaptureSelect Biotin Conjugates

The following biotin conjugated affinity ligands were used in this study:

| | |
|---|---|
| <p>Biotin anti-IgG-Fc (Hu) BAC Cat. No. 710.0822.100</p> | <ul style="list-style-type: none"> ▪ Binds to CH3 domain of human IgG-Fc ▪ Binds all 4 human IgG subclasses ▪ Human-specific (Hu) |
| <p>Biotin anti-LC-kappa (Hu) BAC Cat. No. 710.0833.100</p> | <ul style="list-style-type: none"> ▪ Binds to constant domain of human kappa light chains (LC) ▪ Human-specific (Hu) |
| <p>Biotin anti-LC-lambda (Hu) BAC Cat. No. 710.3080.100</p> | <ul style="list-style-type: none"> ▪ Binds to constant domain of human lambda light chains ▪ Human-specific (Hu) |
| <p>Biotin anti-IgG-CH1 (in development)</p> | <ul style="list-style-type: none"> ▪ Binds to CH1 domain of human IgG ▪ Binds all 4 human IgG subclasses ▪ No binding to bovine, mouse and rat IgG ▪ Cross-binds with dog and cat IgG |

In all experiments, the biotin conjugates were loaded onto Streptavidin (SA) Biosensors using an Octet® system. After loading, a baseline was established by incubating the biosensors for 2.5 minutes in PBS buffer prior to antigen incubation. All binding studies were performed in kinetic mode, allowing further analysis of kinetic parameters. In a first experiment, the binding selectivity of the different CaptureSelect affinity ligands was analyzed using a broad panel of monoclonal and polyclonal antibodies including different human antibody isotypes, IgG subclasses and antibody fragments. Octet® Protein L Biosensors were also included in this study.

As shown in Table 1, the binding selectivity of each CaptureSelect affinity ligand correlated well with the binding profiles observed on the Octet® system. Biotin anti-IgG-Fc only showed binding reactivity towards human IgG antibodies (all subclasses) and IgG-Fc but not to any other species tested. No cross-binding towards bovine and mouse IgG was observed, confirming its selectivity for human IgG only. The same pattern was found for biotin anti-IgG-CH1, except that it showed reactivity for human IgG-derived Fab fragments and not for Fc. Both anti-LC-kappa and anti-LC-lambda conjugates clearly demonstrated explicit selectivity for either LC-kappa or LC-lambda containing antibody formats, respectively. Besides binding to light chains present in intact antibodies, reactivity was observed for free human light chains as well (Bence Jones). The Protein L Biosensors also showed the

expected binding profiles, however, for one human IgG1 kappa monoclonal antibody (Mab) no significant binding was observed. It should be noted that Protein L is restricted to specific subclasses of the human VL domain (i.e., VL-kappa 1, 3 and 4) and does not bind to the human VL-kappa 2 subclass. The tested human IgG1 Mab may have comprised a VL-kappa 2 domain, explaining the lack of binding to Protein L. In this respect, both anti-LC-kappa and anti-LC-lambda affinity ligands are directed against the constant domain of a human light chain (CL) instead of the variable domain (VL). Since the amino acid sequence of the constant domains is more conserved than for VL, less variance can be expected in the binding of different antibody formats compared to Protein L.

The CaptureSelect biotin conjugates were loaded onto Streptavidin (SA) Biosensors at a concentration of 5 µg/mL in 200 µL PBS for 10 minutes (biotin anti-IgG-CH1 was loaded at 10 µg/mL) using a shake speed of 1000 rpm followed by a washing step in PBS for 2.5 minutes to set a baseline. All antibody target samples (10 µg/mL in PBS) were then allowed to bind to the functionalized biosensors (including Protein L Biosensors) for 10 minutes, followed by a 10-minute dissociation step in PBS. All human IgG monoclonal antibodies were purchased from Sigma; Human IgM and IgA, polyclonal human IgG Fab and Fc fragments, polyclonal bovine- and mouse IgG were from Jackson Immunochemistry; Human IgD and IgE from Calbiochem; free human kappa and lambda light chains (Bence Jones) were purchased from BioDesign.

Table 1: Binding selectivity of CaptureSelect Biotin conjugates.

| CaptureSelect biotin conjugates | | | | | | |
|---------------------------------|-------------------|-------------------------|---------------------|---------------------------|----------------------------|-----------|
| Antibody target | Isotype/subclass | Biotin anti-IgG-Fc (Hu) | Biotin anti-IgG-CH1 | Biotin anti-LC-kappa (Hu) | Biotin anti-LC-lambda (Hu) | Protein L |
| IgG subclasses | Hu IgG-1 kappa | ■ | ■ | ■ | - | - |
| | Hu IgG-2 lambda | ■ | ■ | - | ■ | - |
| | Hu IgG-3 kappa | ■ | ■ | ■ | - | ■ |
| | Hu IgG-4 lambda | ■ | ■ | - | ■ | - |
| Ab isotypes | Hu IgM kappa | - | - | ■ | - | ■ |
| | Hu IgD kappa | - | - | ■ | - | ■ |
| | Hu IgE kappa | - | - | ■ | - | ■ |
| | Hu IgA (poly) | - | - | ■ | ■ | ■ |
| Ab fragments | Hu IgG Fab (poly) | - | ■ | ■ | ■ | ■ |
| | Hu IgG Fc (poly) | ■ | - | - | - | - |
| | Free Hu LC kappa | - | - | ■ | - | ■ |
| | Free Hu LC lambda | - | - | - | ■ | - |
| Species | Bovine IgG | - | - | - | - | - |
| | Mouse IgG | - | - | - | - | ■ |

Variance in Binding Reactivity of Biotin Anti-LC-kappa Versus Protein L for a Range of Antibody Formats

For this purpose, additional human LC-kappa containing antibody formats were tested for binding to biotin anti-LC-kappa and Protein L Biosensors. As shown in Figure 3, a higher variance in binding reactivity can be observed for Protein L compared to biotin anti-LC-kappa. Although good reactivity was found for isotypes IgM, A, D and E, only two out of four LC-kappa containing IgG Mabs showed significant binding with Protein L. This again could be due to the presence of a VL kappa 2 domain within the set of antibodies tested. However, very low binding signals were observed for human IgG Fab fragments as well (both polyclonal and recombinant). Although a Fab fragment is only one third of the size of an IgG molecule, one would have expected more or less comparable binding signals with the biotin anti-LC-kappa biosensors. On the other hand, both the free kappa light chain samples (Bence Jones and recombinant) did show proper binding with Protein L. In this respect, results obtained with the biotin anti-LC-kappa biosensors revealed a more consistent binding profile, showing less variance in binding within the broad panel of LC-kappa containing antibody formats tested (as also observed for biotin anti-LC-lambda). Results indicate that affinity ligands that target the constant domain of human light chains provide a more generic tool for detection and characterization of antibody formats compared to those such as Protein L, which target the variable domain.

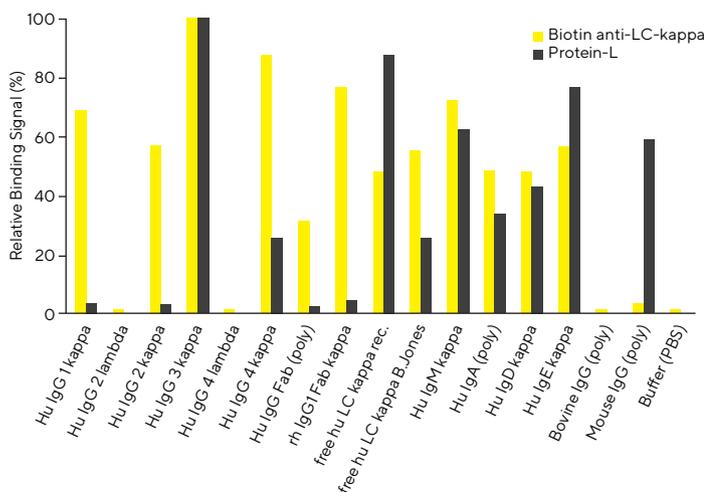


Figure 3: Variance in binding reactivity towards a broad panel of kappa light chain containing antibody formats analyzed on biosensors immobilized with biotin anti-LC-kappa versus Protein L.

Binding analysis was performed as previously described. The residual binding signals after 10 minutes of dissociation were determined for the whole panel of antibody samples tested. For both biotin anti-LC-kappa (Hu) and Protein L the highest binding signals were observed for the human IgG3 kappa antibody (i.e., 2.103 nm and 5.475 nm for anti-LC-kappa (Hu) and Protein L, respectively). To visualize the variance in binding reactivity within this broad panel of LC-kappa containing antibody formats, all binding signals were compared to that produced by human IgG3 kappa, which was set as 100%.

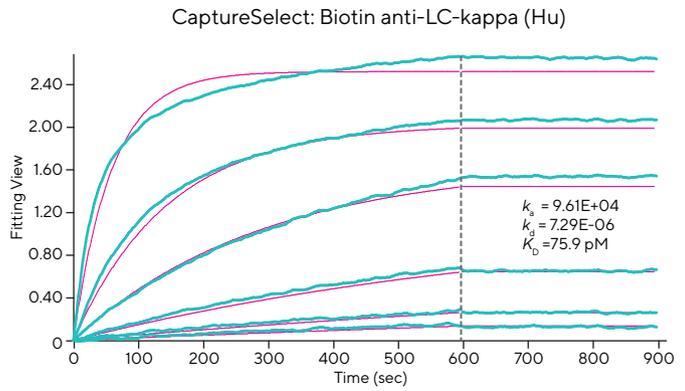
Dose Response Curves

A fully human recombinant IgG1 kappa monoclonal antibody and its corresponding Fab fragment were tested at concentration series ranging from 0.5 µg/mL up to 50 µg/mL. Figure 4 displays the kinetic data from the corresponding curve fits.

Human IgG1 samples on biotin anti-LC-kappa and anti-IgG-CH1 biosensors demonstrated proper association and dissociation curves for the concentration range of IgG tested. Due to bivalent binding that occurs through either the CH1 domain or the constant domain on the kappa light chain that is present twice in an IgG format, very low dissociation rate constant (k_d) values were obtained, as depicted in Figure 4 (i.e., $2.64 \times 10^{-5} \text{ s}^{-1}$ and $7.29 \times 10^{-6} \text{ s}^{-1}$ for anti-IgG-CH1 and anti-LC-kappa, respectively). Proper binding curves for human IgG1 on biotin anti-IgG-Fc biosensors were also generated; however, since this affinity ligand interacts with the Fc domain in a monovalent manner, a higher dissociation rate was found compared to anti-LC-kappa and anti-IgG-CH1 (i.e., $5.01 \times 10^{-4} \text{ s}^{-1}$). Nevertheless, it does show that this set of CaptureSelect biosensors can find their use in quantitation of human IgG antibodies on the Octet® platform. Note that none of these affinity ligands cross-bind to bovine IgG and as such can be used for human IgG detection in the presence of FCS containing media.

Biotin anti-LC-kappa and anti-IgG-CH1 biosensors displayed proper dose response curves for human Fab fragments as well. However, since Fab only contains one light chain and one CH1 domain, no bivalent binding can occur with either of these affinity ligands. Therefore, the calculated k_d values for Fab were significantly higher compared to those for human IgG. These ligands can still be valuable for Fab quantitation purposes, although final detection limits will likely be higher than for measuring whole IgG antibodies. Fab binding was also observed for Protein L, although at a significantly lower binding affinity compared to biotin anti-LC-kappa (data not shown).

Recombinant Human IgG1 Kappa Antibody: Whole Molecule (150 kDa)



Recombinant Human IgG1 Kappa Antibody: Fab Fragment (50 kDa)

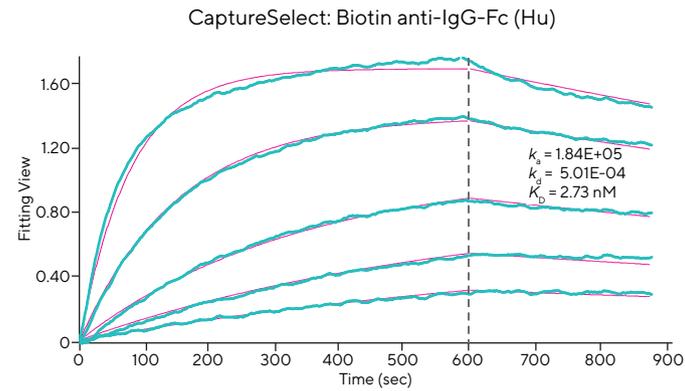
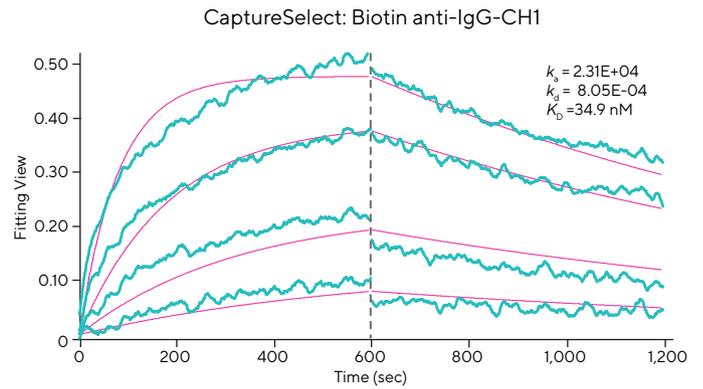
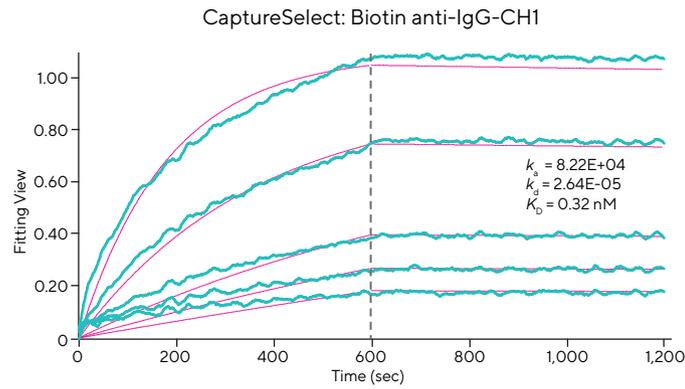
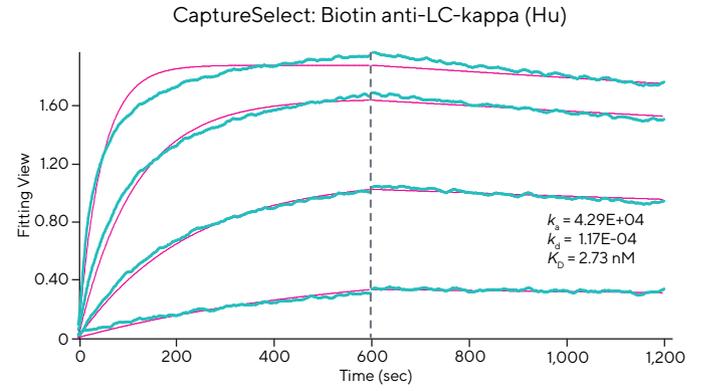


Figure 4: Dose response curves of a recombinant human IgG1 kappa antibody and its corresponding Fab fragment on different antibody capturing biosensors. The CaptureSelect biotin conjugates were loaded onto Streptavidin (SA) Biosensors as previously described. A dilution series of a recombinant human IgG1 kappa monoclonal antibody (whole molecule and Fab fragments thereof) were allowed to bind for 10 minutes, followed by a 10-minute dissociation step (kinetic mode). Binding curves are displayed for each of the biosensors tested. Using the Octet® Analysis Studio Software, kinetic data were calculated.

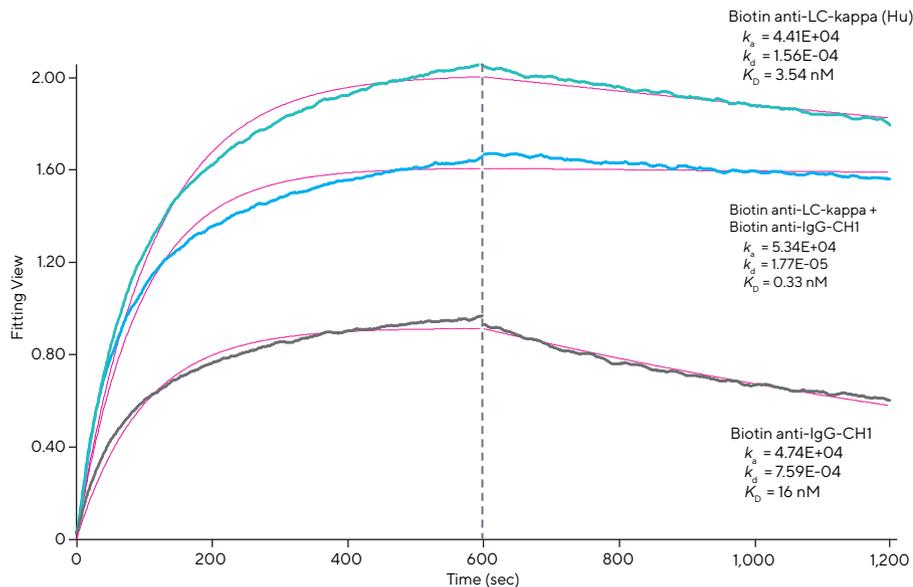


Figure 5: Introducing bivalency to capture human IgG Fab kappa fragments by loading a mixture of biotin anti-IgG-CH1 and biotin anti-LC-kappa (Hu) on one Streptavidin (SA) Biosensor. The CaptureSelect biotin conjugates anti-LC-kappa and anti-IgG-CH1 were immobilized onto Streptavidin (SA) Biosensors at 5 and 10 $\mu\text{g}/\text{mL}$, respectively. One biosensor was immobilized with a mixture of both biotin conjugates using above concentrations for each affinity ligand (15 $\mu\text{g}/\text{mL}$ in total). Binding analysis was performed using a human IgG1 Fab fragment at a concentration of 10 $\mu\text{g}/\text{mL}$. The Octet® Analysis Studio Software was used to calculate the kinetic values for each biosensor. Results are displayed in the graph next to a schematic representation of a “bi-specific” biosensor enabling bivalent capture of a human Fab fragment.

IgG and Fab Capture for Screening Purposes

For numerous applications, it is advantageous to have high-affinity binding agents showing very low dissociation rate values, resulting in a stable complex between the binding agent and its antigen. Especially for screening antibodies against antigen to determine binding characteristics, the availability of high-affinity capture biosensors is key. When capturing human IgG antibodies, the previous results show that a stable complex can be achieved using biotin anti-LC-kappa and anti-IgG-CH1 biosensors (based on bivalent binding). For biotin anti-LC-kappa for instance, hardly any loss of bound IgG could be observed after 10 minutes of dissociation. The k_d value as calculated for biotin anti-IgG-CH1 also serves well for generating a stable captured IgG surface.

When capturing Fab fragments, higher dissociation rates were observed for both affinity ligands, which preferably should be in the 10^{-5} s⁻¹ or lower range instead of 10^{-4} s⁻¹. However, since a human IgG Fab fragment possesses one constant domain on the light chain and one CH1 domain on the heavy chain, simultaneous binding of these domains could again facilitate bivalent binding to a biosensor surface. This would then also result in a stable Fab surface with low dissociation. As proof of concept, one Streptavidin (SA) Biosensor was loaded with a mixture of biotin anti-LC-kappa (at 5 $\mu\text{g}/\text{mL}$) and biotin-anti-IgG-CH1 (at 10 $\mu\text{g}/\text{mL}$) and compared for Fab binding with biosensors immobilized with each of the biotin conjugates separately. The resulting

data shown in Figure 5 indicate a decrease in dissociation rate for the mixed biosensor compared to (e.g., the monospecific anti-LC-kappa biosensor), demonstrating that bivalent binding can occur through binding of two separate domains on a human Fab fragment. Since both ligands bind to the opposite site of the actual antigen binding site of a Fab (Fv domain), one can expect that antigen binding characteristics of the captured Fab are not altered. A further decrease in dissociation rates may be achieved by further optimizing ligand mixtures for immobilization. Note that this principle can also be used for human Fab fragments that possess a lambda light chain by combining biotin anti-LC-lambda with biotin anti-IgG-CH1.

Effect of Free Light Chains on Measuring Human Fab Fragments in the Octet® System

When producing recombinant monoclonal antibodies and Fab fragments, one often has to deal with over-expression of free light chains. For quantitation of IgG antibodies, this problem can easily be circumvented by using affinity ligands that target the IgG Fc domain and do not show any cross-binding towards antibody light chains (e.g., biotin anti-IgG-Fc or Protein A). However, for detection and quantitation of human Fab fragments, the majority of currently available binding agents target epitopes that reside on the antibody light chain (e.g., VL-kappa by Protein L or light chain constant domains by biotin anti-LC-kappa and biotin anti-LC-lambda). Quantitation of Fab fragments then becomes problematic since cross-binding

to free light chain contaminants will also occur, resulting in unreliable outcomes. However, by using an affinity ligand that targets the CH1 domain on the heavy chain of a Fab fragment, this problem can be eliminated. To demonstrate this concept, two different biosensors (biotin anti-IgG-CH1 and Protein L) were used to determine the effect of free light chains on Fab quantitation. For this purpose a human IgG1 Fab sample (at 10 µg/mL) was spiked with levels of free kappa light chains (recombinant) ranging from 0.5 µg/mL up to 50 µg/mL. As shown in Figure 6, no significant difference in Fab binding signals was observed for the biotin anti-IgG-CH1 biosensors with addition of free light chains up to a level of 10 µg/mL. When spiking higher concentrations of free light chains (> 25 µg/mL) some non-specific binding was observed, probably caused by the stickiness of free light chains at these high concentrations (possibly due to the unpaired cysteine residues present on monomeric LC). Unlike anti-IgG-CH1, however, the Protein L Biosensors clearly indicated co-binding of free light chains, in fact at a higher binding rate than for the tested Fab fragment. It should be noted that the spiked free kappa light chains were different from the light chain present on the IgG1 Fab fragment tested. Since Protein L demonstrated variances in binding affinity for different types of light chains, this may have boosted its effect in this experimental setup. Nevertheless, co-binding of free light chains does occur and will clearly have an effect on Fab quantitation assays, when using Protein L (or biotin anti-LC-kappa and anti-LC-lambda). Therefore, introducing biotin anti-IgG-CH1 for specific detection of intact Fab fragments may circumvent the need for currently applied sandwich-based assays.

Sample Treatment Using CaptureSelect Anti-IgG-CH1 Resin

As described in the previous section, the use of affinity ligands directed against epitopes that are not present on antibody light chains can be advantageous for detection of intact antibodies (and in particular Fab fragments) in samples contaminated with free light chains. An even bigger challenge can be developing assays that enable specific detection and quantitation of free light chain contaminants. Currently available affinity ligands directed against light chains do not discriminate between free light chains and light chains present in intact antibody formats. However, as shown in Figure 7, this problem can be solved by implementing a simple sample pretreatment step using BAC's CaptureSelect anti-IgG-CH1 resin (cat. no.191.3120.10). Like biotin anti-IgG-CH1, this resin specifically captures intact human IgG and Fab fragments thereof without cross-binding to free light chains. By incubating a sample with this resin, all intact human IgG or IgG Fab fragments will be depleted, leaving all free light chains in the non-bound fraction. This non-bound fraction can then be collected for quantitation of light chains using a standard curve obtained by biotin anti-LC-kappa biosensors, for example.

In a first experiment, intact human IgG Fab fragments were incubated with the anti-IgG-CH1 resin, resulting in no detectable Fab fragments in the non-bound fraction as determined by SDS-PAGE and biotin anti-LC-kappa biosensors in the Octet® system (Figure 7). Results from the second experiment confirmed selectivity of the resin for intact Fab, leaving all free light chains in the non-bound fraction. In the last experiment a mixture of both was applied, resulting in excellent separation of free light chains (non-bound) from the intact Fab material via elution. In this setup, small spin columns were used for depleting 100 µg Fab with 100 µL resin. Since the binding capacity of the resin for Fab exceeds 5 mg/mL, higher amounts of Fab could be depleted. Furthermore, converting this assay into a 96-well plate format could facilitate sample analysis on free light chains in a high throughput mode.

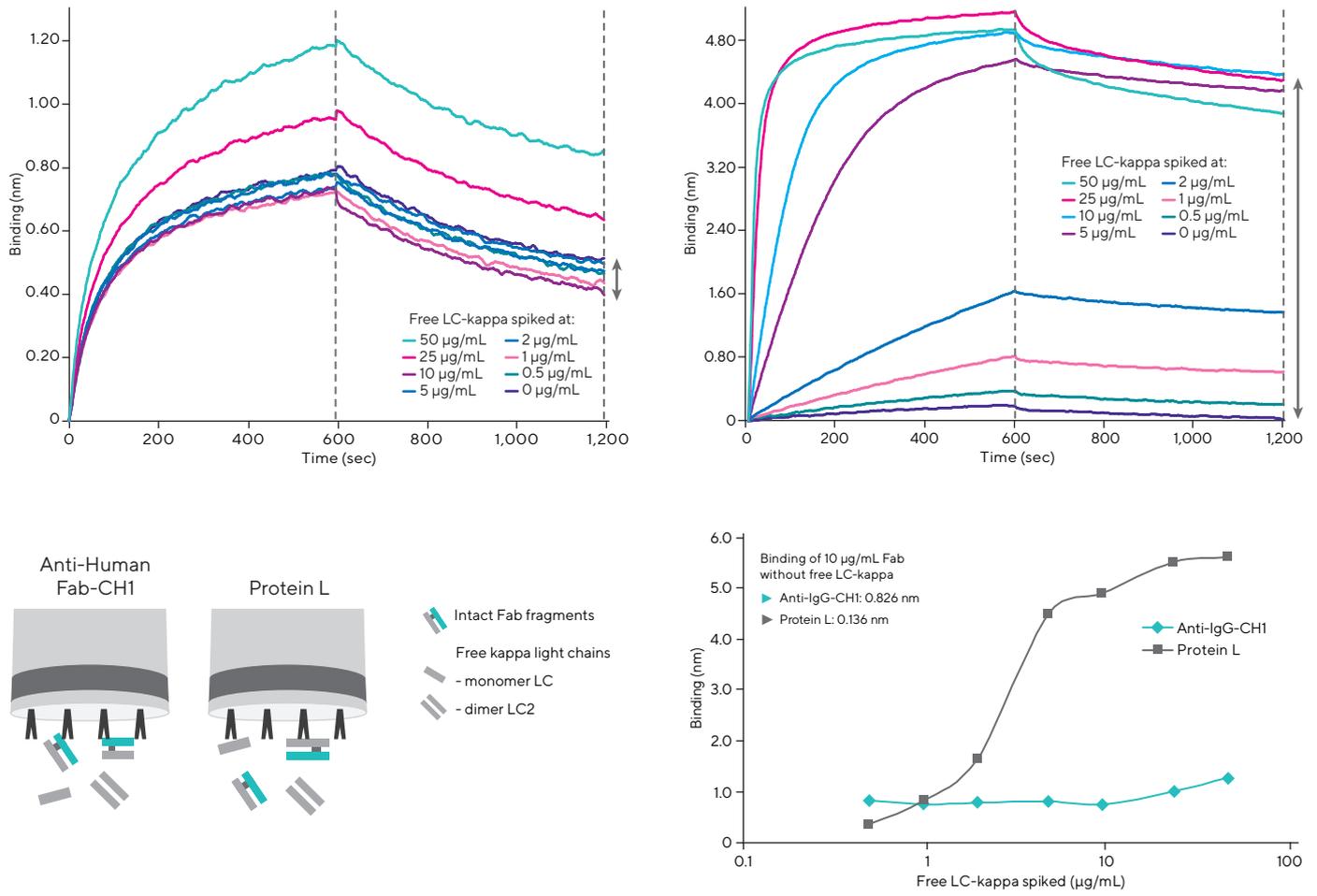
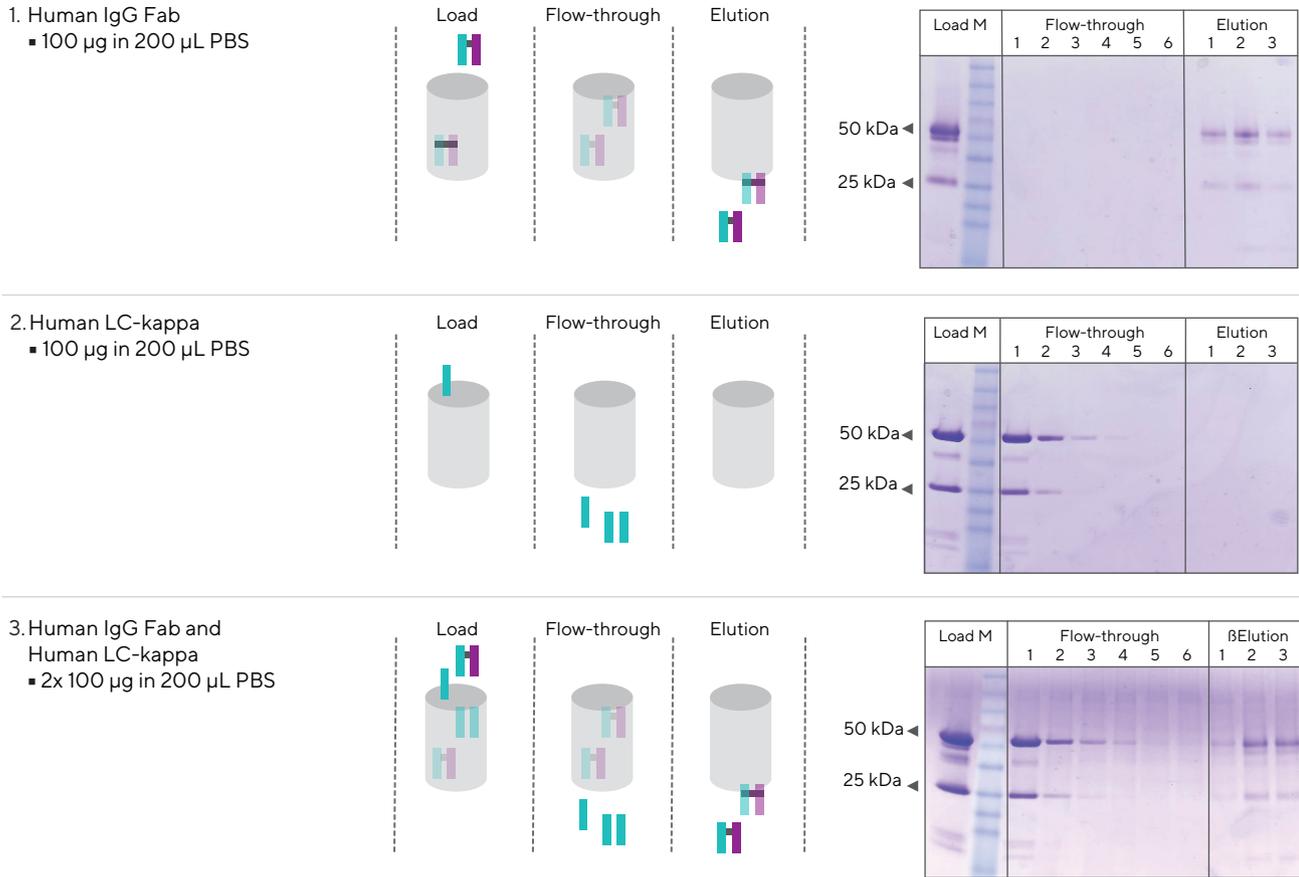
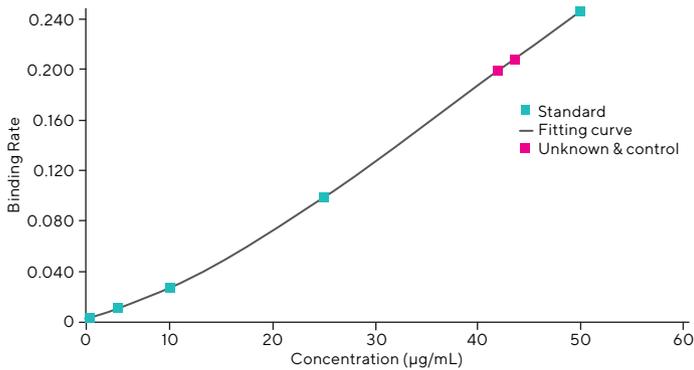


Figure 6: Effect of free light chains on quantitation of human Fab fragments using biotin anti-IgG-CHI versus Protein L. Top: A human Fab fragment derived from a recombinant human IgG1 kappa antibody was analyzed on different antibody capturing biosensors (biotin anti-IgG-CHI and Protein L) at a concentration of 10 µg/mL in PBS. In order to determine the effect of free kappa light chains on the quantitation of intact Fab fragments, different levels of a recombinant human LC-kappa domain (free light chains) ranging from 0.5 µg/mL up to 50 µg/mL were spiked into the Fab sample (10 µg/mL). Binding analysis was performed as previously described. Bottom right: Graphical representation of binding to anti-IgG-CHI and Protein L. Bottom left: Effect of free light chains on quantitation of human Fab fragments using biotin anti-IgG-CHI versus Protein L. Bottom right: The corresponding association and dissociation curves. Binding signals (after 10 minutes association) of all spiked samples are displayed in the lower figure for both biotin anti-IgG-CHI and Protein L.

Sample loaded on anti-IgG-CH1 resin:



Human LC-kappa standard curve:
 Biotin anti-LC-kappa (Hu) biosensor



Quantitation of free human LC-kappa in non-bound fractions
 after sample treatment with CaptureSelect anti-IgG-CH1 resin

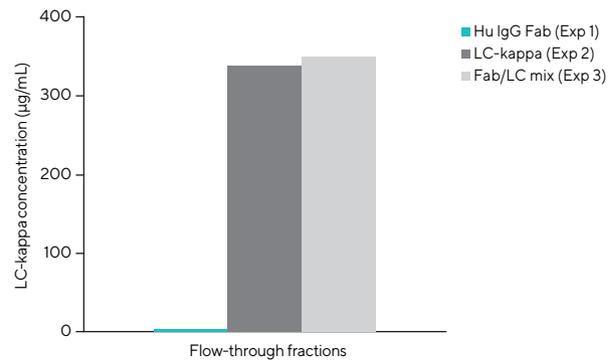


Figure 7: Separating intact human IgG Fab fragments from free human kappa light chains using CaptureSelect anti-IgG-CH1 affinity resin. Top: Samples containing polyclonal human IgG Fab fragments (Jackson Immunochemistry) and/or human kappa light chains (Bence Jones, BioDesign) were prepared in 200 µL PBS and mixed with 100 µL CaptureSelect anti-IgG-CH1 resin (spin columns, Mobitec). The spin columns were tumbled for 1 hour at room temperature. The non-bound fraction (flow-through 1) was collected by spinning for 1 minute at 150 x g followed by 5 washing steps with 400 µL PBS. Bound Fab fragments were eluted using 200 µL 0.1 M glycine pH 2 (1 minute at 1000 x g). Load, flow-through and elution fractions were analyzed by SDS page (CBB stained). Bottom: Levels of free human kappa light chains in the non-bound fractions (flow-through fractions 1) were determined in the Octet® system using an anti-LC-kappa standard curve obtained by a biotin anti-LC-kappa (Hu) biosensor.

Conclusion

CaptureSelect biotin conjugates demonstrated excellent compatibility with Streptavidin (SA) Biosensors and can serve as a valuable tool for detection, quantitation and characterization of any antibody format on Sartorius' Octet® platform. Octet® systems quantify protein concentrations and monitor protein-protein and other biomolecular interaction kinetics (k_a , k_d and K_D), enabling informed research and development decisions earlier in the process, while their high throughput enables accelerated timelines. The microfluidics-free Dip and Read format enables use of crude media and a simple and easy user interaction.

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