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APPLICATION NOTE

Rapid Kinetic and Antagonistic Profile Screening of Hybridoma Supernatants, a COVID-19 Case Study

This Application Note was developed in collaboration with Takis Biotech, Rome, Italy.

Key Takeaways

- Perform high-throughput capture kinetics and competition characterization on hybridoma samples.
- Screen 1,800 clones using only 70 µg of sample.
- Analyze broad kinetic ranges from a single concentration series of samples.
- Quickly view and select the most suitable clones with user-friendly analysis software.

Introduction

The rapid global spread of infectious agents necessitates the urgent need to identify therapeutic candidates quickly. However, the modern drug discovery process is challenged by time constraints from the analysis, characterization, selection and development of lead therapeutic candidates.

Here we present the rapid kinetic and functional screening of 1,800 hybridoma cell culture supernatant samples containing SARS-Cov-2 receptor-binding domain (RBD) specific antibodies generated from immunized mice. Following the full kinetic characterization of the antibodies, a selection of high affinity antibodies antagonizing the interaction between the RBD and Angiotensin-Converting Enzyme 2 (ACE2), which is required for viral entry[1], has been performed using the Carterra LSA platform. The whole process including data evaluation, analysis and clone selection has been performed within a work week highlighting the benefits implementing high-throughput SPR (HT-SPR) in early drug discovery. Carterra's LSA platform with proprietary Kinetics and Epitope software is enabling drug discovery and contract research organizations (CROs) to streamline the discovery process by generating deep information content data. This is resulting in making decisions earlier in the discovery process thereby facilitating stronger selection of therapeutic antibody candidates to further investigate.

This COVID-19 study has been performed in collaboration with Takis Biotech whom we thank for supplying the 1,800 hybridoma culture supernatant containing potentially COVID-19 neutralizing antibodies.

Method

Surface Preparation of the Capture Lawn (Anti Mouse-Fc mAb, Capture Reagent on the LSA)

Antibodies were captured via their FC domain using immobilized anti mouse-Fc mAb, which was coated as a lawn onto an HC200M sensor chip (Carterra, p/n 4287) using standard EDC/sulfo-NHS-mediated amine coupling method in a running buffer of HBSTE (10 mM Hepes pH7.4, 150 mM NaCl, 3 mM EDTA, 0.01% Tween-20 (Carterra p/n 3631). The LSA single flow cell was used to prepare the lawn, which involved (1) activating the chip with a 10 minutes injection of a mixture of 0.133 M EDC + 0.033 M sulfo-NHS in 0.1 M MES pH 5.5 (Carterra p/n 3626), (2) coupling 75 µg/ml goat anti mouse-Fc mAb (Jackson ImmunoResearch #115-005-071) in 10 mM sodium acetate pH 4.5 (Carterra p/n 3802) for 12 minutes and (3) blocking excess reactive esters with a three minutes injection of 1 M ethanolamine HCl pH 8.5 (Carterra p/n 3632). This yielded final coupled levels of about 5,000 RU.

Surface Preparation of the Array (Hybridoma Supernatants)

1,800 hybridoma cell culture supernatants were supplied in 96-well plates. Samples for capture were prepared in 384-deep-well plate by performing a five-fold dilution using HBSTE running buffer (final volume 220 µL). A total of five 384-well plates were prepared. Samples were flow printed for 15 minutes in batches of 96 supernatants in parallel using the 96-channel printhead, which passed a fixed volume of sample (200 µl) back-and-forth across the chip surface, thereby enriching the capture of antibodies onto discrete spots. An entire 384-array of spotted antibodies was prepared by serially docking the printhead onto each of the four nested print group locations. At the end of the antibody capture step, samples are flown back in their well of origin authorizing to re-use the same sample solutions to repeat the measurement or to perform further kinetic characterization with additional antigens.

Capture Kinetics

The target antigen RBD was supplied as a purified recombinant monomer and prepared in running buffer of HBSTE + 0.5 mg/ml BSA as an eight points three-fold dilution series with concentration starting at 1 uM and final volume of 300 µL. Analytes were delivered in the single flow cell in ascending concentration, allowing a 5 minutes association time and a 15 minutes dissociation time. Six binding cycles of buffer alone were injected before the antigens samples to stabilize the surface and provide blanks for double-referencing the data. The last RBD antigen injection (1 µM) was followed by an ACE2 protein injection at 500 nM in order to determine the antagonistic profile of each antibody. Binding data were analyzed in Carterra's Kinetics data analysis software to determine kinetic rate and affinity constants by fitting a simple Langmuir model to the double-referenced data.

Results

Chip Type

For screening purposes a high capacity chip (HC200M) was used. High surface density combined with bi-directional sample delivery across spots on a chip surface allows for the enrichment of capture levels, even from low titer antibody samples from low expressing clones.

Non-Regenerative Kinetics

The simultaneous kinetic characterization of 384 antibodies required 14 µg of RBD antigen. The measurement of the whole panel of 1,800 samples was performed using 70 µg of antigen as well as 44 µL of each culture supernatant.

A 384-mAb panel using 460 pM to 1 µM monomeric antigen as analyte, is shown as a tile view in **Figure 1**. Each panel represents the binding responses (colored by analyte concentration, with a blue/green palette) and global fit (in red) obtained for the antigen interacting with a single mAb. Ligands with low or no binding were automatically flagged by the software and are shown as grey and the ones displaying deviation from the fit model are shown in yellow.

Figure 1. Capture kinetics was performed on 384 ligands simultaneously using the LSA. The specific monomeric antigen was injected as analyte from 0.46 nM to 1 µM over the entire ligand set.

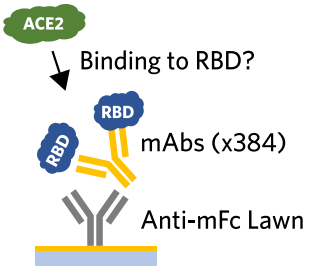
Antibody Affinity Selection Using Iso-affinity Plot

Spike proteins are on the surface of the viral particles, and the RBD domain is responsible for the initial binding interaction to the ACE2 protein on the host cell. Therefore, antibodies that bind very specifically and with high affinity to the RBD and block the ACE2/RBD interaction can be used to neutralize SARSCoV-2 (the virus behind COVID-19).

The iso-affinity plot is a powerful tool to visualize the overall kinetic measurement results of a whole panel of ligands. It depicts the relationship between the association rate constant (k_a, Y-axis) and the dissociation rate constant (k_d, X-axis) all mAb measurements. The diagonal lines represent lines of equal or "iso" affinity (K_D). The dots represent the measurement for one clone. The Kinetic software includes options allowing to list the clones in a defined region of the iso-affinity plot. A capture window is drawn by manually defining the end values of the k_a, k_d and K_D. In our case we selected clones with an affinity <380pM, displaying a k_d <1x10^{-4}s^{-1} and a k_a >5x10^{-4} M^{-1} s^{-1} **(Figure 2)**. The selected values of these parameters were defined according to the affinity of the ACE2/RBD interaction (44nM,2), by selecting clones which display an affinity more than 100-fold higher than the ACE2/RBD interaction. **(Figure 3)**.

Antibody Competition Profile

In order to assess the ability of the clones to block the binding of ACE2 to RBD, an injection of 500 nM of ACE2 following the last injection of RBD was performed **(Figure 4)**. The binding signals recorded provides insight on if the clones compete with ACE2 for the same binding region on RBD **(Figure 5A)**. For blocking clones, no additional binding signal is observed **(Figure 5B)**, showing that these antibodies block binding of ACE2 to RBD and could be potentially be used as virus neutralizing agents.



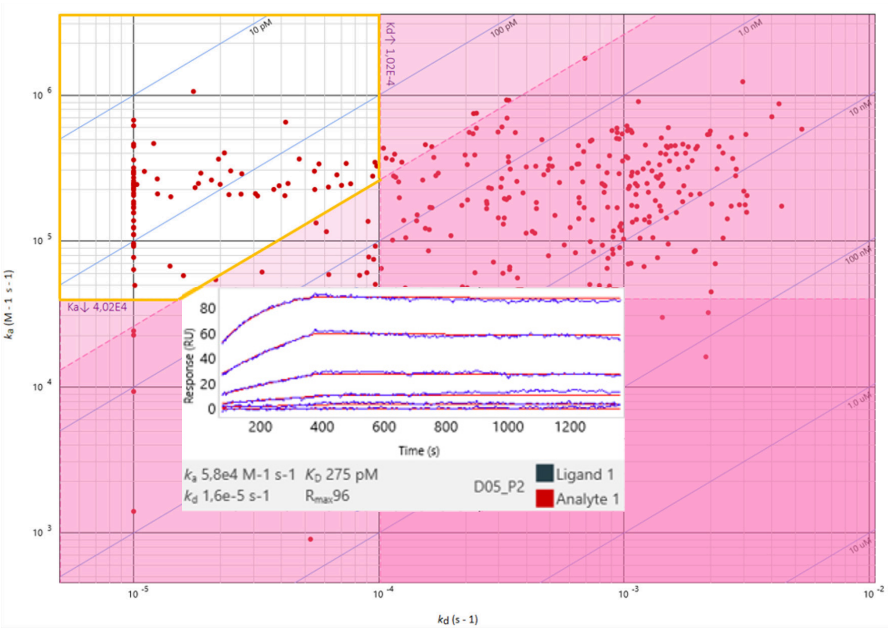


Figure 2: Iso-affinity plot. Clicking on one dot opens a popup window with the sensorgram curves for the selected clone allowing to visually assess the data quality. Note that a k_d value of 1x10^{-5} (s^{-1}) was used as a limit in the analysis, for clones showing barely any detectable dissociation within the allowed dissociation phase (of 15 min), as much longer dissociation phases would need to have been monitored to provide sufficient signal decay to accurately estimate slower dissociation rates, which is beyond the scope of a capture-based kinetic format.

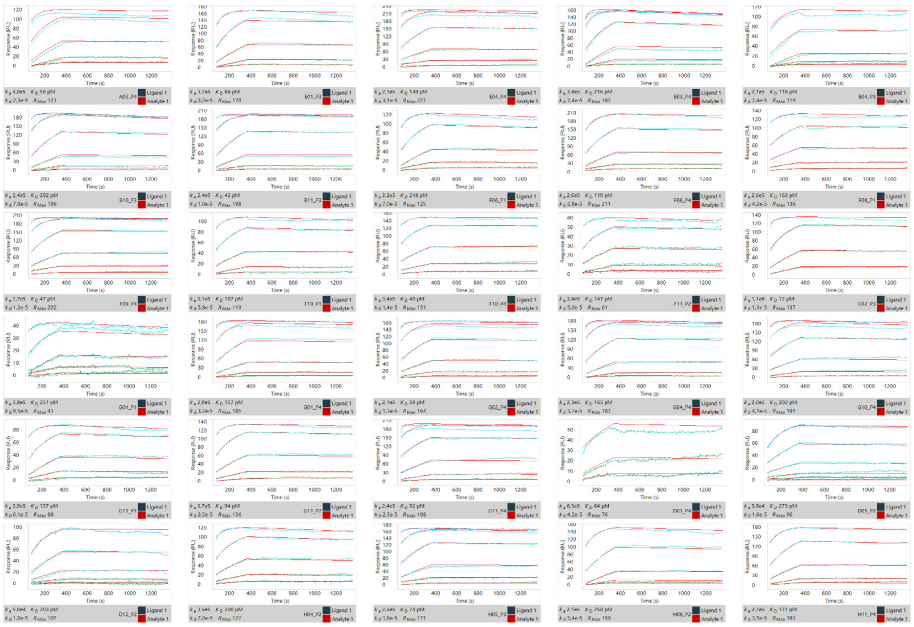


Figure 3. Sensorgrams of the selected clones from the first 384 characterized clones. These data have been extracted from the Excel results file generated by the data analysis software.

Figure 4. (Left) Assessment of the ability of antibodies to inhibit the binding of ACE2 to RBD.

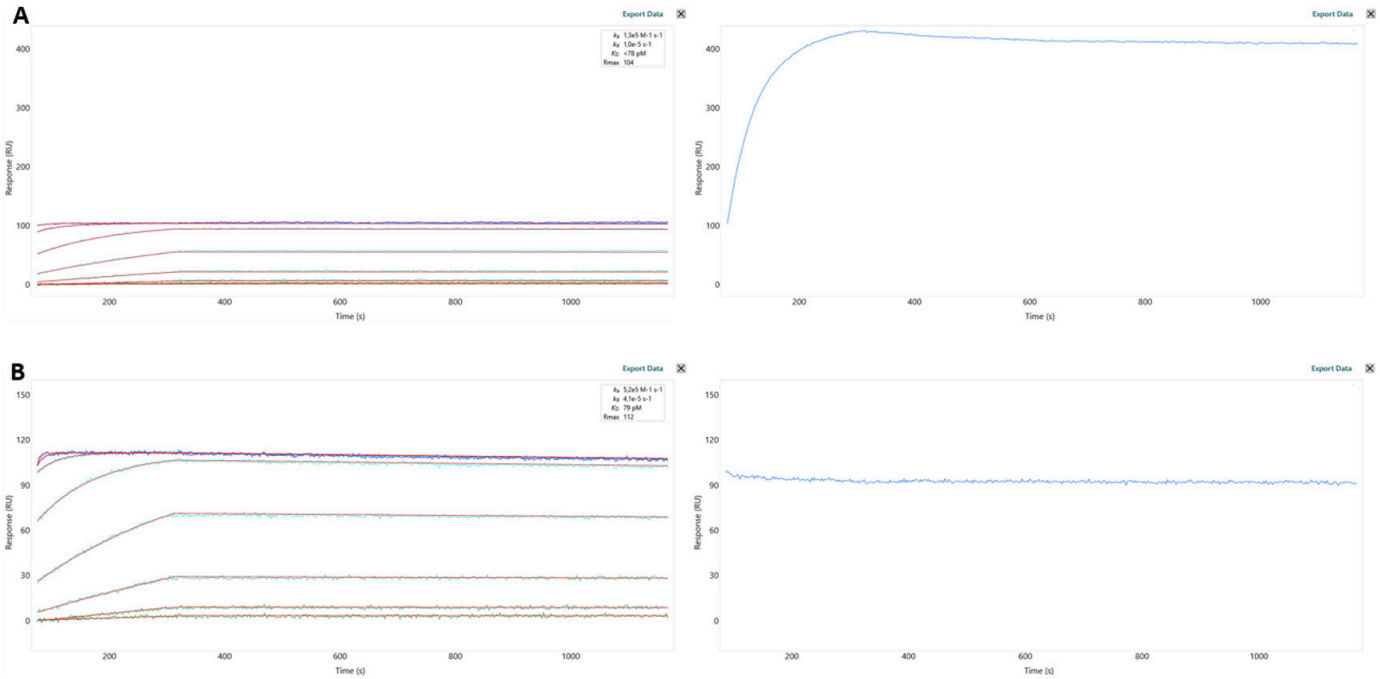


Figure 5. Signals recorded for a RBD titration (left) followed by a 500 nM ACE2 injection (right). Antibody A displays a high affinity for RBD but does not block the binding of ACE2 to RBD. Antibody B displays a high affinity for RBD and blocks the binding of ACE2 to RBD.

Summary

In the present study, we were able to characterize the kinetics of 1,800 samples in 48 hours with 70 µg of antigen. Additionally, evaluation of large data sets and performing clone selection is quick and easy using Carterra's powerful and intuitive Kinetics software. The whole screening campaign, including assay measurement and data evaluation, was completed within a week. The Carterra LSA enables the kinetic characterization of biomolecular interactions in a 384-array format and is well suited to be integrated into an early discovery workflow. Non-regenerative capture kinetic experiments performed by HT-SPR take significantly less time and consume dramatically less antigen than conventional biosensor approaches.

References

1. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. *Cell Mol Immunol*. 2020, 17, 613
2. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature* 2020, 581, 221

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