Detection and Binding Kinetics of SARS-CoV-2 Antibody with SARS-CoV-2 Spike Protein Receptor Binding Domain

Summary

- Kinetic binding of SARS-CoV-2 monoclonal antibody (mAb) to the SARS-CoV-2 spike protein receptor binding domain (RBD) was measured using the OpenSPR-XT™, with both the SARS-CoV-2 in buffer mAb, and the SARS-CoV-2 mAb in diluted serum.
- The K_D for the interaction in serum was determined to be 5.18e-10 M which is on the same order of magnitude as the value obtained for the sample in buffer, demonstrating the OpenSPR-XT™'s ability to detect low concentrations of antibody in complex samples.
- Using a secondary antibody amplification technique, a picomolar limit of detection (LOD) for the primary antibody was obtained.

Overview

The analysis of binding kinetics with SPR plays a critical role in the understanding of COVID-19. This includes basic research for understanding the viral infection mechanisms, the development of therapeutics & vaccines, and the realization of effective diagnostic tools. Although RT-PCR is the primary method of COVID-19 testing currently used, it requires dedicated labs and trained operators, and the supply chain is struggling to keep up with test demand. Direct viral detection through the use of high affinity antibodies could offer an alternative screening approach due to its speed and simplicity. Also, serological testing for COVID-19 specific antibodies can be used as a diagnostic tool for those that no longer have the virus, and could be used to indicate immunity. Presence of viral-specific antibodies in the host can be used as a diagnostic tool to aid the medical practitioners in determining the effectiveness of vaccines as well as identify high-risk targets, such as those with under-responsive immune systems that may need more intensive treatments.

In this application note, the OpenSPR-XT™ was used to detect and measure the binding kinetics of SARS-CoV-2 monoclonal antibody to the SARS-CoV-2 spike protein RBD. A direct binding assay was performed to measure the kinetics of this interaction, in a normal buffer background as well as by spiking the antibody into 50% serum. The LOD for the primary antibody was also optimized through secondary antibody amplification. The binding signal obtained indicates that the presence of the SARS-CoV-2 antibody could be detected down to picomolar concentrations. The OpenSPR-XT™ is an affordable and user-friendly benchtop SPR instrument that enables the measurement of label-free binding kinetics of diverse biomolecular interactions.

Materials and Equipment

- OpenSPR-XT instrument
- OpenSPR Carboxyl sensor
- Amine Coupling Kit
- Tracedrawer Kinetic Analysis Software
- Ligand: SARS-CoV-2 Spike Protein Receptor Binding Domain (RBD): SinoBiological, CAT#: 40150-V08B2
- Primary Antibody: Rabbit Anti Spike Protein Monoclonal Antibody (mAb): SinoBiological, CAT#: 40150-R007
- Secondary Antibody: Donkey Anti-Rabbit Monoclonal Antibody (mAb): Sigma, CAT#: SAB3700861-2MG
- Normal Rabbit Serum: Jackson Immunoresearch, CAT#: 011-000-10
- Running Buffers: pH 7.4 PBS-T, PBS-T + 1% Bovine Serum Albumin (BSA)

Procedure

1. Following the start-up procedure in the software, the OpenSPR-XT™ instrument was set up, using PBS-T as the initial running buffer.
2. Sensor surface was prepared following the wizard steps in the OpenSPR software.

3. The ligand was immobilized on the EDC/NHS activated surface at a concentration of 10 µg/ml and a flow rate of 20 µL/min on channel 2 only (designated as the response channel).
4. BSA was immobilized on both channels as a blocker at a flow rate of 20 µL/min.
5. Remaining COOH groups were blocked with the OpenSPR blocking solution.
6. The instrument was primed in PBS-T + 1% BSA as the analysis running buffer.
7. The primary antibody was prepared in the analysis running buffer at a concentration of 150 nM, and further diluted in 3-fold serial dilutions.
8. The rabbit serum was diluted 2-fold in the analysis running buffer and used to prepare a 150 nM sample of the primary antibody, which was further diluted in analysis running buffer in 3-fold serial dilutions.
9. The secondary antibody samples were prepared in the analysis running buffer at a concentration of 150 nM.
10. The primary antibody was injected over the ligand at 50 µL/min (2 min association, 5 minute dissociation) in order of increasing concentration.
11. The secondary antibody was injected over the primary antibody at 50 µL/min (2 min association, 5 min dissociation) at a constant concentration of 150 nM.
12. The ligand was regenerated with an injection of pH 1.5 glycine-HCl at 150 µL/min before each subsequent primary antibody injection.
13. Steps 10-12 were repeated for the primary antibody in 50% (1:1 with analysis buffer) serum samples, with a dissociation of 10 minutes for the primary and secondary antibody injections.

Results and Discussion

Figure 1 shows the immobilization of the SARS-CoV-2 spike protein RBD on the OpenSPR-XT carboxyl sensor, with approximately 2500 RU of immobilization.

The binding and kinetic fits of the SARS-CoV-2 antibody and the same antibody in diluted serum to the immobilized receptor domain are presented in *Figure 2* and *Figure 3*, respectively. In both cases, the data was fit to a one-to-one binding model in Tracedrawer. The calculated kinetic constants are presented in *Table 1*, and show similar values for the antibody in diluted serum, and the antibody samples in buffer.

The specificity of the binding was confirmed using Human IgG as a negative control, which showed no binding to the ligand. This demonstrates the high affinity and specificity of this antibody, and OpenSPR-XT's ability to detect binding and measure kinetics for antibodies in serological samples.

For applications in diagnostics, the ability to measure antibody concentrations similar to those found in serologial samples is a necessity. Secondary antibody amplification was therefore used to demonstrate a method to further improve the LOD for the primary antibody. This is a technique where multiple secondary antibodies bind to the Fc region of a single primary antibody. This increased avidity leads to a secondary antibody binding signal larger than that of the primary, and in turn, to an improved LOD of the primary antibody. Figure 4 displays an example of a primary antibody plus secondary antibody amplification cycle. The secondary antibody increases the responses by 285% for this concentration.

The secondary antibody response was plotted against the primary antibody concentration for each curve and fitted using a logarithmic model. This fit determined that by using secondary antibody amplification, concentrations of the primary antibody could be detected into the picomolar range, yielding a tenfold improvement in LOD for the primary antibody compared to the direct assay.

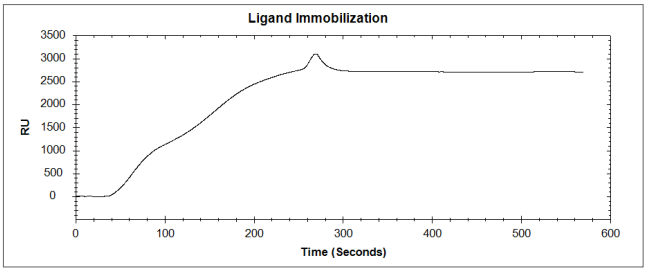

Figure 1: Ligand immobilization of the SARS-CoV-2 spike protein RBD on an OpenSPR carboxyl sensor

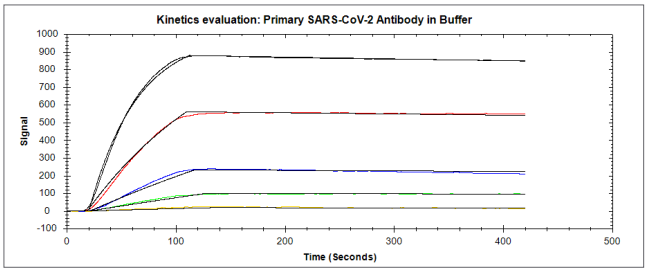

Figure 2: Binding of the primary antibody in analysis running buffer to the immobilized ligand at concentrations of 150 nM, 50 nM, 16.7 nM, 5.56 nM and 1.85 nM. The solid black lines represent the one-to-one kinetic model fits.

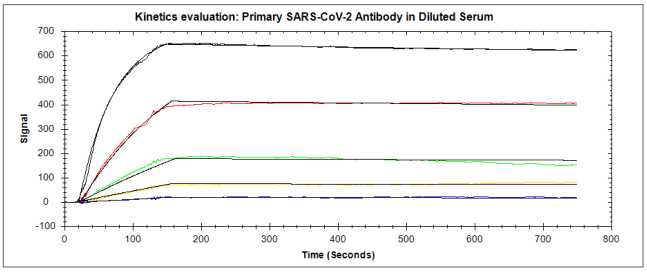

Figure 3: Binding of the primary antibody in 50% serum to the immobilized ligand at concentrations of 150 nM, 50 nM, 16.7 nM, 5.56 nM and 1.85 nM. The solid black lines represent the one-to-one kinetic model fits.

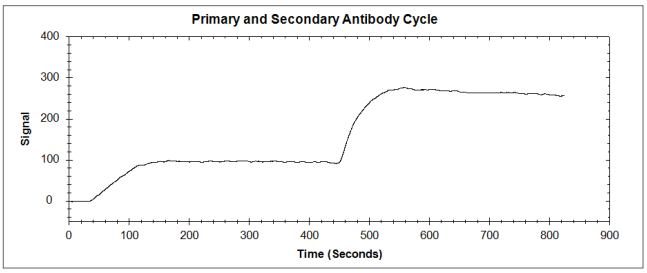

Figure 4: 5.56 nM primary antibody with secondary antibody amplification. The secondary antibody binding to the primary yields a 285% increase in total signal.

Table 1: Kinetic values measured using OpenSPR™ data with the TraceDrawer™ analysis software

Parameter	SARS-CoV-2 mAb in buffer	SARS-CoV-2 mAb in Serum
k_a [1/M*s]	1.51e5 (±2.17e0)	1.30e5 (±3.94e0)
k_d [1/s]	1.20e-4 (±1.60e-5)	6.75e-5 (±2.00e-5)
K_D [M]	7.93e-10 (±1.06e-10)	5.18e-10 (±1.53e-10)

Conclusions

This study demonstrates that the OpenSPR-XT™ can be used to measure binding kinetics of SARS-CoV-2 mAb to its cognate viral target in serological samples, with similar results to those obtained using an antibody sample in running buffer. It was also proven that the presence of the SARS-CoV-2 mAb can be directly detected in low nano-molar concentrations, and that this LOD can be improved tenfold, down to picomolar concentrations via secondary antibody amplification. This supports the use of the OpenSPR-XT™ for applications in diagnostics and vaccine development, specifically in the context of COVID-19.