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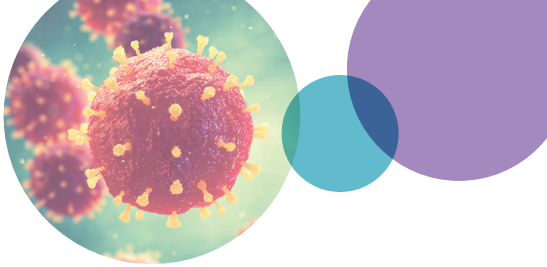

Octet Bio-Layer Interferometry systems: Advancing development of coronavirus vaccine and therapeutics

Introduction

The World Health Organization declared the new COVID-19 outbreak as a pandemic on the 11th of March 2020. Millions of people have been infected worldwide with the virus, and hundreds of thousands of deaths reported so far. The rapidly evolving epidemiology of the pandemic and absence of a licensed therapeutic or vaccine for the disease has accelerated the need to develop an intervention. At the heart of the search for a vaccine are modern biochemical and analytical technologies that can be deployed for the rapid screening of the many potential candidates to determine those with the best chances of success. Since the COVID-19 genome sequence was first shared in January 2020, Octet® data has been featured in several breakthrough publications related to coronavirus biology, vaccine, and antiviral therapeutic development. Octet BLI platforms are helping accelerate SARS CoV-2 research by providing scientists:

- High-throughput kinetic interaction analysis solutions to select and characterize antigenic targets, vaccine and therapeutic candidates fast
- Fast and flexible platform for antibody epitope characterization and coverage determination
- Efficient workflows with a wide range of biosensors tailored for vaccine and biotherapeutic development research
- Versatile solutions in upstream and downstream vaccine development and manufacturing such as vaccine potency, stability, and titer measurements
- Intuitive, easy-to-use data analysis software tools

Octet instruments utilize Bio-Layer Interferometry (BLI) technology that measures light interference as a result of binding on a sensing surface (biosensors) to monitor biomolecular interactions. The detection is reported in real-time as binding responses. The instrument operates in a microfluidics-free environment where samples are housed in 96- or 384-well plates. Assay workflow involves a simple Dip and Read™ measure, where the disposable biosensor is dipped into samples presented in microwell plates, and interactions are detected in real-time. This workflow is advantageous over microfluidics based technologies in that it enables specific capture and analysis of target molecules in complex matrices such as cell culture supernatants, serum, plasma, and other media, circumventing the need to purify antibodies and other recombinant proteins for screening without the risk of sample clogging. These features enable users to easily vary assay and biosensor conditions and reuse precious samples that result in fast and low-cost assay optimization. Additionally, users can choose from a range of tailored affinity capture and immobilization chemistries suitable for vaccine and biotherapeutic development research. Widely used, ready-to-use biosensor chemistries are listed in Table 1.

Biosensor	Description	Application
AHC	Anti-Human Fc-Capture	Capturing human IgGs or human Fc-fusion proteins from purified or unpurified media for kinetic or epitope binning analysis with various analytes.
AMC	Anti-Mouse Fc-Capture	Capturing mouse IgGs or mouse Fc-fusion proteins from purified or unpurified media for kinetic or epitope binning analysis with various analytes.
SA	Streptavidin	Immobilizing biotinylated molecules for all kinetic or epitope binning analysis.
HIS1K	Anti-Penta-HIS	Capture of His-tagged proteins from purified or unpurified media for kinetic analysis with target analytes. Quantitation of His-tagged proteins in buffer, media or diluted lysate. Biosensor is pre-coated with Penta-His antibody.
FAB2G	Anti-Human Fab-CH1 2nd Generation	Kinetic analysis of human Fab fragments and IgG with target antigen, Fc receptors, or other analytes. Quantitation of Fab and IgG.
NTA	Ni-NTA	Quantitation of HIS-tagged proteins in buffer or diluted matrix, capturing of HIS-tagged proteins for kinetic analyses with various analytes.
Protein A	Fc-Capture	Capturing IgGs and Fc fusion proteins from various species including human.

Table 1: Widely used biosensor chemistries for antibody-based kinetic analysis, receptor binding and epitope binning assays. See full selection of biosensor chemistries.

Octet instruments have been widely deployed in various vaccine development research programs (HIV[1,2], influenza[3], Ebola[4], novel coronavirus[5-15]) and in evaluating vaccine agents such as nanoparticles[16], virus-like particles (VLPs)[17] and bispecific antibodies[18]. Octet systems also provide solutions beyond lead candidate selection to bioprocess development and manufacturing. This review summarizes the use of Octet assays in some recent advances in coronavirus research and vaccine development efforts.

Determination of coronavirus receptor binding mechanisms and cross-reactivity

The 2019-nCoV or SARS CoV-2 shares genetic and morphologic features with other coronavirus families, particularly from the betacoronavirus genus (SARS-CoV 2003). SARS CoV-2 makes use of a glycosylated, homotrimeric class I fusion spike (S) protein to gain entry into host cells via the human angiotensin converting enzyme-2 (ACE2) receptor[19]. The SARS CoV-2 S glycoprotein bears significant structural homology with SARS-CoV compared to other major coronaviruses such as the MERS-CoV. The S-protein is known to exist in a metastable pre-fusion conformation that undergoes rearrangements to fuse the viral membrane with the host cell membrane. Binding of the S1 subunit to host-cell receptors triggers destabilization of the pre-fusion trimer and conformational change in the protein initiates fusion with the host cell membrane through the S2 subunit. In order to engage the host-cell receptor, the receptor-binding domain (RBD) of S1 would need to undergo hinge-like transitions that either hides or displays the receptor-binding regions which are termed as *down* and *up* conformations respectively, providing accessibility to receptor-binding and presenting itself as a key antigenic target for vaccine development.

One of the first CryoEM structures of the SARS CoV-2 S trimer in the pre-fusion conformation was revealed by Wrapp *et al.* where they showed that while the overall structure of SARS CoV-2 S protein was mostly similar to SARS-CoV S, differences existed between the down conformations at the RBD[12].

Another cryo-EM structure of SARS CoV-2 spike glycoprotein complex resolved by Walls *et al.* showed that that the SARS-CoV-2 S glycoprotein harbors a furin cleavage site at the boundary between the S1/S2 subunits, which is processed during biogenesis and differentiates this virus apart from SARS-CoV and other SARS-related CoVs[10]. Using Octet binding assays, they showed that the receptor-binding domains of SARS-CoV-2 S and SARS-CoV S engaged the human ACE2 with similar binding affinities. Similar observations were reported by Joyce *et al.* using a high-resolution crystal structure of the SARS-CoV-2 S RBD[5]. Additionally, in this work antibodies that were known to interact with SARS-CoV and MERS CoV receptor binding domains (RBD) were tested for SARS-CoV-2 binding activity using an Octet kinetic assay. Only two antibodies, 240CD and CR3022 out of the pool, displayed low nanomolar binding affinities against the SARS-CoV 2 RBD. These two antibodies, however, competed for the same SARS-CoV-2 RBD binding site according to an Octet cross-competition assay. Cross-competition or epitope binning assays can be used to segment monoclonal antibodies (mAbs) into bins based upon the antigen region or epitope bound by each antibody (Figure 1). Furthermore, an Octet competition assay revealed that 240CD or CR3022 antibody binding to SARS CoV-2 RBD did not perturb ACE2 recognition. Crystallography data revealed that CR3022 engaged the RBD at a site that is conserved in both SARS CoV and SARS CoV-2 explaining the cross-reactivity. However, a comparison of SARS-CoV and MERS-CoV epitope regions from previous structural data to SARS CoV-2 identified in this work revealed that CR3022 was binding to a novel epitope within SARS CoV-2 S. A specific RBD knockout mutant intro-

Epitope binning formats and assay guidance on Octet instrument

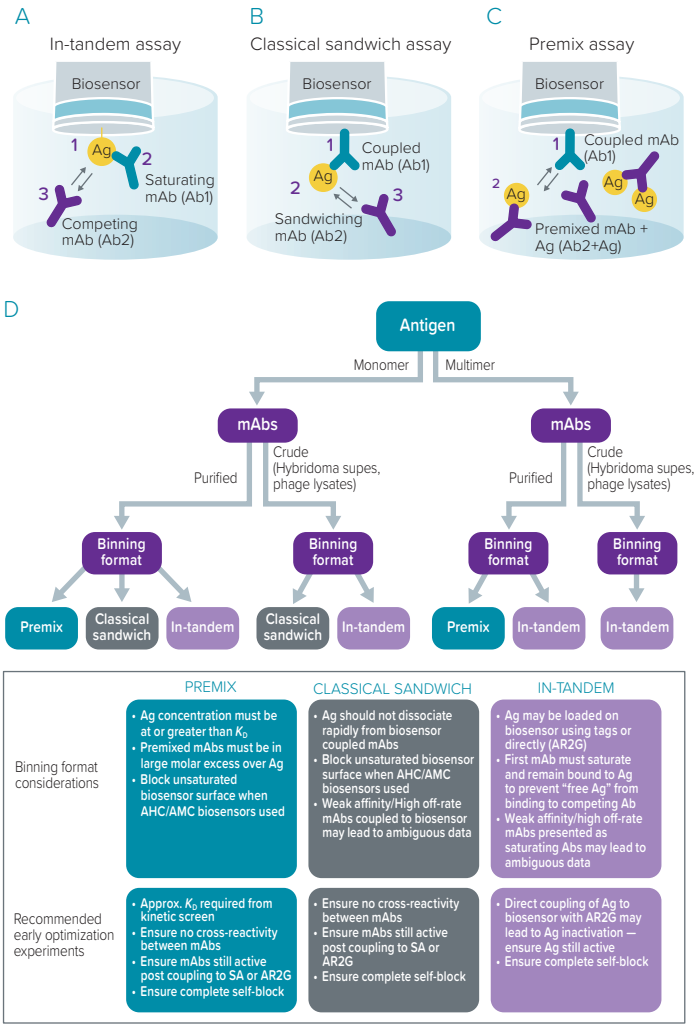

Figure 1: Assay formats of epitope binning assays and recommendation guide to select the most suitable epitope binning assay format. Octet systems enable multiple assay formats for cross-competition assessment and provide dedicated high-throughput software analysis tools for epitope binning. (A) In Tandem Assay: Antigen is immobilized on the biosensor, followed by the binding of the saturating mAb (Ab1) and competing mAb (Ab2), respectively. (B) Classical Sandwich Assay: One mAb is immobilized on the biosensor (Ab1), antigen is captured using this mAb and then a second sandwiching mAb is tested (Ab2).(C) Premix Assay: One mAb is immobilized on the biosensor (Ab1), the biosensor is then exposed to a premix solution containing the antigen and a large molar excess of the second mAb (Ab2). (D) Guidance for selecting the most suitable epitope binning assay format. Please read the Octet assay guide to learn more about developing and analyzing cross-competition screens.

duced within this epitope region (glycan sequon at position 384) eliminated binding to both CR3022 and 240CD antibodies according to Octet data confirming shared epitopes between both antibodies.

To understand the SARS CoV-2 spike conformations with respect to receptor binding and cell entry, structures of CR3022 binding to trimeric units of SARS-CoV-2, SARS-CoV, and MERS-CoV were modeled [5]. Data indicated that CR3022 epitopes were obstructed by adjacent spike promoters when the RBD is in the *down* conformation but were more accessible in the up confirmation. Octet assays observed CR3022 binding to non-stabilized S-glycoprotein (S1) conformations and much weaker binding to S2 P trimers. To assess whether minimal proteolytic action or receptor binding could increase the availability of the obscure CR3022 epitope, S2 trimers were treated with trypsin or incubated with ACE2. Incubation of S2 P trimer with human ACE2 did not dramatically affect CR3022 binding. However, trypsin treatment of the S2 P conformation resulted in increased binding levels similar to non-stabilized S glycoprotein binding, and the level of binding was titratable with increasing proteolytic action, inferring to the cryptic nature of the CR3022 binding epitope. Similar observations were reported by Yuan *et al.*, using the crystal structure of neutralizing antibody CR3022 bound to SARS-CoV-2 RBD[14]. Octet binding data showed that despite high sequence conservation between SARS-CoV and SARS CoV 2 RBDs, CR3022 FABs binds SARS-CoV with ~100-fold higher affinity than to SARS CoV-2 RBD possibly driven by the non-conserved residues between the two epitopes mentioned above. An *in vitro* microneutralization assay indicated higher neutralization activity with SARS-CoV, but not SARS-CoV-2 for CR3022, a result that is consistent with lower RBD binding affinities. Overall, these structural studies provide insight into how SARS CoV-2 can be targeted by the humoral immune response and revealed a conserved, but cryptic epitope shared between SARS CoV-2 and SARS CoV that can potentially be utilized to create cross-protective nAbs.

Neutralization antibody development

Neutralizing antibodies (nAbs) are likely to be one of the most effective treatments against coronavirus infections among the current therapeutic options and are in urgent need. Several nAbs targeting SARS-CoV RBD, have been shown to exhibit significant *in vivo* antiviral activity by reducing virus titers in animal models. However, the availability of nAbs with cross-reactivity could be key for sustained utilization as therapeutics that can stand against virus mutations that are typical of single standard RNA viruses.

Due to the ~80% sequence similarity between the two Spike (S) proteins of SARS CoV and SARS CoV-2, understanding whether antibodies raised from SARS-CoV spike (S) protein immunization would retain cross-reactivity to the new SARS-CoV-2 will offer important insights and guidance to therapeutic antibody and prophylactic vaccine development.

Tian *et al.*, in a pioneering study, expressed and purified the CoV-2 RBD and tested the binding of several SARS-CoV specific neutralizing antibodies that were known to target the RBD and exert potent neutralization activities against SARS-CoV [9]. SARS-CoV nAbs, m396, CR3014, CR3022 and the MERS-CoV nAb m336 were tested for SARS-CoV-2 RBD recognition (Figure 2). While most antibodies did not show appreciable binding to the SARS CoV-2 RBD, CR3022 that was isolated from a SARS CoV patient bound with a 6.3 nM affinity (k_{on} of 1.84×10^5 Ms^{-1} and k_{off} of 1.16×10^{-3} s^{-1}).

Octet BLI competition assays showed that CR3022 did not compete for the ACE2 binding site in SARS-CoV-2 RBD, indicating binding to an epitope different from the ACE2 binding site (Figure 2B). Similarly, Wrapp *et al.* investigated another set of previously published SARS-CoV RBD binding antibodies, S230, m396, and 80R with SARS CoV 2 S protein[12]. Octet binding assays indicated a lack of significant binding activity possibly due to the recognition through different epitopes compared to SARS CoV. Sun *et al.* reported that several pAbs and mAbs generated by immunizing mouse and rabbits with SARS-CoV S1 or RBD proteins did not show favorable cross-neutralization activities in SARS-CoV-2 pseudoviruses (PSV). However, these antibodies exhibited potent neutralization potencies against SARS CoV virus and high-affinity binding to SARS CoV RBD (K_D ~9–200 pM)[8]. These results indicate that antibodies that exhibit SARS-CoV neutralization potencies or RBD binding is not necessarily a prerequisite for activity in SARS CoV-2 despite high sequence homologies at the RBD and suggests the necessity to develop novel antibodies that specifically target SARS CoV-2 RBDs.

Wang *et al.* reported the discovery of a human monoclonal antibody with neutralization potencies for SARS-CoV-2 and SARS-CoV virus activity [11]. 47D11, derived from a screen that consisted of a collection of supernatants that contained SARS S hybrodoma derived from immunized transgenic mice, showed cross-reactivity and neutralization activity against both SARS CoV and SARS CoV-2 pseudotyped VSV infections. Octet studies showed that 47D11 binds the SARS CoV S_{ecto} domain with higher affinity (K_D = 0.745 nM) relative to SARS CoV-2 S_{ecto} domain (K_D = 10.8 nM) although similar binding affinities were reported for binding to the SARS CoV and SARS CoV-2 RBD binding domains, further validating the differences in epitope accessibility between the two virus receptor binding motif (RBM) conformations. Additionally, as in other cases reported thus far, 47D11 did not compete with the SARS CoV or SARS CoV-2 RBD, implying neutralization through a mode that is different from receptor blocking. This cross-neutralizing antibody reportedly targets a communal epitope and offers potential for prevention and treatment of SARS CoV-2.

Pinto *et al.* screened for nAbs from a SARS CoV survivor[7]. Eight mAbs from the screen bound to both SARS CoV and SARS CoV-2 S protein-transfected CHO cells, out of which mAbs S303, S304, S309, and S315 recognized both SARS CoV and SARS CoV-2 RBDs. Octet data indicated S309 binding to both S domains with similar nanomolar affinities and also showed

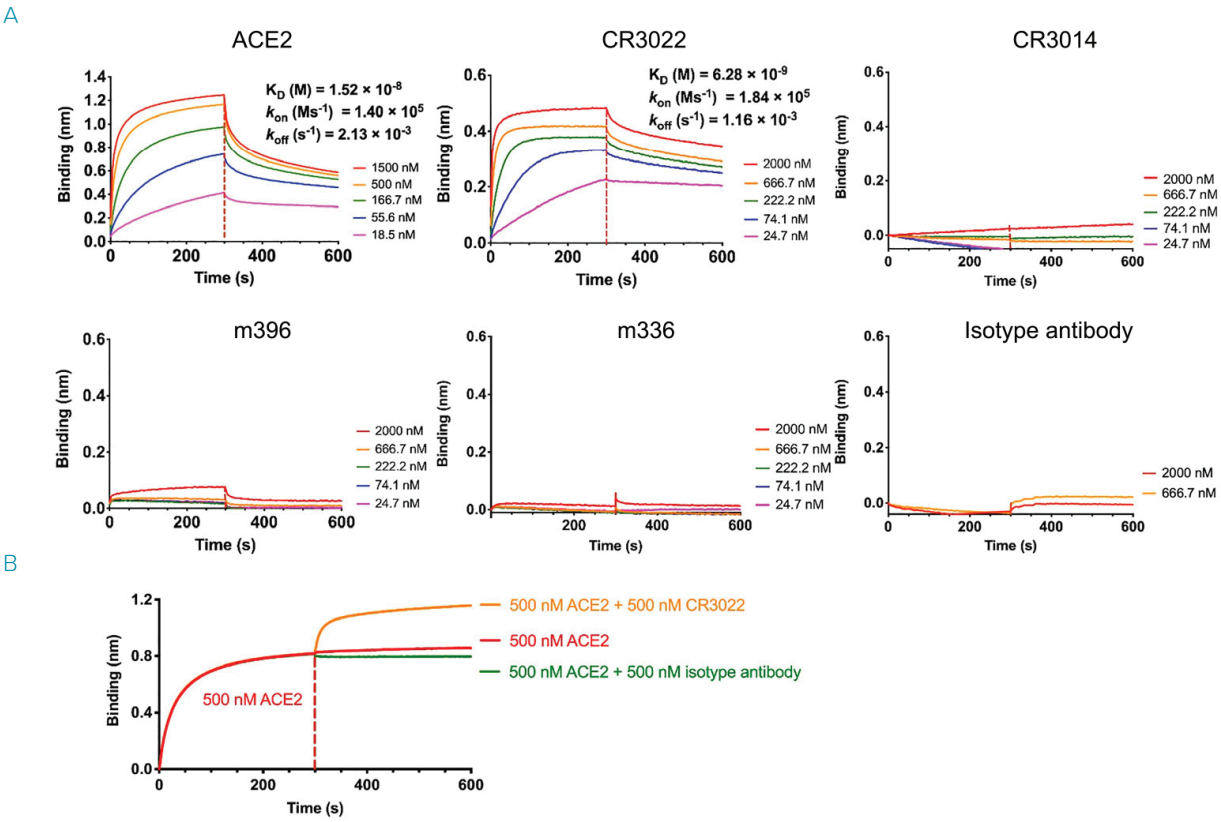

Figure 2: Characterizing of SARS CoV antibody interactions to SARS CoV-2 RBD. (A) Binding profiles of SARS CoV-2 RBD to ACE2 and antibodies and (B) competition of CR3022 and ACE2 with SARS CoV-2 RBD measured by Octet. For the competition analysis, ACE2 were immobilized onto HIS1K biosensors, followed by binding to either ACE2, CR3022 + ACE2 or isotype antibody control of ACE2. Figure is reproduced from reference 9.

comparable neutralization potencies against both SARS CoV and CoV-2 pseudoviruses and authentic SARS-CoV-2. According to structural data, S309 recognizes a protein/glycan epitope on the SARS CoV-2 RBD distinct from the receptor-binding motif (RBM) and seems to be accessible to both up and down states. Octet BLI competition assays showed that binding of S309 Fab and IgG forms to SARS CoV-2 RBD did not discourage ACE2 receptor binding to SARS CoV-2 S protein. To gain more insight on the epitopes recognized by the panel of mAbs, an Octet epitope binning assay was carried out to map the antigenic sites present on the SARS CoV and, SARS-CoV-2 RBDs. Octet epitope binning analysis identified four antigenic regions within the RBD of SARS CoV where the mAbs were targeting. Based on these findings, mAb combinations that targeted different antigenic regions were tested for neutralization potencies and possible synergistic effects where data indicated that either S304 or S315 in combination with S309 enhanced neutralization potencies against SARS CoV-2. S309 identified in this work showed broad neutralization activity against multiple sarbecoviruses showing promise as an effective therapeutic candidate.

Pan *et al.* used the SARS CoV-2 RBD domain to produce neutralization antibodies in horse antisera[6]. F(ab')$_2$s isolated from horse antisera reportedly showed neutralization activity against SARS CoV-2 with an EC_{80} of ~25 µg/mL. Octet BLI kinetic analysis also confirmed SARS CoV-2 receptor binding to F(ab')$_2$ with an affinity of 76 nM. Affinity purification of F(ab')$_2$ from antisera improved neutralization activity against SARS CoV-2 (EC_{80} = 0.18 µg/mL) and increased binding affinity (0.76 nM) measured by Octet BLI, highlighting RBD-specific F(ab')$_2$ as a potential therapeutic candidates for SARS CoV-2.

Wu *et al.* reported the generation and testing of single-domain antibodies targeting SARS CoV-2 RBD[13]. The panning using SARS-CoV-2 RBD and S1 as antigens resulted in the identification of antibodies targeting five types of neutralizing or non-neutralizing epitopes on SARS-CoV-2 RBD. They tested 18 human single-domain antibodies in competition binding assays using the Octet system and found that they could be divided into three competition groups (groups A, B, or C) that did not show any competition with each other. The single domain antibody n3130 identified from the library using SARS-CoV-2 S1 as the panning antigen showed potent neutralization activity with both pseudotyped and live virus and strong binding to SARS CoV-2 S1 and RBD domains. This work also reports the unique immunogenic profiles of SARS-CoV-2 RBD compared to that of SARS-CoV and MERS-CoV, which may have important implications for the development of effective vaccines against SARS CoV-2.

Design and validation of a peptide inhibitor to block viral entry

In addition to protective antibodies, peptide and small molecule inhibitors can also be used as viable inhibitory strategies to target the SARS CoV-2 RBD to prevent virus entry. Zhang *et al.* investigated the design and development of a 23-mer peptide inhibitor (SBP1) discovered based on the ACE2 PD α1 helix that spans the binding interface according to the crystallographic structure of the SARS-CoV-2 S receptor–ACE2 protein complex[15]. Octet BLI was utilized to the test target specificity of the peptide binding to SARS CoV-2 RBD. SPB1 bound the RBD with an affinity of 47 nM (k_{on} = 4.69 × 10^4 M^{-1} s^{-1} and k_{off} = 2.2 × 10^{-3} s^{-1}) where this binding affinity is comparable to that of the full-length ACE2 binding to SARS CoV-2 RBD. SBP1 could, therefore, potentially cover spike proteins on the SARS-CoV-2 surface and outcompete the binding for ACE2 as a novel therapeutic intervention strategy.

Summary

Affinity characterization, neutralization, and competition studies are essential in the selection of high affinity and potentially neutralizing lead candidates for therapeutic and vaccine development against the novel SARS CoV-2. Kinetic analysis further describes the components of association and dissociation that comprise the overall affinity, while cross-competition assays allow for the identification of more optimal candidates. In addition to the studies illustrated above, researchers have also utilized Octet assays to characterize and investigate other members of the coronavirus or associated species where the observations and results are invaluable towards implementing and framing current therapeutic strategies for SARS CoV-2. Table 2 provides a summary of selected studies illustrating the use of Octet assays on current and historical coronavirus research[20-27]. In all these studies, the Octet system's ease-of-use combined with the availability of a variety of ready-to-use biosensor chemistries has enabled users to significantly reduced time to results and advance the continued search for vaccines and therapeutics for the current pandemic.

Analyte(s)	Immobilized ligand(s)	Virus species	Assay focus	Biosensor	Reference
ACE2	Fc-tagged SARS CoV-2 RBD	SARS CoV-2	Cross-reactivity and receptor binding	AHC	12
ACE2	SARS-CoV-2 S_B SARS-CoV S_B	SARS CoV-2	Receptor binding	HIS1K	10
mAb, MERS S protein, VHH	SARS CoV-2 S1, SARS CoV-2 RBD	SARS CoV-2	VHH and mAb binding assessment, Epitope binning	AR2G	13
Fab, IgG	SARS CoV-2 S protein, SARS CoV-2 RBS	SARS CoV-2	FAB, IgG binding characterization	HIS1K	14
ACE2, SARS-CoV RBD	SARS-CoV-specific neutralizing antibodies	SARS CoV-2	Cross-reactivity binding	SA	9
Antibodies	Biotin-Recombinant SARS-CoV S1 protein	SARS CoV-2	Neutralization antibody assessment	SA	8
FAB, ACE2, SARS S protein	mAb, ACE2	SARS CoV-2	Receptor binding, antibody reactivity, competition assays	AHC, HIS1K	5
SARS CoV-2 RBD	Inhibitor Peptide	SARS CoV-2	Peptide inhibitor development	SA	15
mAb	S1B and SARS S_{ecto} domains	SARS CoV and SARS CoV-2	Antibody reactivity assessment	HIS1K	11
SARS CoV-2 RBD, SARS CoV RBD, mAbs, FAB	mAbs, SARS CoV-2 RBD, SARS CoV RBD	SARS CoV and SARS CoV-2	Antibody binding characterization, epitope binning, competition binding	Protein A, HIS2, HIS1K	7
Coronavirus S proteins	Biotin-9OAc6SLN	SARS CoV and MERS CoV	Receptor binding	SA	20
MERS-5HB fusion inhibitor	Biotin-peptide (MERS-HR2P)	MERS CoV	Protein Inhibitor development	SA	21
mAb, MERS S protein	mAb, MERS S protein	MERS CoV	Epitope binning and Receptor binding	SAX, Protein A	22
MERS-CoV S	mAbs	MERS CoV	Antibody reactivity measurements, competition assays	AHC, HIS1K	23
MERS-CoV NTD, mutants	mAb	MERS CoV	Antibody reactivity measurements	AHC	24
MERS S-protein	mAbs	MERS CoV	Antibody reactivity measurements	AHC	25
RBD/S1/S2 antigen	Vaccine-induced mouse monoclonal IgGs	MERS CoV	Antibody reactivity and epitope binning	AHC, HIS1K	26
FABs	MERS-CoV RBD	MERS CoV	Epitope binning	HIS1K	27

Table 2: Examples of Octet BLI assays in coronavirus research and vaccine development.

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