BioReliance®

Pharma & Biopharma
Manufacturing &
Testing Services

Technical Note

Surface Plasmon Resonance-based assays for monitoring SARS-CoV-2 surface glycoprotein protein binding interactions

The envelope glycoprotein or "spike" from the novel coronavirus (SARS-CoV-2) mediates the virus binding to Angiotensin-coverting enzyme 2 (ACE2) for cellular entry and consequently is a prime target for therapeutic development, including antibody-based therapeutics. Monoclonal or polyclonal antibody (mAb or pAb) targeting the receptor binding domain (RBD) of the spike protein may inhibit the interaction with ACE2 and hence neutralize SARS-CoV-2 infectivity by blocking cell entry. Using Surface Plasmon Resonance (SPR), the binding kinetics between the spike protein and neutralizing antibody candidates can be aggessed and thus predict whether these candidates can inhibit the spike-ACE2 interaction.

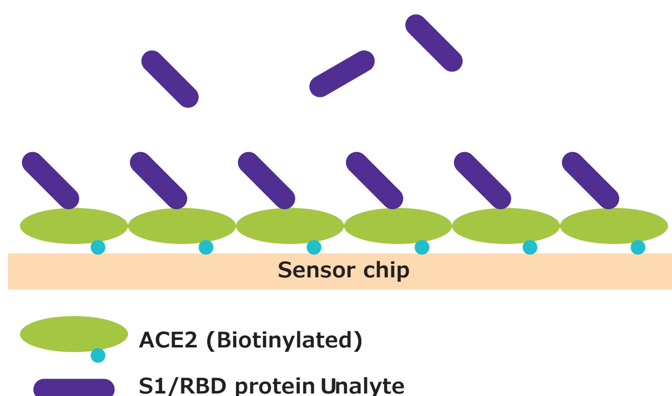


Figure 1: Illustration of ACE2 – S1/RBD binding assay

In this technical note, we investigate the interactions between the following;

- The spike glycoprotein binding with ACE2
- Spike glycoprotein and anti-spike antibodies (mAb/pAb)
- The spike glycoprotein binding with mAb/pAb to inhibit the interaction with ACE2

Spike glycoprotein-ACE2 binding assay

The ACE2 – S1/RBD binding study was performed using commercial recombinant biotinylated ACE2 via a capture approach, where ACE2 was captured on the surface as ligand and either spike S1 domain or receptor binding domain (RBD) recombinant protein was used as the analyte.

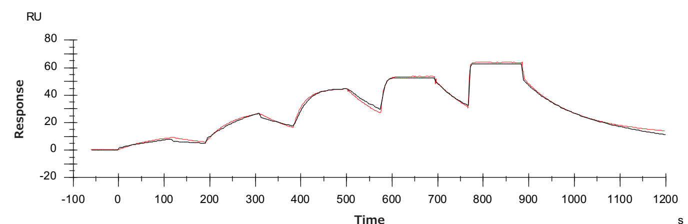


Figure 2: Representative sensorgram of ACE2 – S1/RBD binding assay (single cycle kinetics). Analyte concentration range 4 – 1000 nM. Generated data was evaluated using a 1:1 binding model.

Table 1 Representative kinetic data for ACE2 – S1 and ACE2-RBD binding

	k_{on} (1/Ms), ×10⁵	k_{off} (1/s), ×10³	K_D (nM)
S1	1.83	2.17	11.9
RBD	6.13	6.19	10. 1

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

MilliporeSigma

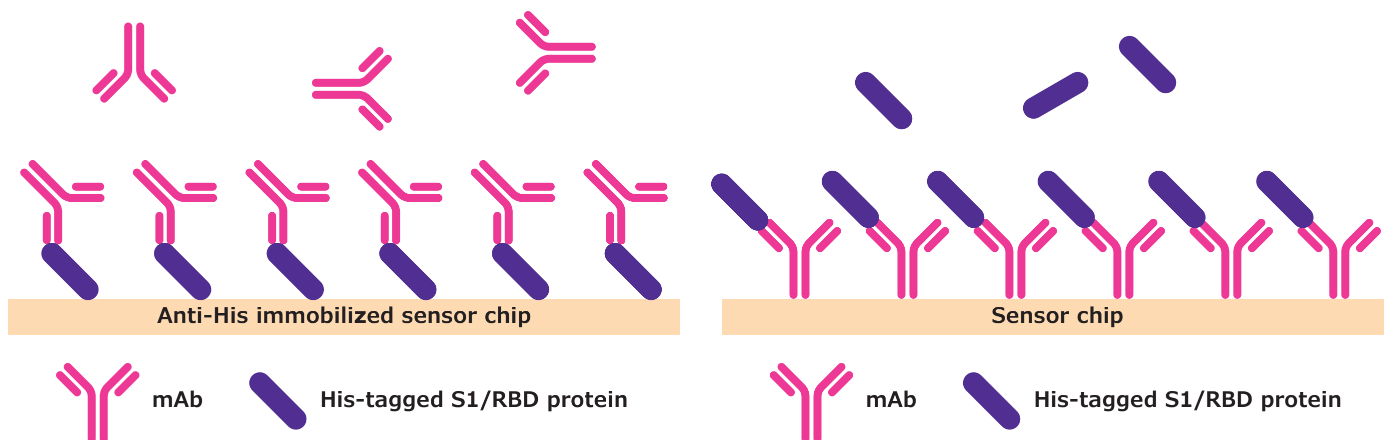


Figure 3: Illustration of mAb – S1/RBD binding assay formats, where the binding interaction was investigated using two assay formats. Spike glycoproteins were captured on the sensor surface via His-tag as ligand, with mAb as analyte (left) or mAb was captured as ligand and spike glycoprotein as analyte (right).

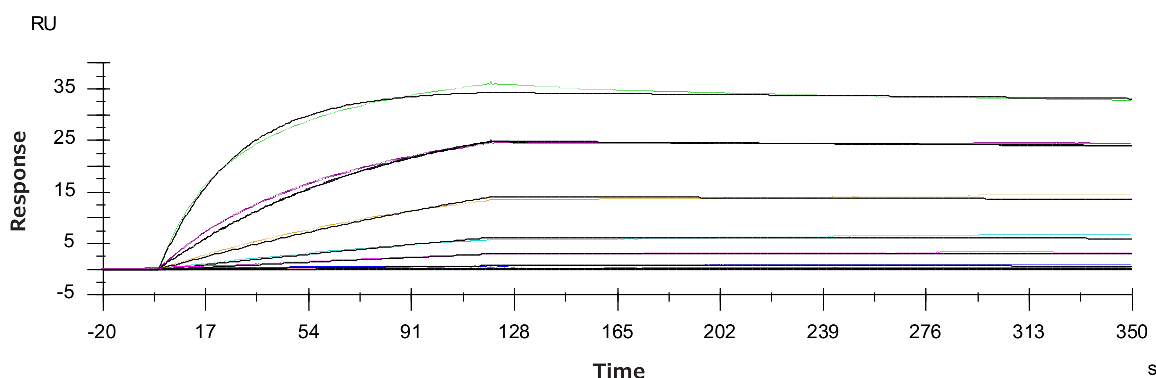


Figure 4: Representative sensorgrams showing the mAb – Spike S1 domain binding interaction. Analyte concentration range 0.1 – 250 nM. Generated data was evaluated using a 1:1 binding model.

mAb-Spike S1/RBD binding assays

mAb-spike interactions were investigated using two different assay formats as illustrated in Figure 3. Recombinant spike S1 domain or receptor binding domain (RBD) recombinant proteins were used as both ligand and analyte and binding to a commercial anti-spike monoclonal antibody was assessed.

Table 2 Kinetic data for mAb – S1/RBD binding

	k_{on} (1/Ms), ×10^5	k_{off} (1/s), ×10^3	K_D (nM)
S1	1.42x10^5	1.96x10^{-4}	1.4
RBD	2.15x10^5	6.67x10^{-4}	3.1
RBD*	4.89x10^5	1.03x10^{-3}	2.1

*RBD as analyte

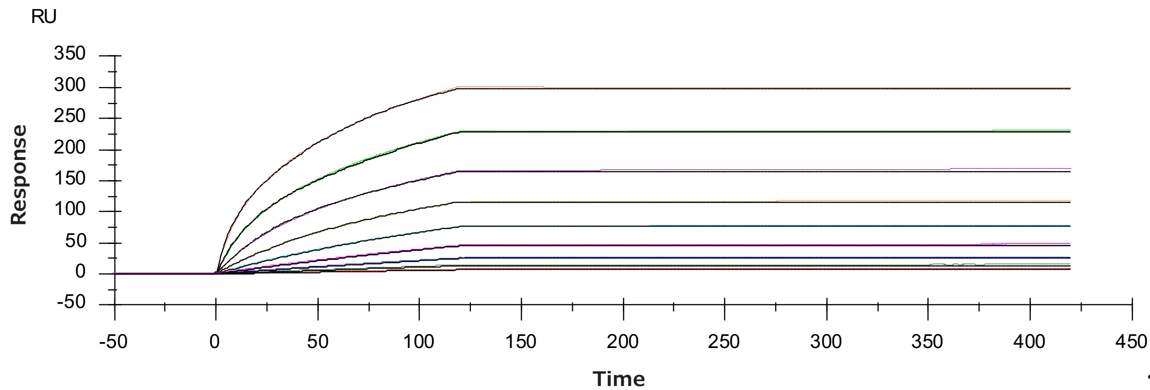


Figure 5: Representative sensorgrams for pAb – RBD interaction.

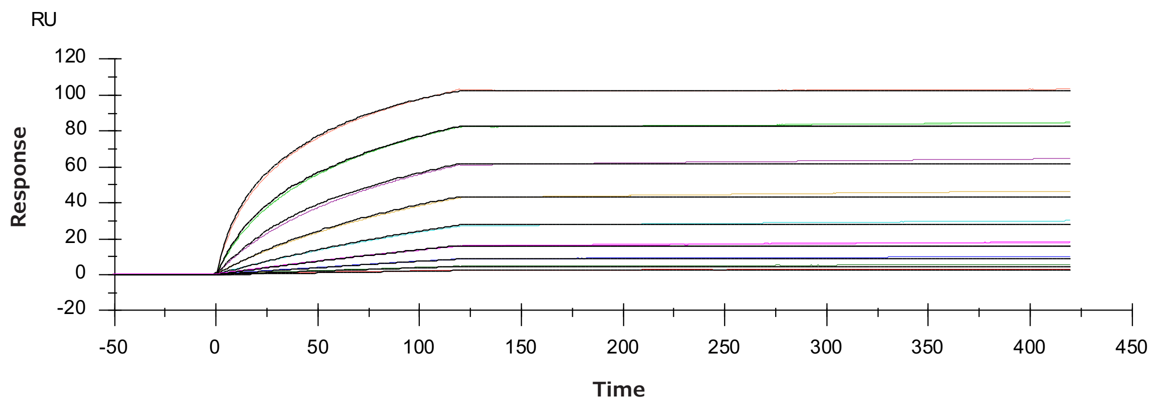


Figure 6: Representative sensorgrams for pAb – S1 interaction.

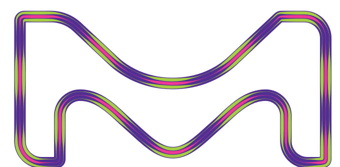
pAb-Spike S1/RBD binding assays

A His-capture approach was used to investigate the pAb (Rabbit anti-SARS CoV-2 Spike) and spike glycoprotein binding. 1 – 250 nM of pAb was analyzed as analyte injected over anti-His captured spike S1 or RBD on the sensor surface. Data generated was evaluated using all pre-defined kinetic models available; 1:1, bivalent analyte, heterogenous analyte, and heterogenous ligand models was performed, however none provided a satisfactory fitting. Poor fitting using the 1:1 binding model was expected since heterogenous pAb will involve a multivalent binding response. Hence, the exact k_{on} and k_{off} are challenging to obtain due to the complexity of multi-binding site utilized by pAb. However, using the 1:1 binding model, the commercial anti-spike pAb we investigated exhibited nanomolar binding affinity to both full length S1 and RBD glycoproteins.

Summary

Binding activity data generated using these assays will allow assessment of the affinities of mAb and pAb therapies toward spike protein. This is essential for characterization, for comparative analysis of anti-spike IgG product candidates, and for assessing their ability to inhibit the critical interaction between spike and host cell receptor ACE2 needed for viral entry *in vivo*.

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany



For additional information, please visit
EMDMillipore.com/your-pc-partner

© 2020 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. MilliporeSigma, the vibrant M and BioReliance are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

MS_TN6952EN Ver. 1.0
33059
09/2020