SARS-CoV-2 Spike RBD Nanobody (CSB-RA33245A2GMY) LSPR Protocol

(Antibody Capture)

1. Sample Information

Sample	SARS-CoV-2 Spike protein RBD His/Sumostar Tag	The SARS-CoV-2 Spike RBD Nanobody
Manufactor	Cusabio	Cusabio
Cat.No.	CSB-YP3324GMY1	CSB-RA33245A2GMY
Molecular Weight (kDa)	70	90
Buffer	20 mM Tris-HCl, 0.5 M NaCl, 6% Trehalose, pH 8.0	NaCl 137mmol/L, KCl 2.7mmol/L, Na2HPO4 10mmol/L, KH2PO4 2mmol/L, 6% Trehalose, pH 8.0
Tag	His/Sumostar Tag	Human IgG1 Fc tag
Purity	87.6%	90%
Concentration(mg/ml)	0.5	2.09
Isoelectric point	6.03	8.26

2. Instrument Parameters

Name	OpenSPR	COOH chip	Analysis method	Analysis software
Manufactor	Nicoya	Nicoya	----------	----------
Cat.No.	----------	SEN-AU-100-10-COOH	----------	----------
Lot.No.	----------	SCB1127	----------	----------
Else	----------	----------	One To One	Trace Drawer

3. Buffer Information

Name	Details	Manufactor
Activator Buffer	EDC (400mM) and NHS (100mM) (1:1)	Sigma
Immobilization Buffer	10mM Sodium Acetate, pH3.5	Sinopharm Chemical Reagent Co., Ltd
Blocking Buffer	Ethanolamine	Sigma
Regeneration Solution	0.05% SDS	BIOSHARP
PBS buffer	----------	Hyclone
80% IPA solution	IPA	Sinopharm Chemical Reagent Co., Ltd
HEPES buffer	10 mM HEPES (HBS-EP buffer)	Beyotime Biotechnology
NaCl	150 mM NaCl (HBS-EP buffer)	Sinopharm Chemical Reagent Co., Ltd
EDTA	3 mM EDTA (HBS-EP buffer)	Sinopharm Chemical Reagent Co., Ltd
Tween-20	0.005% Tween-20 (HBS-EP buffer)	Sigma

4. Assay Protocol

※Chip Preparation

Install COOH chip (Nicoya, SEN-AU-100-10-COOH, SCB1127) according to OpenSPRTM instrument standard operating procedures. Inject HBS-EP buffer pH7.4 at maximum flow rate of 150 μL/min to reach the signal baseline, and then load 200μL 80% IPA to empty bubbles for 10 seconds at flow rate of 150 μL/min. Finally, wash the sample loop with HBS-EP

buffer for 1 min and empty it with air. After reaching the signal baseline, the flow rate of the HBS-EP buffer can be adjusted to 20 μL/min. 200 μL EDC/NHS buffer is injected to activate the COOH chip for 4min at 20 μL/min.

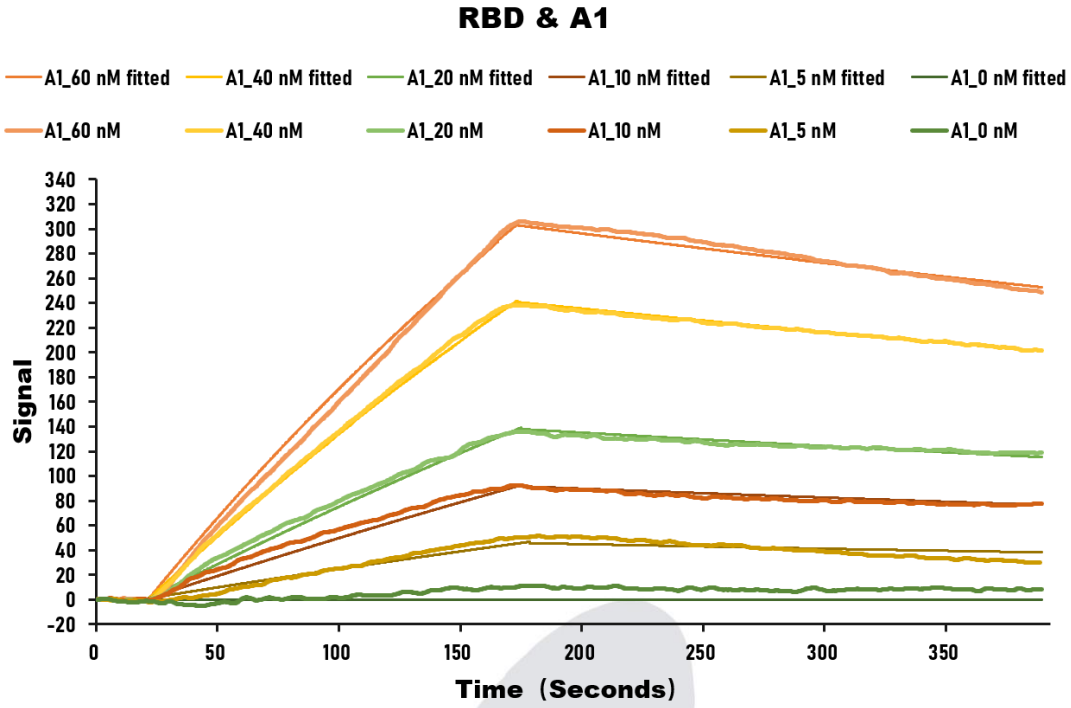
※Ligand Capturing

Dilute the ligand protein SARS-CoV-2 Spike protein RBD His/Sumostar Tag (CSB-YP3324GMY1) by Immobilization Buffer to 0.5mg/ml, and inject 200 μL of the solution to the sample channel at flow rate of 20 μL/min for 4min. 200 μL blocking Buffer, which is not coupled with ligand protein, is loaded to block the –COOH for 4min. The sample loop is then flushed with HBS-EP buffer pH 7.4 and emptied with air while observing whether the signal baseline is stable.

※Running Analyte

Make sure that the signal baseline is stable and dilute the SARS-CoV-2 Spike RBD Nanobody (CSB-RA33245A2GMY) with HBS-EP buffer to 6 concentrations (60, 40, 20, 10, 5 nM, 0 nM). The SARS-CoV-2 Spike RBD Nanobody (CSB-RA33245A2GMY) is injected in ascending order to the sample channel at the flow rate of 20 μL/min at 25℃ for an association phase of 240s, followed by 180s dissociation. The association and dissociation procession are both performed in HBS-EP buffer. In order to remove the ligand and any bound analyte, the –COOH chip surface should be regenerated completely by 0.25% SDS at the flow rate of 150 μL /min after interaction with analyte.

Binding SARS-CoV-2 Spike protein RBD His/Sumostar Tag with A1 Antibody in LSPR Assay



SARS-CoV-2 Spike protein RBD His/Sumostar Tag (CSB-YP3324GMY1) captured on COOH chip can bind SARS-CoV-2 Spike RBD Nanobody (CSB-RA33245A2GMY) with an affinity constant of 28.2nM as detected by LSPR Assay.

Kinetic Affinity

Capture Ligand	SARS-CoV-2 Spike protein RBD His/Sumostar Tag (CSB-YP3324GMY1)
Ligand Capture concentration	0.5mg/ml
Capture Ligand Level (RU)	900
Analyte	SARS-CoV-2 Spike RBD Nanobody (CSB-RA33245A2GMY)
Analyte concentration	0~60nM
Ka (1/(M*S))	2.41×10^{4}
Kd (1/S)	6.08×10^{-4}
KD (M)	2.82×10^{-8}