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Antibody characterizations by BioLayer Interferometry (BLI)

In 1 collection

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DOI

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COLLECTIONS

Bivalent binding of a fully human IgG to the SARS-CoV-2 spike proteins reveals mechanisms of potent neutralization

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PARENT PROTOCOLS

Part of collection

Bivalent binding of a fully human IgG to the SARS-CoV-2 spike proteins reveals mechanisms of potent neutralization

MATERIALS TEXT

- OctetRED96 System with Data Acquisition Software version 9.0.0.4
- Anti-Human IgG Fc (AHC) sensors (ForteBio Cat no. 18-5060)
- Amine Reactive 2nd Generation (AR2G) sensors (ForteBio Cat no. 18-5092)
- Black Microplates, 96-well F-bottom (Greiner Cat no. 655209)
- Human Fc (Calbiochem Cat no. 401104-5MG)
- Kinetics buffer: phosphate-buffered saline (PBS) buffer supplemented with 0.1% Tween-20 and 0.1% Bovine serum albumin (BSA)
- 1M ethanolamine pH 8.5 (ForteBio Cat no. 18-1071)
- 400 mM 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride (EDC) (20X) (ThermoFisher Cat no. 22980)
- 200 mM N-hydroxysuccinimide (NHS) (20X) (ThermoFisher Cat no. 24500)

- 10 mM sodium acetate pH 6.0 (ForteBio)
- 10 mM sodium acetate pH 4.0 (ForteBio)
- 10 mM Glycine pH 2.3-2.7
- Purified IgG
- Purified Tag-less RBD
- Purified Fc-RBD and Fc-RBD mutants
- Purified Fab clones
- Purified ACE2-Fc

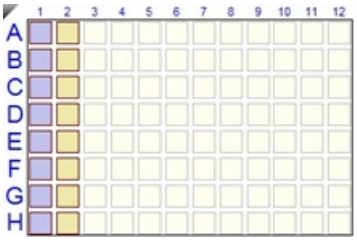
SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Avidity binding by BioLayer Interferometry (BLI)

1 Avidity Binding by BioLayer Interefometry

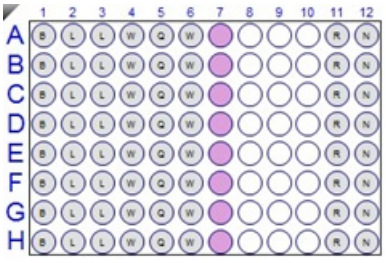
1.1 Hydrate each biosensor with 200 µl of kinetics buffer in the sensor tray for at least 15-20 min prior to every run. For each avidity measurement, run a reference sensor in parallel.



Sensor tray layout

1.2 Set up the sample plate with each well containing 200 µl of reagent as described below:

- **Column 1:** Buffer – Kinetics buffer
- **Column 2:** Loading – 1 µg/ml of Fc-RBD or Fc-RBD mutant diluted in kinetics buffer
- **Column 3:** Loading – Kinetics buffer (for ref sensor loading)
- **Column 4:** Wash – Kinetics buffer
- **Column 5:** Quench – 0.25 mg/ml of Human Fc
- **Column 6:** Wash/Dissociation – Kinetics buffer
- **Column 7:** Association Sample – Purified IgG clone in two-fold dilutions starting from 25 nM, diluted in kinetics buffer. Ensure that the last well in row H contains 0 nM (ie. Kinetics buffer only) to serve as the reference well.
- **Column 11:** Regeneration – 10 mM Glycine pH 2.3
- **Column 12:** Neutralization – PBST 0.1%



Sample tray layout

Citation: Rabiatul Adawiyah, Patricia Ng, Bei Wang, Cheng-I Wang (08/01/2020). Antibody characterizations by BioLayer Interferometry (BLI). https://dx.doi.org/10.17504/protocols.io.bi39kgr6

1.3 The assay protocol is defined in the data acquisition software as below. Replicate steps as “new assay” for the reference sensor, taking note to change the loading sample plate column. Plate temperature and shaking speed is set to 25°C and 1000 rpm respectively.

		Steps	Sample plate column	Time
IgG assay run	1.	Baseline	1	60s
	2.	Loading with Fc-RBD or mutants	2	600s (10 min)
	3.	Blocking with Human Fc	5	600s (10 min)
	4.	Wash to remove excess	4	600s (10 min)
	5.	Wash/Baseline	6	120s (2 min)
	6.	Association with IgG	7	360s (6 min)
	7.	Dissociation	6	600s (10 min)
	8.	Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)
	9.	Baseline	1	30s
Ref sensor run	1.	Baseline	1	60s
	2.	No Loading	3	600s (10 min)
	3.	Blocking with Human Fc	5	600s (10 min)
	4.	Wash to remove excess	4	600s (10 min)
	5.	Wash/Baseline	6	120s (2 min)
	6.	Association with IgG	7	360s (6 min)
	7.	Dissociation	6	600s (10 min)
	8.	Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)
	9.	Baseline	1	30s

Assay definition

1.4 Run the assay according to the protocol set. Each avidity measurement (including reference) should take about 1 hr 40 min.

1.5 Once completed, process the data according to the following parameters in the analysis software (v. 9.0.0.4):

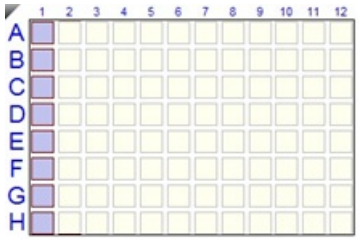
o **Step 1:** Data Selection – Sensor selection. Select Sample plate row H as reference well and Sensor tray column 2 as reference sensor.
o **Step 2:** Subtraction – Select “Double reference” to subtract both reference well and reference sensor.
o **Step 3:** Align Y-axis to “Baseline” and set time range as the last 10 secs of the Baseline step before association.
o **Step 4:** Inter-step correction to remove any bulk shifts or artefacts for a better line fit.
o **Step 5:** Process data with *Savitzky-Golay Filter.*
oAfter processing, calculate K_D based on the 1:2 bivalent model fitting.

1.6 Repeat measurement for each IgG clone and RBD mutant. Regenerated sensors can be used for another 1 or 2 more times, depending on whether the signal reached baseline levels after regeneration and whether loading signal with 1 µg/ml Fc-RBD remains consistent with previous runs. Otherwise, use fresh sensors.

Citation: Rabiatul Adawiyah, Patricia Ng, Bei Wang, Cheng-I Wang (08/01/2020). Antibody characterizations by BioLayer Interferometry (BLI). https://dx.doi.org/10.17504/protocols.io.bi39kgr6

2 ACE2 competition assay by BioLayer Interferometry (BLI)

2.1 To immobilize ACE2-Fc onto AR2G sensors, first hydrate each biosensor with 200 µl of water in the sensor tray for at least 15-20 min prior to the run.

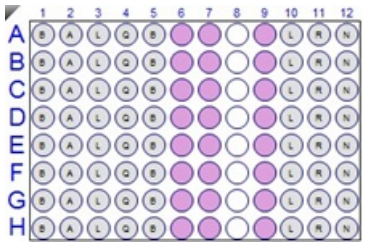


Sensor tray layout

2.2 Prepare EDC:NHS activation buffer (20 mM EDC + 10 mM NHS in water). Note that, after mixing, the activation buffer will only be viable for about 1 hour. It is best to prepare them fresh right before starting the run.

2.3 Set up the sample plate with each well containing 200 µl of reagent as described below:

- **Column 1:** Buffer – Water
- **Column 2:** Activation – EDC:NHS buffer
- **Column 3:** Loading – 10 µg/ml ACE2-Fc in 10mM sodium acetate pH 4.0
- **Column 4:** Quench – 1M Ethanolamine pH 8.5
- **Column 5:** Buffer – Kinetics buffer
- **Column 6:** Association 1 – 5 µg/ml Tag-less RBD, prepared in kinetics buffer
- **Column 7:** Association 2 – Panel of IgG clones, 10 µg/ml each, prepared in kinetics buffer
- **Column 9:** Association 1 – Kinetics buffer (This serves as a control to ensure there is no cross-reactivity between ACE2 and the antibodies in the absence of antigen).
- **Column 10:** Loading – Kinetics buffer
- **Column 11:** Regeneration – 10 mM Glycine pH 2.3
- **Column 12:** Neutralization – PBST 0.1%



Sample plate layout

2.4 Set up the assay definition as below. Plate temperature and shaking speed is set to 25°C and 1000 rpm respectively. Replicate steps of Cycle 1 and “append to current assay” to use the same sensor in Cycle 2 as reference to check for any non-specific binding.

Citation: Rabiatul Adawiyah, Patricia Ng, Bei Wang, Cheng-I Wang (08/01/2020). Antibody characterizations by BioLayer Interferometry (BLI). https://dx.doi.org/10.17504/protocols.io.bi39kgr6

		Steps	Sample plate column	Time
Immobi-lization	1.	Baseline in water	1	60s
	2.	Activation (EDC:NHS)	2	300s (5 min)
	3.	Loading 10 ug/ml of ACE2-Fc in 10mM sodium acetate pH 4.0	3	600s (10 min)
	4.	Quench (1M Ethanolamine)	4	300s (5 min)
	5.	Baseline in water	1	30s
Cycle 1	6.	Baseline in kinetics buffer	5	60s
	7.	Association 1 (with 5 ug/ml Tagless RBD)	6	600s (10 min)
	8.	Association 2 (with panel of IgG clones)	7	300s (5 min)
	9.	Dissociation	5	300s
	10	Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)
Cycle 2 (ref)	11.	Baseline in kinetics buffer	5	60s
	12.	Association 1 (with empty buffer ie. No antigen)	9	600s (10 min)
	13.	Association 2 (with panel of IgG clones)	7	300s (5 min)
	14.	Dissociation	5	300s
	15.	Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)

Assay definition

2.5 Run the assay according to the protocol set.

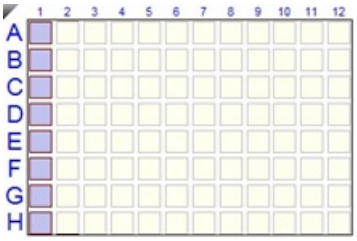
2.6 After the run is complete, extract and process the raw data:

- **Step 1:** Find the timepoints of interest to select data for analysis.
- **Step 2:** Normalize Cycle 1 data (set Y-value to 0).
- **Step 3:** Normalize Cycle 2 data (set Y-value to 0).
- **Step 4:** Subtract normalized Cycle 2 data from normalized Cycle 1 data.
- **Step 5:** Plot subtracted data on Prism.

Epitope binning by BioLayer Interferometry (BLI)

3 Epitope binning by BioLayer Interferometry (BLI)

3.1 Epitope binning consists of first immobilizing antibody 5A6 onto AR2G biosensors, followed by a classical sandwich assay with consecutive associations to target RBD and a panel of second antibodies. To start, hydrate each biosensor with 200 µl of water in the sensor tray for at least 15-20 min prior to the immobilization run.



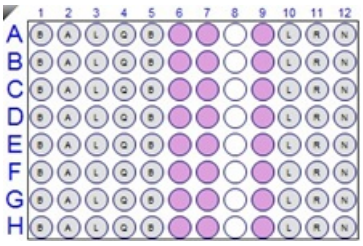
Sensor tray layout

Citation: Rabiatul Adawiyah, Patricia Ng, Bei Wang, Cheng-I Wang (08/01/2020). Antibody characterizations by BioLayer Interferometry (BLI). https://dx.doi.org/10.17504/protocols.io.bi39kgr6

3.2 Prepare EDC:NHS activation buffer (20 mM EDC + 10 mM NHS in water). Note that, after mixing, the activation buffer will only be viable for about 1 hour. Thus, it is best to mix them right before starting the run.

3.3 Set up the sample plate with each well containing 200 µl of reagent as described below:

- **Column 1:** Buffer – Water
- **Column 2:** Activation – EDC:NHS buffer
- **Column 3:** Loading – 7.5 µg/ml 5A6 antibody in 10mM sodium acetate pH 6.0
- **Column 4:** Quench – 1M Ethanolamine pH 8.5
- **Column 5:** Buffer – Kinetics buffer
- **Column 6:** Association 1 – 5 µg/ml Tag-less RBD, prepared in kinetics buffer
- **Column 7:** Association 2 – Panel of IgG clones, 10 µg/ml each, prepared in kinetics buffer. Include 5A6 and at least one irrelevant IgG as controls to ensure self-blocking (i.e. 5A6-5A6 sandwich should not have any binding signal) and no non-specific binding to irrelevant proteins.
- **Column 9:** Association 1 – Kinetics buffer (This serves as another control to ensure there is no cross-reactivity between antibodies in the absence of antigen).
- **Column 10:** Loading – Kinetics buffer
- **Column 11:** Regeneration – 10 mM Glycine pH 2.3
- **Column 12:** Neutralization – PBST 0.1%



Sample plate layout

3.4 Set up the assay protocol for the immobilization run as below. Plate temperature and shaking speed is set to 25°C and 1000 rpm respectively.

	Steps	Sample plate column	Time
1.	Baseline in water	1	60s
2.	Activation (EDC:NHS)	2	300s (5 min)
3.	Loading 7.5 ug/ml of Purified 5A6 IgG in 10mM sodium acetate pH 6.0	3	600s (10 min)
4.	Quench (1M Ethanolamine)	4	300s (5 min)
5.	Baseline in water	1	30s

Assay definition - Immobilization

3.5 Run the assay according to the protocol set. Immobilization of 5A6 IgG should take about 20 min.

Citation: Rabiatul Adawiyah, Patricia Ng, Bei Wang, Cheng-I Wang (08/01/2020). Antibody characterizations by BioLayer Interferometry (BLI). https://dx.doi.org/10.17504/protocols.io.bi39kgr6

3.6 Once immobilization is successful, prepare to run the sandwich assay according to the assay definition below. Replicate steps of Cycle 1 and "append to current assay" to use the same 5A6-immobilized sensor in Cycle 2 as reference to check for any non-specific binding. Repeat cycles in the sandwich assay as necessary to screen against the full panel of IgGs.

		Steps	Sample plate column	Time
Cycle 1	1.	Baseline in water	1	30s
	2.	"Loading" (empty buffer – input proper Loading Sample ID for analysis eg. 5A6)	10	30s
	3.	Baseline in kinetics buffer	5	60s
	4.	Association 1 (with 5 ug/ml Tagless RBD)	6	600s (10 min)
	5.	Association 2 (with panel of IgG clones)	7	300s (5 min)
	6.	Dissociation	5	300s
	7.	Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)
Cycle 2 (reference)	8.	Baseline in water	1	30s
	9.	"Loading" (empty buffer – input proper Loading Sample ID for analysis eg. 5A6)	10	30s
	10.	Baseline in kinetics buffer	5	60s
	11.	Association 1 (with empty buffer ie. No antigen)	9	600s (10 min)
	12.	Association 2 (with panel of IgG clones)	7	300s (5 min)
	13.	Dissociation	5	300s
	14.	Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)

Assay definition - Sandwich

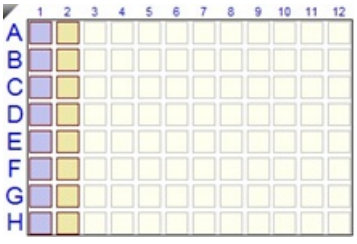
3.7 After the run is complete, extract and process the raw data:

- **Step 1:** Find the time points of interest to select data for analysis.
- **Step 2:** Normalize Cycle 1 data (set Y-value to 0).
- **Step 3:** Normalize Cycle 2 data (set Y-value to 0).
- **Step 4:** Subtract normalized Cycle 2 data from normalized Cycle 1 data.
- **Step 5:** Plot subtracted data on Prism.

Fab affinity measurement by BioLayer Interferometry (BLI)

4 Fab affinity measurement by BioLayer Interferometry (BLI)

4.1 Hydrate each biosensor with 200 µl of kinetics buffer in the sensor tray for at least 15-20 min prior to every run. For each Fab affinity measurement, run a reference sensor in parallel.



Sensor tray layout

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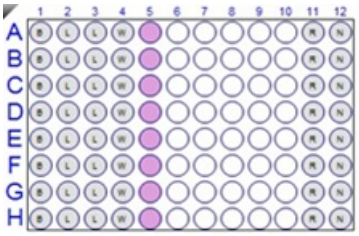
4.2 Set up the sample plate with each well containing 200 µl of reagent as described below:

- **Column 1:** Buffer – Kinetics buffer
- **Column 2:** Loading – 1 µg/ml of Fc-RBD diluted in kinetics buffer
- **Column 3:** Loading – Kinetics buffer (for ref sensor loading)
- **Column 4:** Wash/Dissociation – Kinetics buffer
- **Column 5:** Association Sample – Purified Fab clone in two-fold dilutions starting from 100 nM, diluted in kinetics buffer. Ensure that the last well in row H contains 0 nM (i.e. Kinetics buffer only) to serve as the reference well.
- **Column 11:** Regeneration – 10 mM Glycine pH 2.3
- **Column 12:** Neutralization – PBST 0.1%



Sample tray layout

4.3 The assay protocol is defined in the data acquisition software as below. Replicate steps as "new assay" for the reference sensor, taking note to change the loading sample plate column. Plate temperature and shaking speed is set to 25°C and 1000 rpm respectively.

	Steps	Sample plate column	Time
Fab assay run	1. Baseline	1	60s
	2. Loading with Fc-TEV-RBD	2	600s (10 min)
	3. Wash/Baseline	4	300s (5 min)
	4. Association with Fab	5	360s (6 min)
	5. Dissociation	4	600s (10 min)
	6. Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)
	7. Baseline	1	30s
Ref sensor run	1. Baseline	1	60s
	2. No Loading	3	600s (10 min)
	3. Wash/Baseline	4	300s (5 min)
	4. Association with Fab	5	360s (6 min)
	5. Dissociation	4	600s (10 min)
	6. Regeneration and Neutralization	11 and 12	5s, 5s (5 cycles)
	7. Baseline	1	30s

Assay definition

4.4 Run the assay according to the protocol set. Each Fab affinity measurement (including reference) should take about an hour.

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4.5 Once completed, process the data according to the following parameters in the analysis software (v. 9.0.0.4):

o **Step 1:** Data Selection – Sensor selection. Select Sample plate row H as reference well and Sensor tray column 2 as reference sensor.
o **Step 2:** Subtraction – Select "Double reference" to subtract both reference well and reference sensor.
o **Step 3:** Align Y-axis to "Baseline" and set time range as the last 10 secs of the Baseline step before association.
o **Step 4:** Inter-step correction to remove any bulk shifts or artefacts for a better line fit.
o **Step 5:** Process data with *Savitzky-Golay Filter.*
o After processing, calculate K_D based on the 1:1 grouped fitting.

4.6 Repeat measurement for each subsequent Fab clones. Regenerated sensors can be used for another 1 or 2 more times, depending on whether the signal reached baseline levels after regeneration and whether loading signal with 1 µg/ml Fc-RBD remains consistent with previous runs. Otherwise, use fresh sensors.

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