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Human and Mouse IgG Subtype Identification Using the Octet® Platform

Technical Note

Scope
This Technical Note describes quantitation and subtype identification assay workflows that use the Octet® BLI systems and Octet® AHC2, AMC2 and AMC Biosensors for characterization of human and mouse IgG proteins.

Abstract

Antibodies are a key component in the humoral response and understanding isotype and subtype identity in addition to binding specificity, affinity, glycosylation may provide novel insights into the design of antibody-based therapeutics and vaccines. The Octet® Bio-Layer Interferometry (BLI) Platform offers easy, label-free, and high-throughput quantitation and characterization of antibody isotype and subtype class identity. This Technical Note highlights the ease of assay design and setup offered by the dip-and-read format on the Octet® BLI system, and provides a step-by-step guide to antibody quantitation and subtype characterization. Human and mouse IgG quantitation and subclass characterization data is shown and discussed for Octet® Anti-human IgG Fc Capture (AHC2), Anti-mouse Fc Capture (AMC), and Anti-murine IgG Capture (AMC2) Biosensors.

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Overview

This technical note describes a rapid assay method for quantitation and subtype identification of human and mouse IgG. The antibodies can be quantified in buffer, serum-free media or lysates using the Octet® AHC2 or AMC/AMC2 Biosensors. Using the same biosensors, the subtype of the antibody can be identified using subtype-specific secondary antibodies. The flexible microplate-based format of the Octet® Bio-Layer Interferometry (BLI) platform allows up to 48 samples to be analyzed on an Octet® R8 BLI system and up to 96 samples on an Octet® RH16 BLI system in 1 hour. On the Octet® RH96 BLI system, 96 samples can be analyzed in about 15 minutes.

Material and Methods

Materials

Instruments:

- Octet® R8 BLI system
- Octet® BLI Discovery and Analysis Studio Software

Biosensors:

- Octet® AMC Biosensors for kinetics assays, Part No. 18-5088 (tray); 18-5089 (pack); 18-5090 (case)
- Octet® AMC2 Biosensors for kinetics and quantitation assays, Part No. 18-5163 (tray), 18-5164 (pack),18-5165 (case)
- Octet® AHC2 Biosensors for kinetics and quantitation assays, Part No. 18-5142 (tray), 18-5143 (pack), 18-5144 (case)

For all Octet® BLI systems:

- 96-well, black, flat bottom microplate, Greiner Bio-One Part No. 655209

Antibody reagents:

- Octet® Sample Diluent (Sartorius Part No. 18-1104)
- Mouse IgG isotype control antibodies: Novus Biologicals, USA (Mouse IgG1, Part No. NBP2-21942-0.5MG; Mouse IgG2a, Part No. NBP1-96778; Mouse IgG2b, Part No. NBP1-96969; Mouse IgG3, Part No. NBP1-96977)
- Human IgG isotype control antibodies: Sino Biological, USA (Human IgG1, Part No. HG1K; Human IgG2, Part No. HG2K; Human IgG4, Part No. HG4K)
- Anti-subtype specific antibodies used in the example data within this technical note:
  - Goat anti-mouse IgG subtype specific antibodies: Bethyl Laboratories (mIgG1, Part No. A90-105A; mIgG2a, Part No. A90-107A; mIgG2b, Part No. A90-109A; mIgG3, Part No. A90-111A)
  - Mouse anti-human subtype specific antibodies: Abcam, USA (Anti-Human IgG1, Part No. ab1927; Anti-Human IgG2, Part No. ab99777; Anti-Human IgG4, Part No. ab1930)
- Unknown samples to be tested: purified IgG samples of interest in buffer

Assay Workflow

A. Prepare the samples and standards
B. Set up the assay protocol
C. Analyze the data

A. Prepare the Sample Plate

Up to 48 samples can be analyzed in one assay on an Octet® R8 BLI system, up to 96 samples in one assay using an Octet® RH16 BLI system, and up to 600 samples using an Octet® RH96 BLI system. The assay requires 1 microplate when using an Octet® R8 BLI system (Figure 1), or 2 plates when using an Octet® RH16 BLI system (Figure 2).

Figure 1
Octet® R8 BLI System Sample Plate Format.

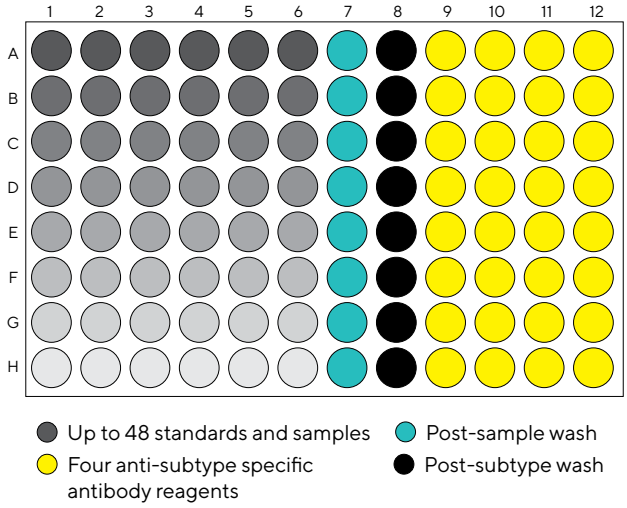
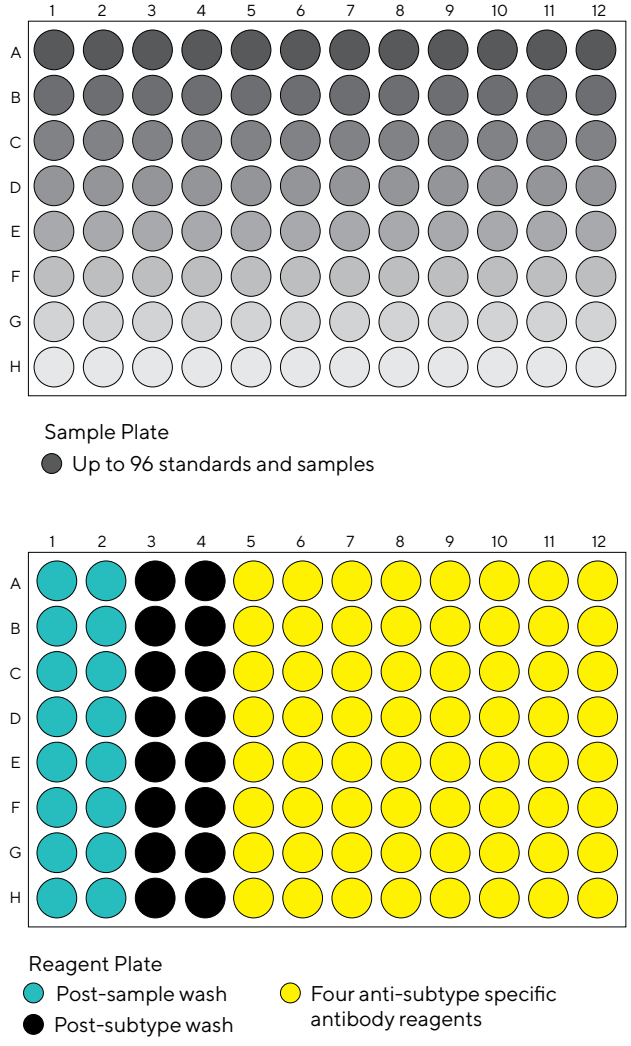


Figure 2
Octet® RH16 BLI System Sample and Reagent Plate Format.



When using an Octet® R8 BLI system, 4 columns of wells in the sample plate will need to be reserved for the anti-subtype specific antibody reagents (such as goat anti-mouse IgG1, goat anti-mouse IgG2a, goat anti-mouse IgG2b, goat anti-mouse IgG3, etc.). When running an Octet® RH16 BLI system, 8 columns of wells on the reagent plate will need to reserved.

Two columns of wells in the sample plate (Octet® R8 BLI system) or 4 columns of wells in the reagent plate (Octet® RH16 BLI system) will need to be reserved for post-sample wash and post anti-subtype antibody reagent wash.

Prepare the Samples

- Most buffer-based samples can be measured undiluted.
- Dilute serum-free media samples a minimum of 1:1 in Sample Diluent.
- Dilute lysates a minimum of 1:5 in Sample Diluent.
- Final volume needed for each solution is 200 µL per well.

Prepare the Standards and Controls

- Prepare standards and controls in a matrix matching as closely as possible to the matrix used for the samples. If the samples have been diluted, then the standards and controls should be prepared in a mixture of blank media and Sample Diluent at the same dilution factor used for the sample.
- Final volume needed for each solution is 200 µL per well. ***Note:*** Best results will be obtained when the matrix for samples, standards, and biosensor hydration match as closely as possible.
- Pipet 200 µL of each standard and sample into a 96-well black flat-bottom plate.

Prepare the Anti-subtype Antibody Reagents

- Prepare anti-subtype antibody reagents in a matrix matching the matrix used for the samples as closely as possible. The working concentration should be 20–40 µg/mL.
- Pipet 200 µL of each anti-subtype antibody reagent into a 96-well black flat-bottom plate (refer to Figure 1 for Octet® R8 BLI system, Figure 2 for Octet® RH16 BLI system).

Prepare the Wash Buffer

- Wash buffer should be the matrix used for sample, standards and biosensor hydration.

B. Set Up the Assay Protocol

Program the Octet® system to run an assay with the steps shown in Table 1. Repeat these steps with new biosensors for each additional column of samples to be tested.
Note: Enter the sample name into the corresponding sensor information field and enter the subtype info in the well ID. This will allow for easy sorting of data to correlate sample names to positive subtypes.

C. Analyze the Data

1. Load data into Octet® Analysis Studio Software.
2. In the preprocessed tab, click on the desired step to be quantitated (first step) from the raw data graph.
   - Click on the **K to Q** function and select **Quantitate Selected Step** button.
   - Click **Yes** in the pop-up to open the data in Quantitation data analysis.
   - The data analysis will automatically switch to Quantitation mode.
3. In the Results tab, select the standard curve equation to be used (typically Dose Response-4PL). To load a previously saved standard curve, select **Load Standards**.
4. Select the binding rate equation (typically Initial Slope).
5. Click **Calculate Binding Rate**. Quantitation results will be displayed automatically in the table.
6. Save the report if desired.
7. The subtype information is read from the binding signal of the anti-subtype antibodies. A positive binding signal indicates that the antibody detected corresponds to that specific subtype present.
   - This can be done from the real-time binding curves saved as jpegs immediately after the run.
   - Alternatively, the data can be processed in the Kinetic analysis module in the Octet® Analysis Studio Software.
     - Process data by aligning the baselines without subtraction, producing data with all subtype binding steps aligned.
     - In the Analysis tab, the table can be sorted by Response to give a read out of the positive subtype binding signals.

Example Assay Performance Data

The figures in the following examples illustrate the assay performance when quantifying human IgG or mouse IgG followed by subtype identification.

Table 1
Assay Steps for Human and Mouse IgG Subtype Identification on the Octet® System.

Step Name	Location	Step Time (sec)	Flow Rate (rpm)	Step Type
Quantitation	Sample column 1	120	400	Loading
Wash	Post-sample wash	30	1000	Baseline
Subtype	Subtype antibody column 1	120	1000	Association
Wash	Post-subtype wash	30	1000	Baseline
Subtype	Subtype antibody column 2	120	1000	Association
Wash	Post-subtype wash	30	1000	Baseline
Subtype	Subtype antibody column 3	120	1000	Association
Wash	Post-subtype wash	30	1000	Baseline
Subtype	Subtype antibody column 4	120	1000	Association

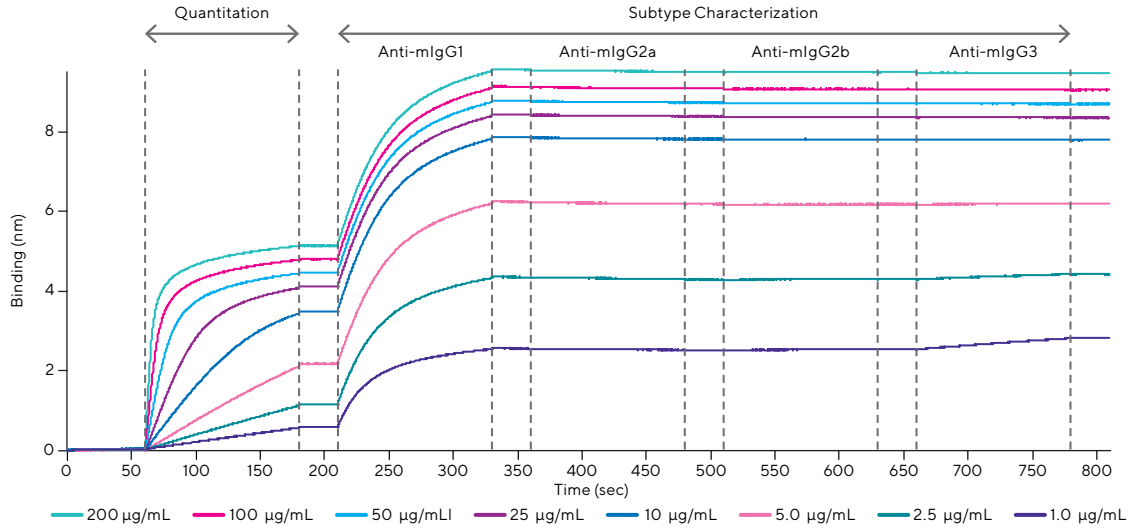
Mouse IgG Quantitation and Subtype Characterization using IgG1 Standards

The data in Figure 3 was generated using Octet® AMC2 Biosensors and an Octet® R8 BLI system. Panel A shows the raw data for a complete calibration range for the IgG1 subtype run at 200, 100, 50, 25, 10, 5, 2.5, and 1 µg/mL in Octet® Sample Diluent (marked 'Quantitation' on the graph) immediately followed by subtype confirmation using the appropriate secondary subtype-specific control antibody (marked 'Subtype Characterization' on the graph). The resulting calibration curve from the quantitation analysis using the IgG1 standards and the subtype results from the kinetic response analysis demonstrate the ability to specifically detect the mouse IgG1 subtype.

Figure 3

Mouse IgG Quantitation and Subtype Characterization Using mIgG1 Standards.

A.



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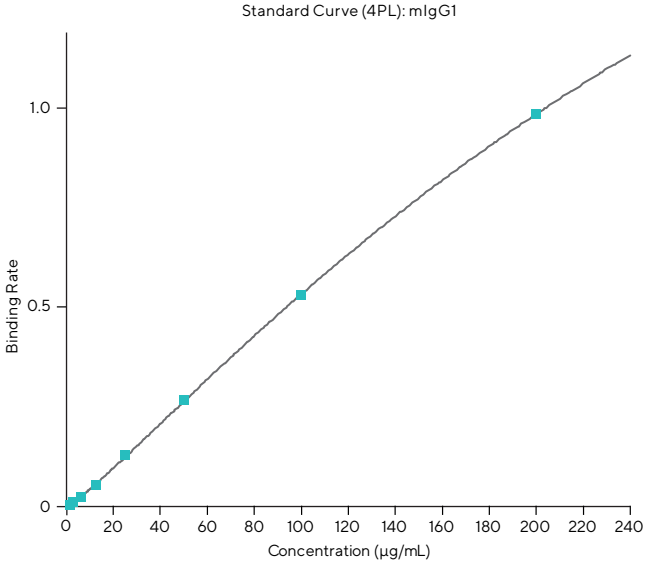

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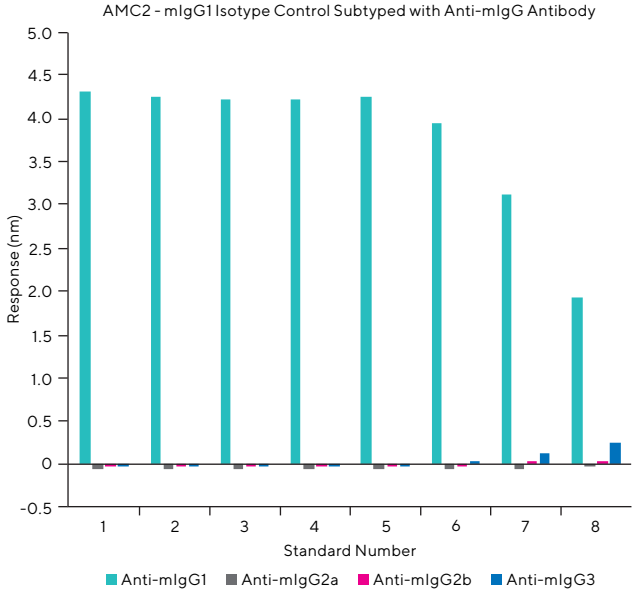


Note. A. mIgG1 dose-response curve using Octet® AMC2 Biosensors. mIgG1 isotype control antibodies were 2-fold serially diluted from 200 µg/mL–1.0 µg/mL to generate a dose-response standard curve. The isotype control antibodies were further tested for subtype using anti-subtype antibodies during the Subtype Characterization steps (marked in the graph). B. mIgG1 standard curve. mIgG1 standard calibration curve was generated by applying a 4PL fitting model to the dose-response data. C. mIgG1 antibody subtype characterization. The mIgG1 isotype antibodies at different concentrations (200 µg/mL–1.0 µg/mL, 2-fold serially diluted) were subtyped with anti-mIgG1/2a/2b and 3 subtype antibodies (20 µg/mL). The assays were performed in Octet® Sample Diluent on an Octet® R8 BLI system at 30°C.

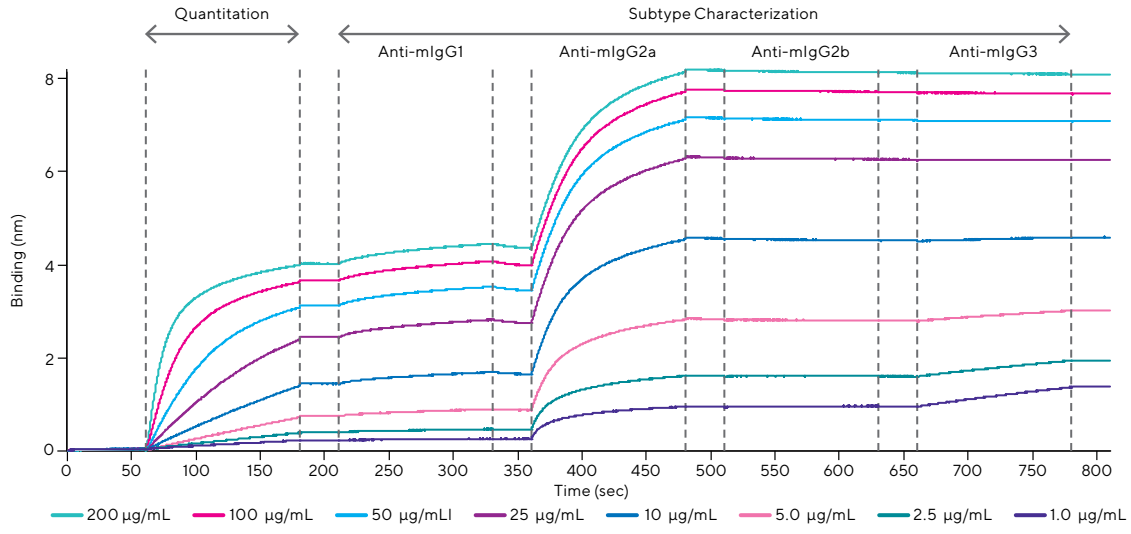
Mouse IgG Quantitation and Subtype Characterization Using IgG2a Standards

The data in Figure 4 was generated using Octet® AMC2 Biosensors and an Octet® R8 BLI system. Panel A shows the raw data for a complete calibration range for the IgG2a subtype run at 200, 100, 50, 25, 10, 5, 2.5, and 1 µg/mL in Octet® Sample Diluent (marked 'Quantitation' on the graph) immediately followed by subtype confirmation using the appropriate secondary subtype-specific control antibody (marked 'Subtype Characterization' on the graph). The resulting calibration curve from the quantitation analysis using the IgG2a standards and the subtype results from the kinetic response analysis demonstrate the ability to specifically detect the mouse IgG2a subtype.

Figure 4

Mouse IgG Quantitation and Subtype Characterization Using IgG2a Standards.

A.



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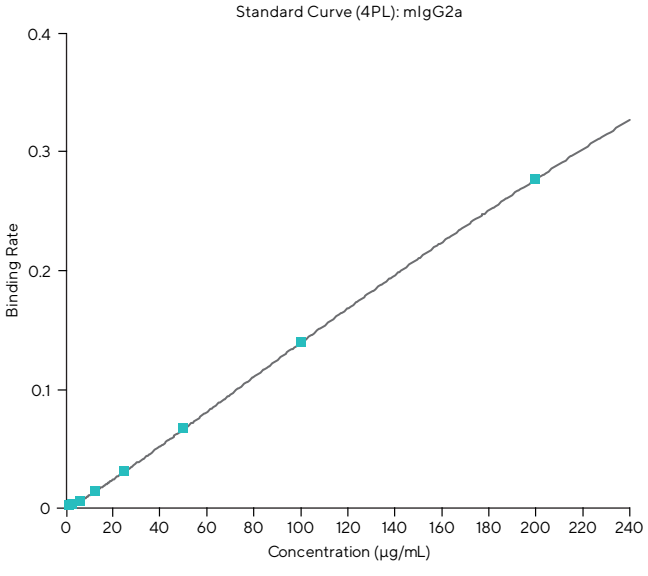

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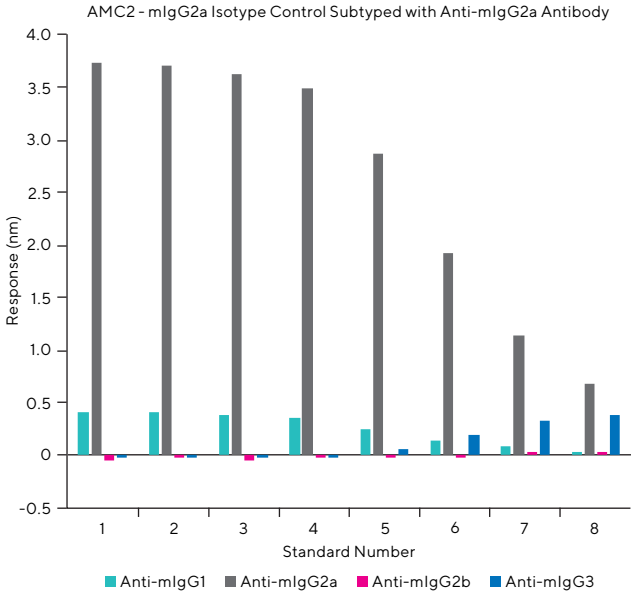


Note. A. mIgG2a dose-response curve using Octet® AMC2 Biosensors. mIgG2a isotype control antibodies were 2-fold serially diluted from 200 µg/mL–1.0 µg/mL to generate a dose-response standard curve. The Isotype control antibodies were further tested for subtype using anti-subtype antibodies during the Subtype Characterization steps (marked in the graph). B. mIgG2a standard curve. mIgG2a standard calibration curve was generated by applying a 4PL fitting model to the dose-response data. C. mIgG2a antibody subtype characterization. The mIgG2a isotype antibodies at different concentrations (200 µg/mL–1.0 µg/mL, 2-fold serially diluted) were subtyped with anti-mIgG1/2a/2b and 3 subtype antibodies (20 µg/mL). The assays were performed in Octet® Sample Diluent on an Octet® R8 BLI system at 30°C.

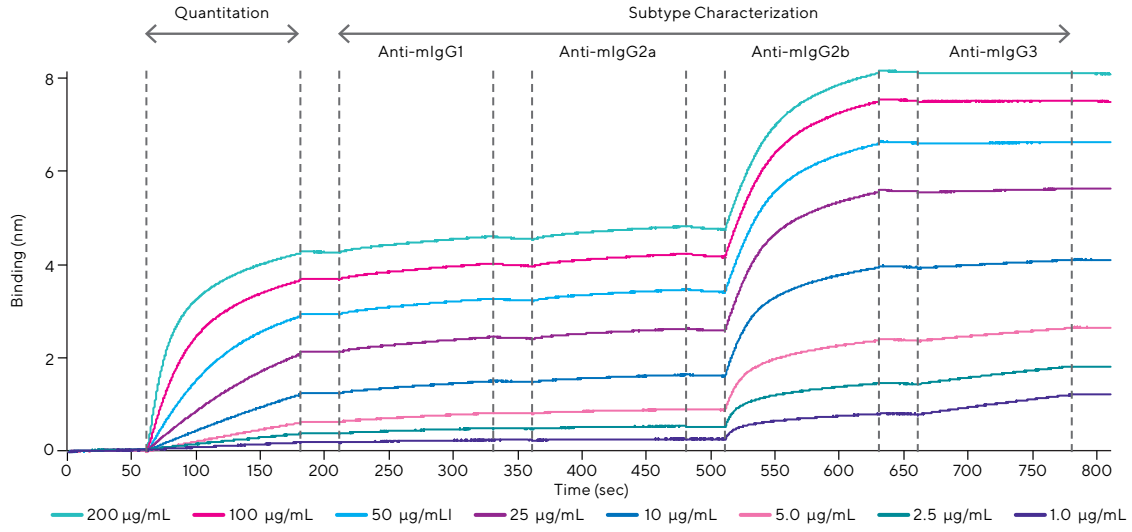
Mouse IgG Quantitation and Subtype Characterization Using IgG2b Standards

The data in Figure 5 was generated using Octet® AMC2 Biosensors and an Octet® R8 BLI system. Panel A shows the raw data for a complete calibration range for the IgG2b subtype run at 200, 100, 50, 25, 10, 5, 2.5, and 1 µg/mL in Octet® Sample Diluent (marked 'Quantitation' on the graph) immediately followed by subtype confirmation using the appropriate secondary subtype-specific control antibody (marked 'Subtype Characterization' on the graph). The resulting calibration curve from the quantitation analysis using the IgG2b standards and the subtype results from the kinetic response analysis demonstrate the ability to specifically detect the mouse IgG2b subtype.

Figure 5

Mouse IgG Quantitation and Subtype Characterization Using IgG2b Standards.

A.



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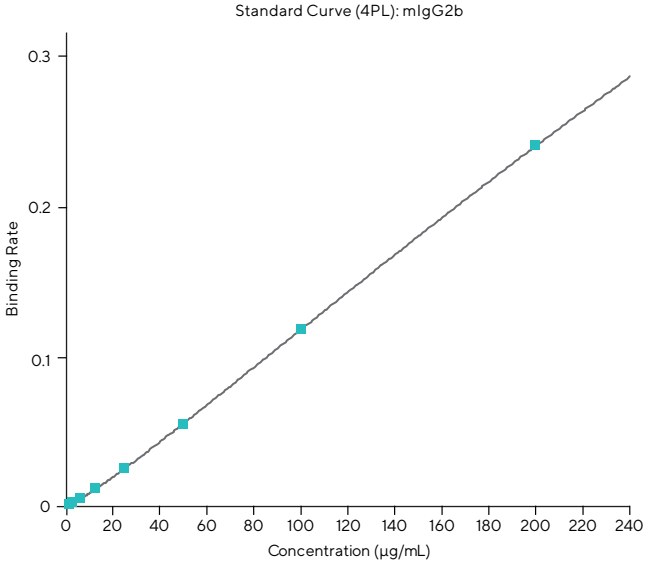

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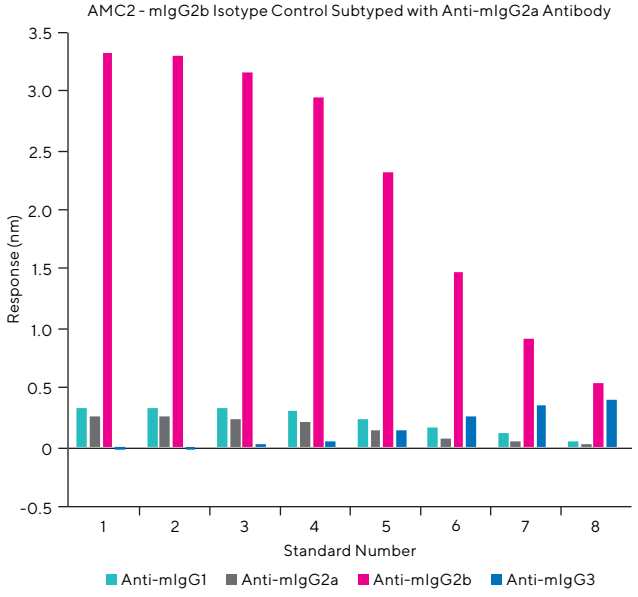


Note. A. mIgG2b dose-response curve using Octet® AMC2 Biosensors. mIgG2b isotype control antibodies were 2-fold serially diluted from 200 µg/mL–1.0 µg/mL to generate a dose-response standard curve. The Isotype control antibodies were further tested for subtype using anti-subtype antibodies during the Subtype Characterization steps (marked in the graph). B. mIgG2b standard curve. mIgG2b standard calibration curve was generated by applying a 4PL fitting model to the dose-response data. C. mIgG2b antibody subtype characterization. The mIgG2b isotype antibodies at different concentrations (200 µg/mL–1.0 µg/mL 2-fold serially diluted) were subtyped with anti-mIgG1/2a/2b and 3 subtype antibodies (20 µg/mL). The assays were performed in Octet® Sample Diluent on an Octet® R8 BLI system at 30°C.

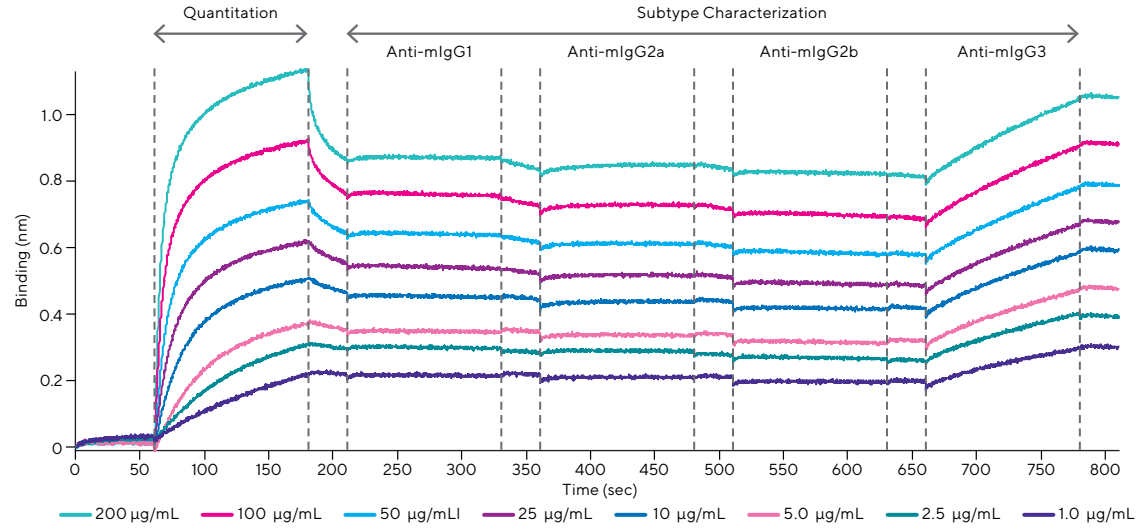
Mouse IgG Quantitation and Subtype Characterization Using IgG3 Standards

The data in Figure 6 was generated using Octet® AMC Biosensors and an Octet® R8 BLI system. Panel A shows the raw data for a complete calibration range for the IgG3 subtype run at 200, 100, 50, 25, 10, 5, 2.5, and 1 µg/mL in Octet® Sample Diluent (marked 'Quantitation' on the graph) immediately followed by subtype confirmation using the appropriate secondary subtype-specific control antibody (marked 'Subtype Characterization' on the graph). The resulting calibration curve from the quantitation analysis using the IgG3 standards and the subtype results from the kinetic response analysis demonstrate the ability to specifically detect the mouse IgG3 subtype.

Figure 6

Mouse IgG Quantitation and Subtype Characterization Using IgG3 Standards.

A.



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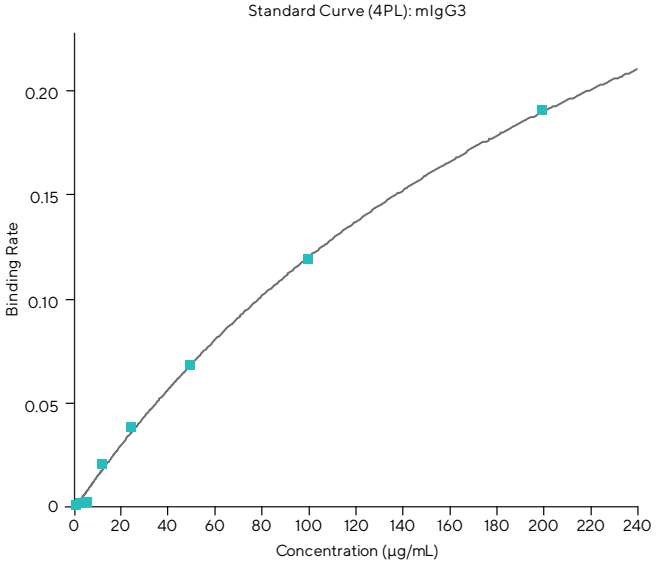

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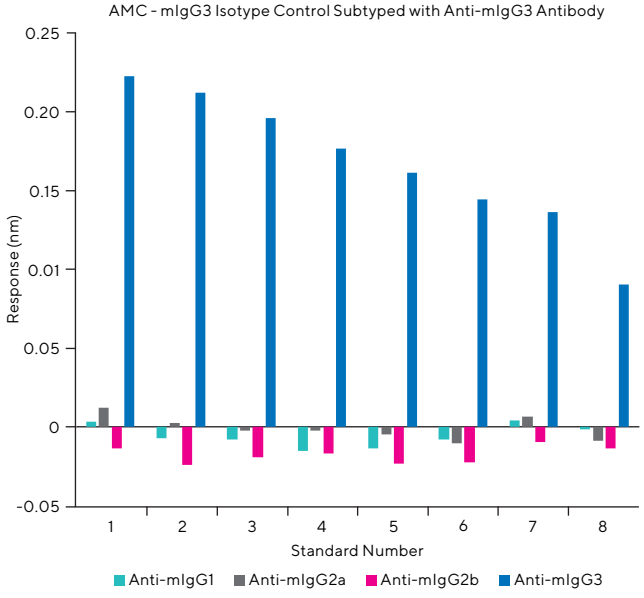


Note. A. mIgG3 dose-response curve using Octet® AMC Biosensors. mIgG3 isotype control antibodies were 2-fold serially diluted from 200 µg/mL–1.0 µg/mL to generate a dose-response standard curve. The Isotype control antibodies were further tested for subtype using anti-subtype antibodies during the Subtype Characterization steps (marked in the graph). B. mIgG3 standard curve. mIgG3 standard calibration curve was generated by applying a 4PL fitting model to the dose-response data. C. Mouse mIgG3 antibody (mIgG3) subtype characterization. The mIgG3 isotype antibodies at different concentrations (200 µg/mL–1.0 µg/mL, 2-fold serially diluted) were subtyped with anti-mIgG1/2a/2b and 3 subtype antibodies (20 µg/mL). The assays were performed in Octet® Sample Diluent on an Octet® R8 BLI system at 30°C.

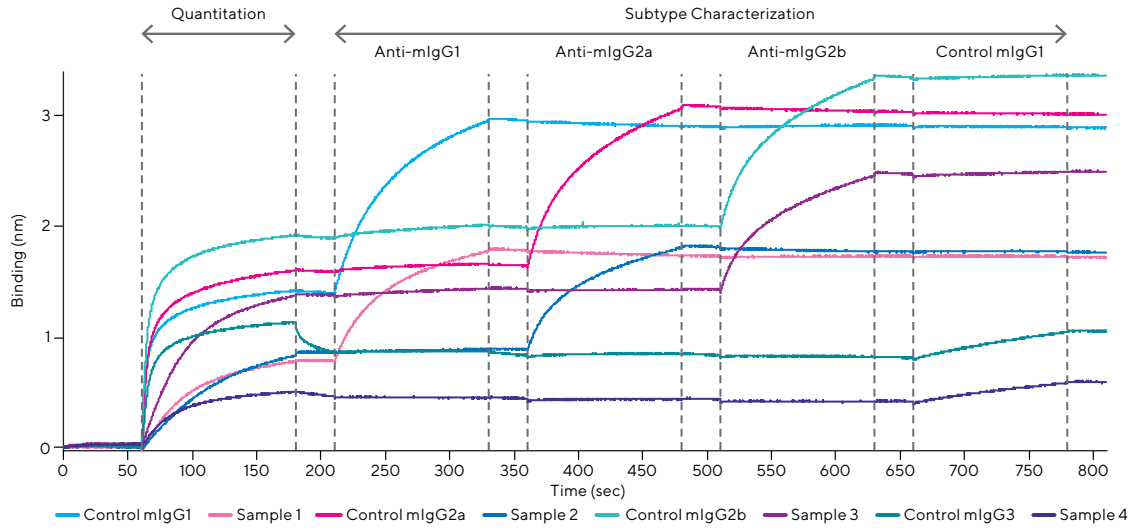
Mouse IgG Quantitation and Subtype Characterization Using mIgG1, mIgG2a, mIgG2b and mIgG3 Standards

The data in Figure 7 presents the results of mIgG quantitation and subtype analysis performed with samples consisting of a mixture of mouse IgG subtypes and concentrations. When analyzed using a saved standard curve, the samples could be both quantitated and subtyped in the same assay.

Figure 7

Mouse IgG Quantitation and Subtype Characterization Using mIgG1, mIgG2a, mIgG2b and mIgG3 Standards.

A.



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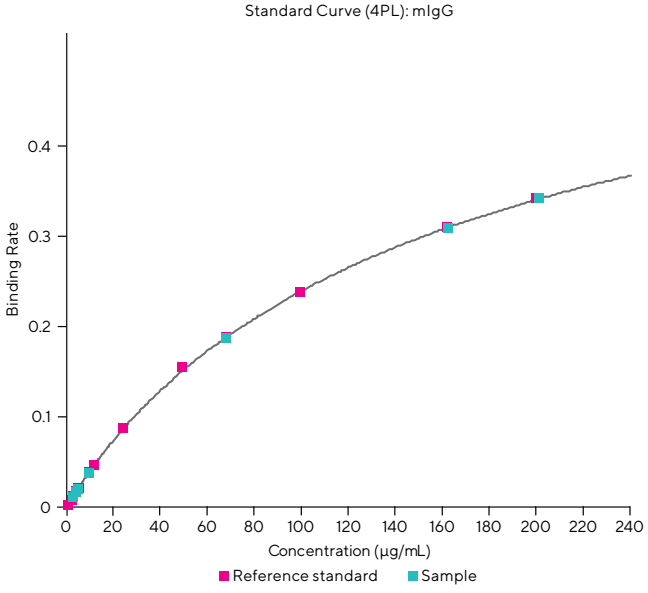

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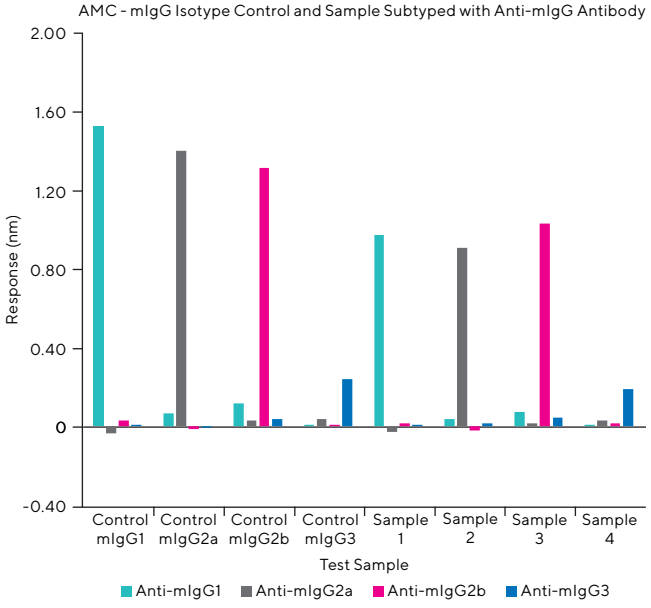


Note. A. Mouse IgG Antibody (mIgG) quantitation and subtyping using Octet® AMC Biosensors. mIgG samples (unknown and isotype control) were quantitated and subtyped using anti-subtype mIgG1, mIgG2a, mIgG2b and mIgG3 antibodies. The mIgG samples were quantitated by interpolating the binding rate to a previously saved mIgG isotype control standard curve. B. Mouse IgG standard curve. mIgG standard curve was generated by applying a 4PL fitting model to mIgG1, mIgG2a, mIgG2b and mIgG3 isotype control antibodies dose-response data. C. Mouse IgG antibody (mIgG) subtype characterization. The mIgG samples (unknown and isotype control antibodies) were subtyped with anti-mIgG1m, mIgG2a and mIgG2b and mIgG3 anti-subtype antibodies (20 µg/mL). The assays were performed in Octet® Sample Diluent on an Octet® R8 BLI system at 30°C.

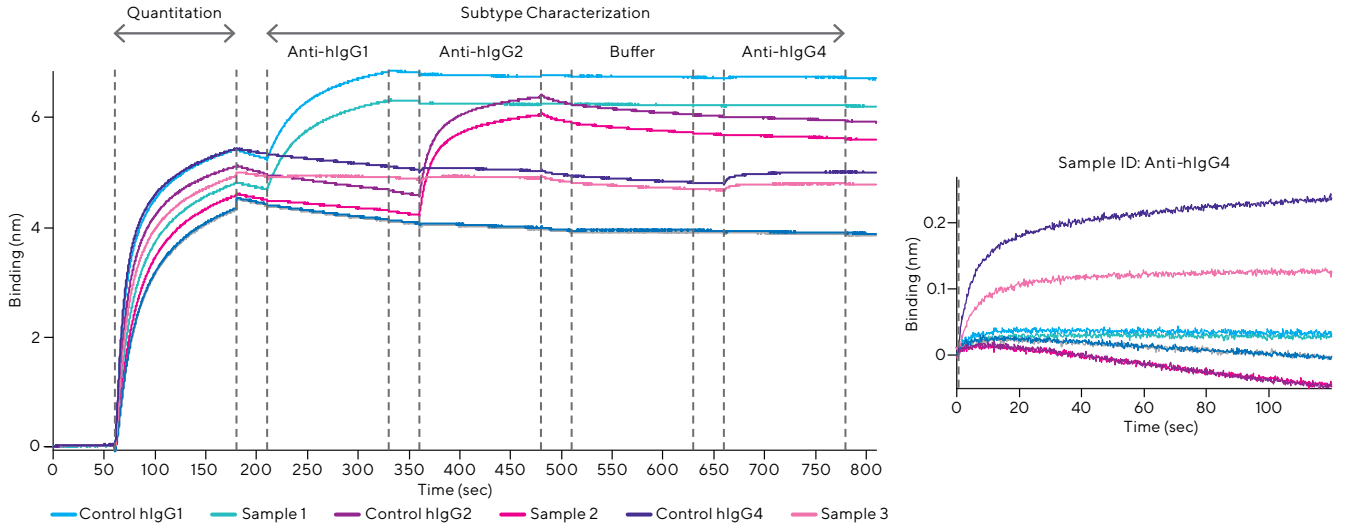
Human IgG Quantitation and Subtype Characterization Using hIgG1, hIgG2 and hIgG4 Standards

The data in Figure 8 was generated using Octet® AHC2 Biosensors and an Octet® R8 BLI system. Samples assayed were a mixture of human IgG subtypes and concentrations. When analyzed using a saved standard curve, the samples could be both quantitated and subtyped in the same assay.

Figure 8

Human IgG Quantitation and Subtype Characterization Using hIgG1, hIgG2 and hIgG4 Standards.

A.



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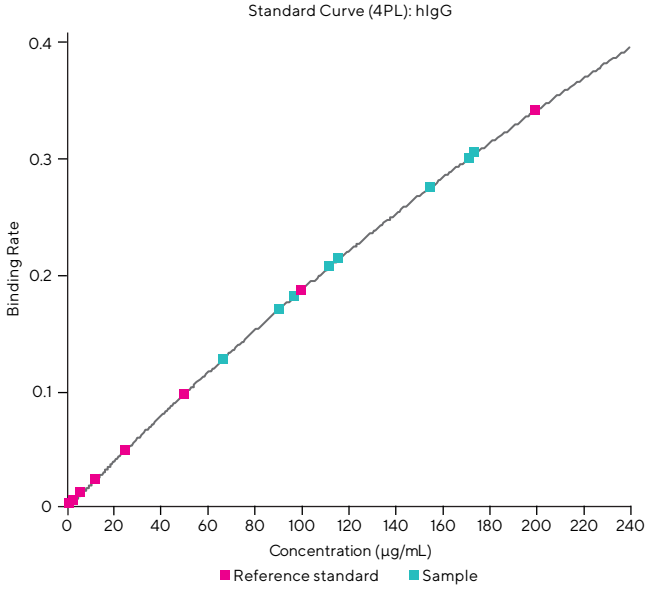

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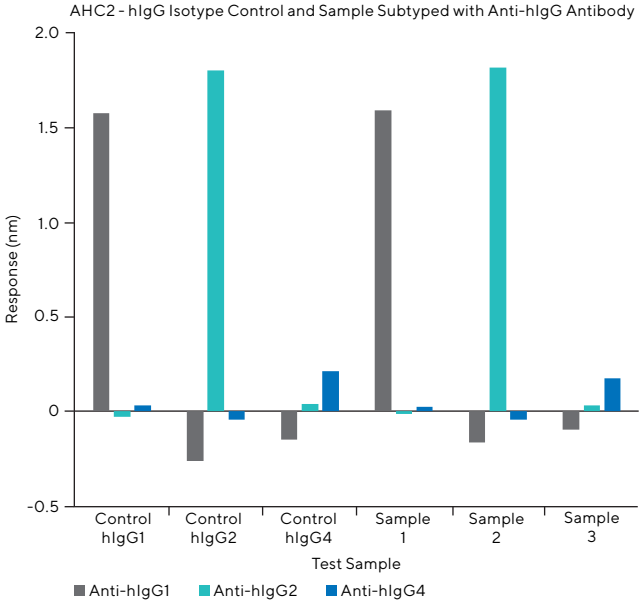

Note. A. Human IgG Antibody (hIgG) quantitation and subtyping using Octet® AHC2 Biosensors. hIgG samples (unknown and isotype control) were quantitated and subtyped using anti-subtype hIgG1, hIgG2, and hIgG4 antibodies. Human IgG3 antibody subtype was not tested. The hIgG samples were quantitated by interpolating the binding rate to a previously saved hIgG isotype control standard curve. B. Human IgG standard curve. hIgG standard curve was generated by applying a 4PL fitting model to hIgG 1, hIgG2 and hIgG4 isotype control antibodies dose-response data. C. Human IgG antibody (hIgG) subtype characterization. The hIgG samples (unknown and isotype control antibodies) were subtyped with anti-hIgG1m, hIgG2 and hIgG 4 anti-subtype antibodies (40 µg/mL). The assays were performed in Octet® Sample Diluent on an Octet® R8 BLI system at 30°C.

References

1. Beyond binding: antibody effector functions in infectious diseases, Lu, L, Suscovich, T, Fortune, S, et al., Nat Rev Immunol, 2018, 18:46–61. doi.org/10.1038/nri.2017.106
2. Considerations for the design of antibody-based therapeutics, Goulet DR and Atkins WM, J Pharm Sci., 2020, 109(1):74-103. doi.org/10.1016%2Fj.xphs.2019.05.031
3. Avidity in antibody effector functions and biotherapeutic drug design, Oostindie, SC, Lazar, GA, Schuurman, J, et al., Nat Rev Drug Discov, 2022, 21:715–735. doi.org/10.1038/s41573-022-00501-8

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