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Human and Mouse IgG Subtype Identification Using the Octet™ Platform

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 ForteBio® Anti-Human IgG Fc or Anti-Murine Fv 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 platform allows up to 48 samples to be analyzed on an Octet QK or Octet RED system and up to 96 samples on an Octet QK384 or Octet RED384 system in 1 hour.

MATERIALS REQUIRED

- Octet instrument running software version 6.1 or later
- Anti-Human IgG Fc (ForteBio part no. 18-5001 [tray]) or Anti-Murine Fv Biosensors (ForteBio part no. 18-5022 [tray])
- Sample Diluent (ForteBio part no. 18-5028)
- Human IgG or Mouse IgG standards for calibration (standards should be as similar to the molecule to be detected as possible, i.e. same species and subtype)
- Unknown samples to be tested: purified, serum-free cell culture supernatants or lysates containing the IgG of interest
- Anti-subtype specific antibodies used in the example data within this technical note:
  - The goat anti-mouse IgG subtype specific antibodies used here are from Bethyl Laboratories (mIgG1, part no. A90-105A; mIgG2a ,part no. A90-107A; mIgG2b, part no. A90-109A; mIgG3, part no. A90-111A).
  - The mouse anti-human IgG subtype specific antibodies used here are from Southern Biotech (hIgG1, part no. 9052-1; hIgG2 ,part no. 9060-1; hIgG3, part no. 9210-1) and Abcam (IgG4, part no. ab1930).
- 96-well, flat bottom, black polypropylene microplate (Greiner Bio-One part no. 655209)

ASSAY WORKFLOW

A. Prepare the samples and standards

B. Set up the assay protocol

C. Analyze the data

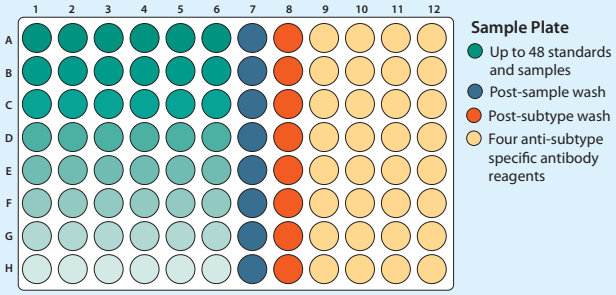

FIGURE 1: Octet QK/RED sample plate format.

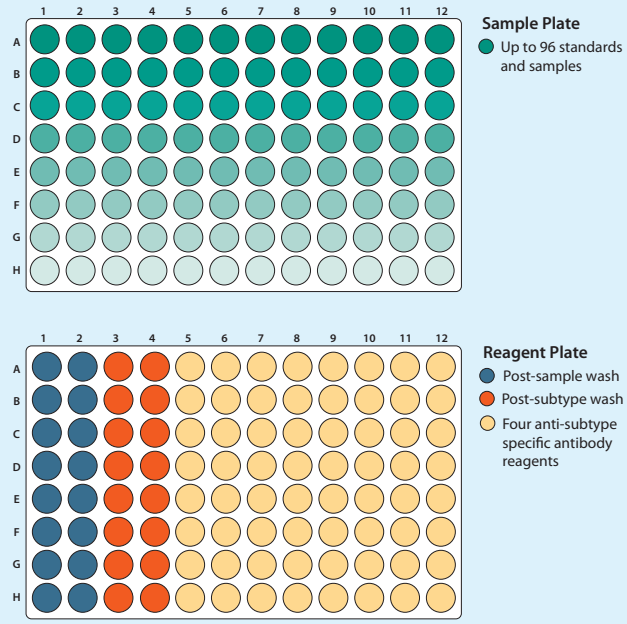

FIGURE 2: Octet QK384/RED384 sample and reagent plate format.

Step Name	Location	Step Time (sec)	Flow Rate (rpm)	Step Type
Quantitation	Sample column 1	120	200	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	Baseline

TABLE 1: Assay steps for human and mouse IgG subtype identification on the Octet system.

A. PREPARE THE SAMPLE PLATE

Up to 48 samples can be analyzed in one assay on an Octet QK or Octet RED system and up to 96 samples in one assay using an Octet QK384 or Octet RED384 system. The assay requires 1 microplate when using an Octet QK or Octet RED system (Figure 1) or 2 plates running an Octet QK384 or Octet RED384 system (Figure 2).

When using an Octet QK or Octet RED 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 QK384 or Octet RED384 system, 8 columns of wells on the reagent plate will need to reserved.

Two columns of wells in the sample plate (QK/RED) or 4 columns of wells in the reagent plate (QK384/RED384) 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 that 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 as the samples.
- 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 that used for the samples as closely as possible. The working concentration should be 20 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 QK/RED, Figure 2 for Octet QK384/RED384)

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 Data Analysis software version 6.1 or later.
2. In tab 2, click on the desired step to be quantitated (1st step) from the raw data graph.
   - Click "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-5PL). 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 subtype antibodies. A positive binding signal indicates that the antibody detected that 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 Kinetic Data Analysis 6.1 or later:
  - 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 shown in the following examples illustrate the assay performance when quantifying human IgG or mouse IgG followed by subtype identification.

Mouse IgG Quantitation and Subtype Characterization using IgG1 Standards

The data in Figure 3 was generated using ForteBio Anti-Murine Fv biosensors and an Octet RED system. The top panel 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 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.

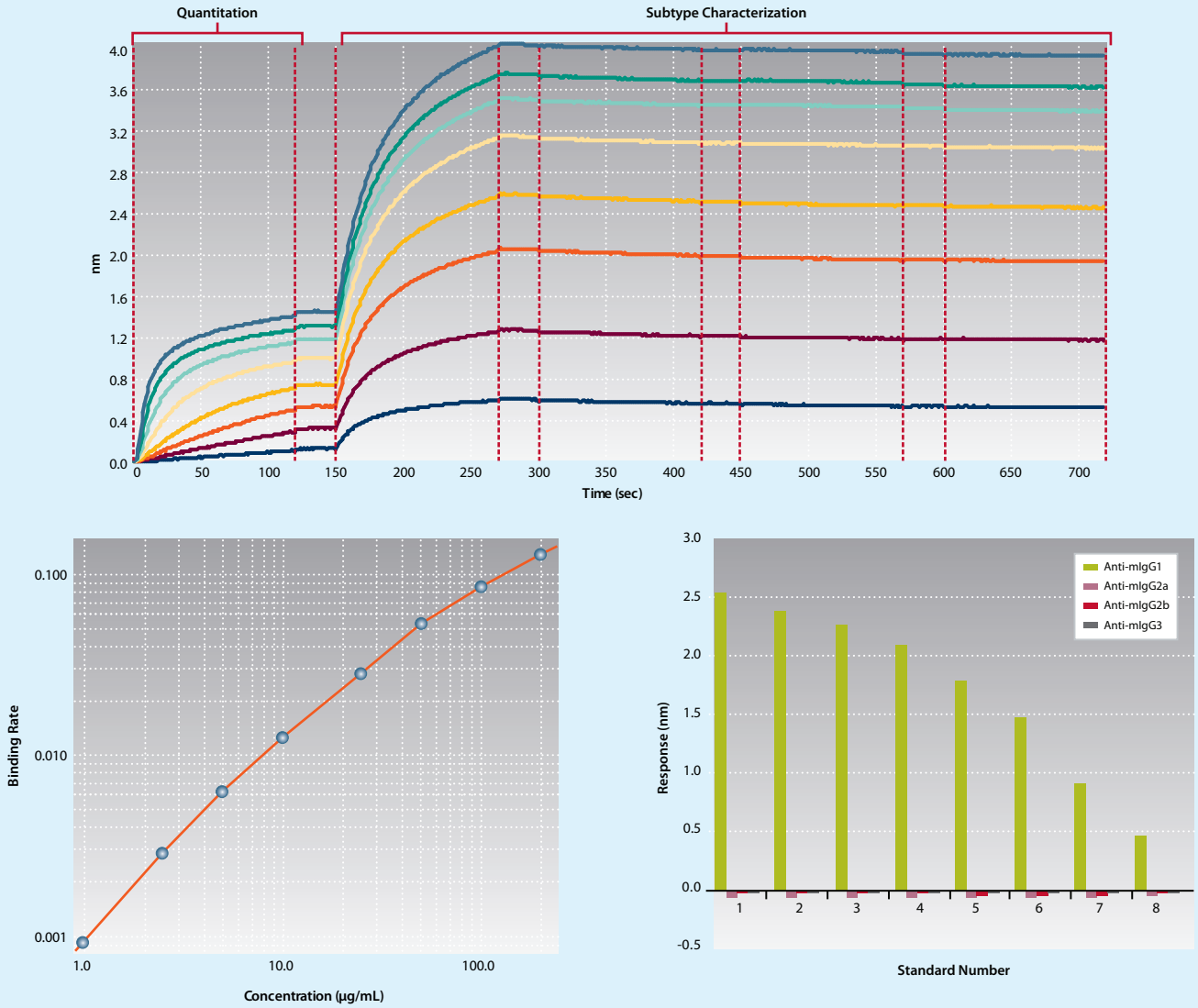

FIGURE 3: Mouse IgG quantitation and subtype characterization using IgG1 standards.

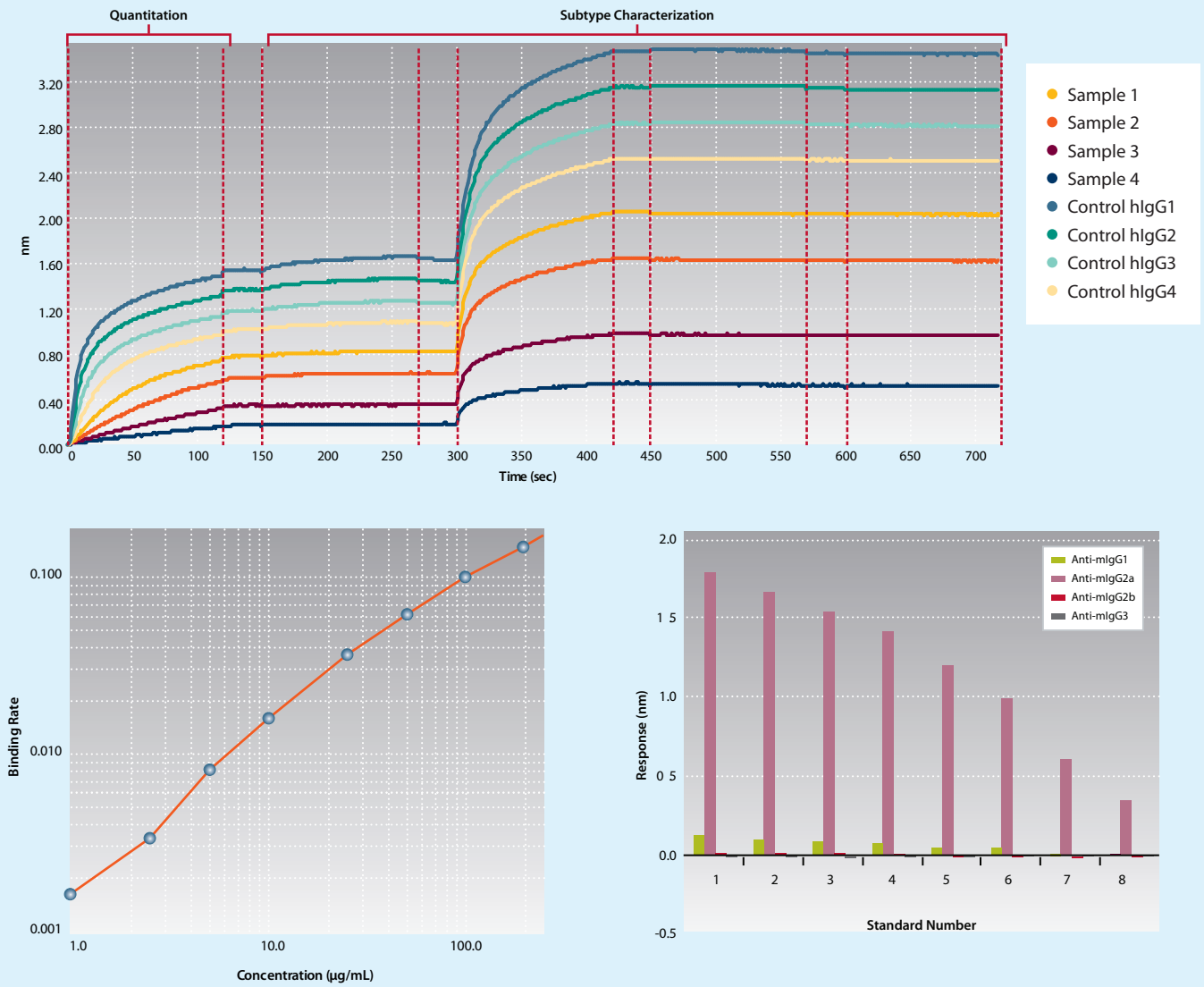

FIGURE 4: Mouse IgG quantitation and subtype characterization using IgG2a standards.

Mouse IgG Quantitation and Subtype Characterization Using IgG2a Standards

The data in Figure 4 was generated using ForteBio Anti-Murine Fv biosensors and an Octet RED system. The top panel 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 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.

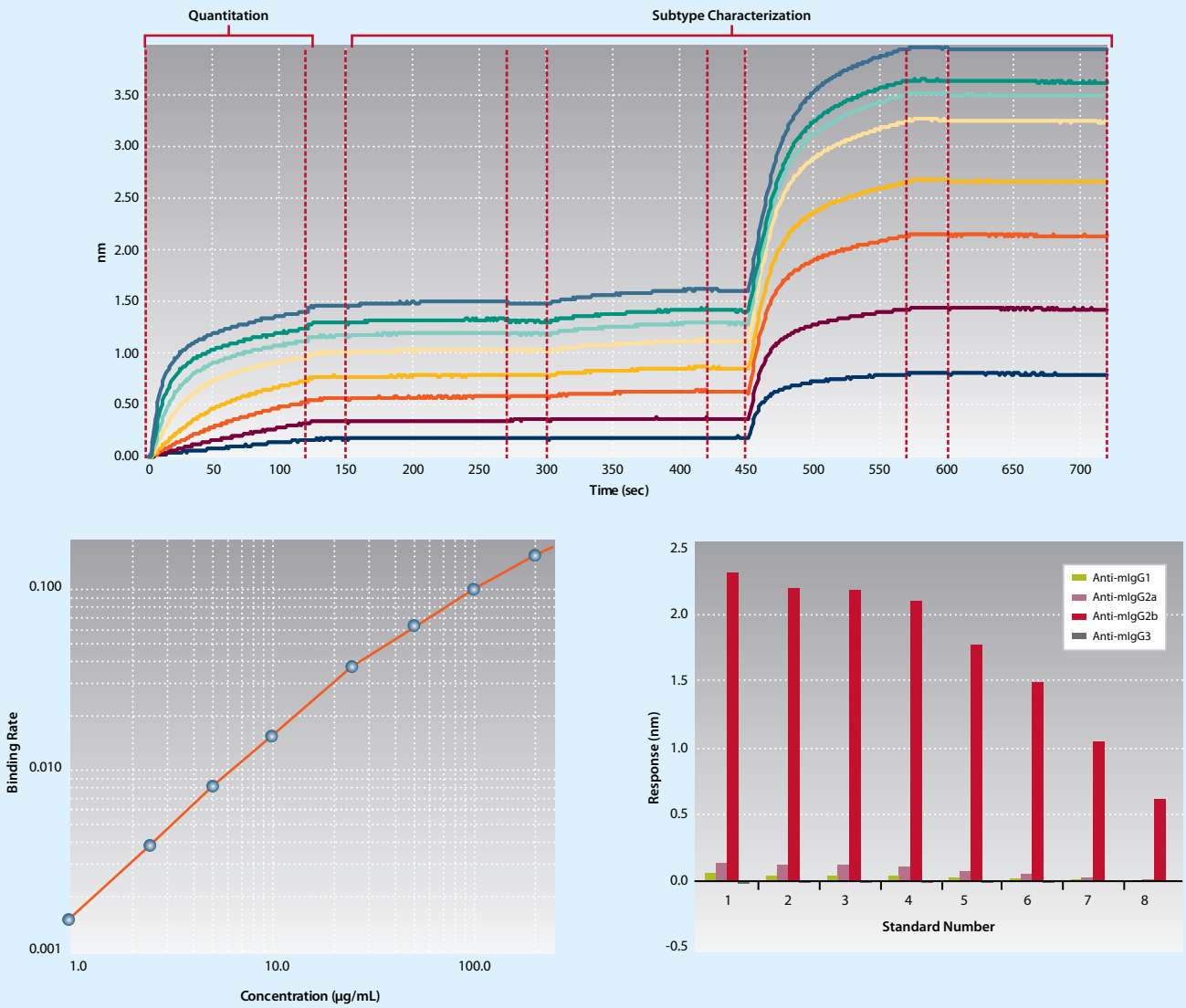

FIGURE 5: Mouse IgG quantitation and subtype characterization using IgG2b standards.

Mouse IgG Quantitation and Subtype Characterization Using IgG2b Standards

The data in Figure 5 was generated using ForteBio Anti-Murine Fv biosensors and an Octet RED system. The top panel 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 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.

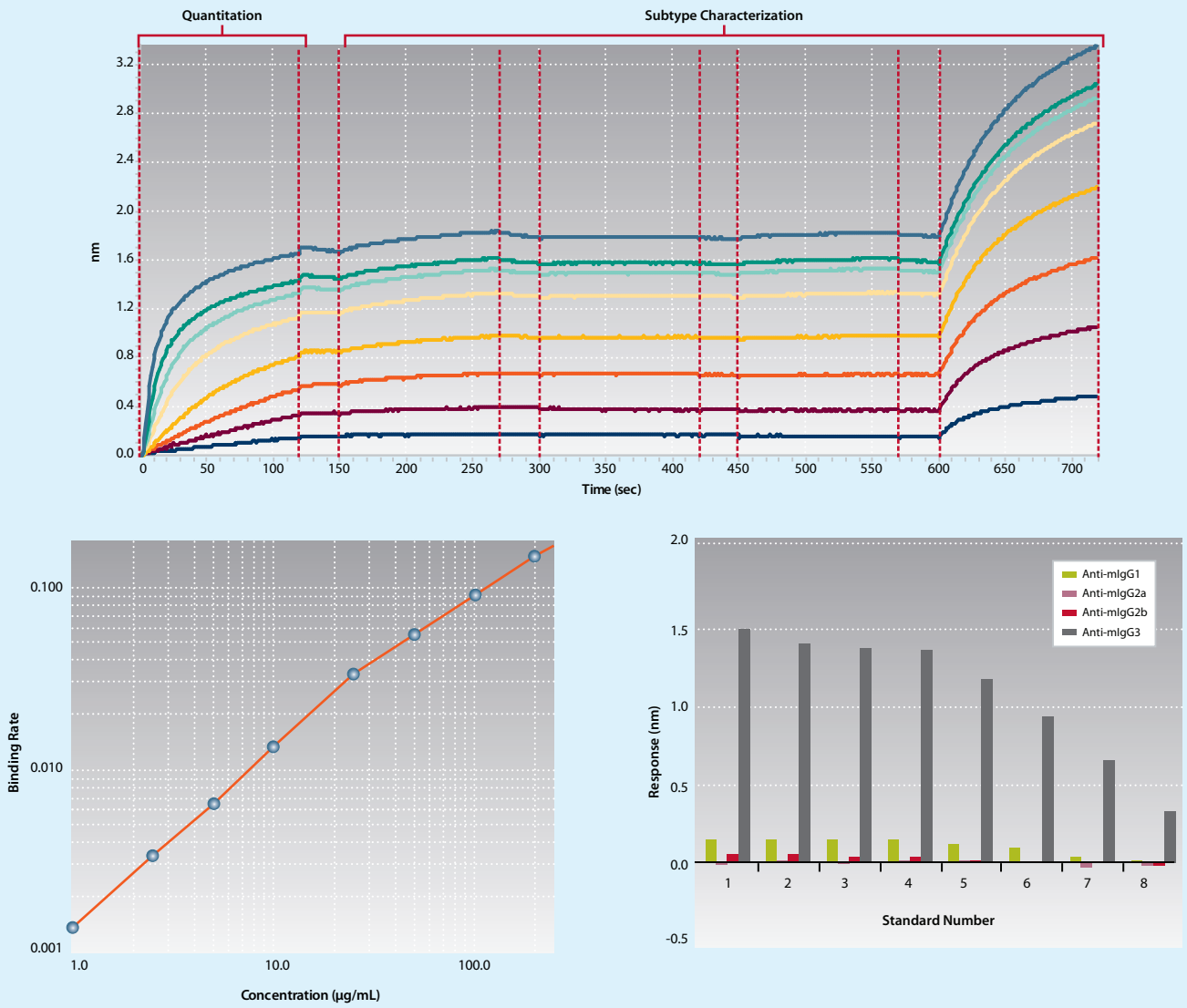

FIGURE 6: Mouse IgG quantitation and subtype characterization using IgG3 standards.

Mouse IgG Quantitation and Subtype Characterization Using IgG3 Standards

The data in Figure 6 was generated using ForteBio Anti-Murine Fv biosensors and an Octet RED system. The top panel 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 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.

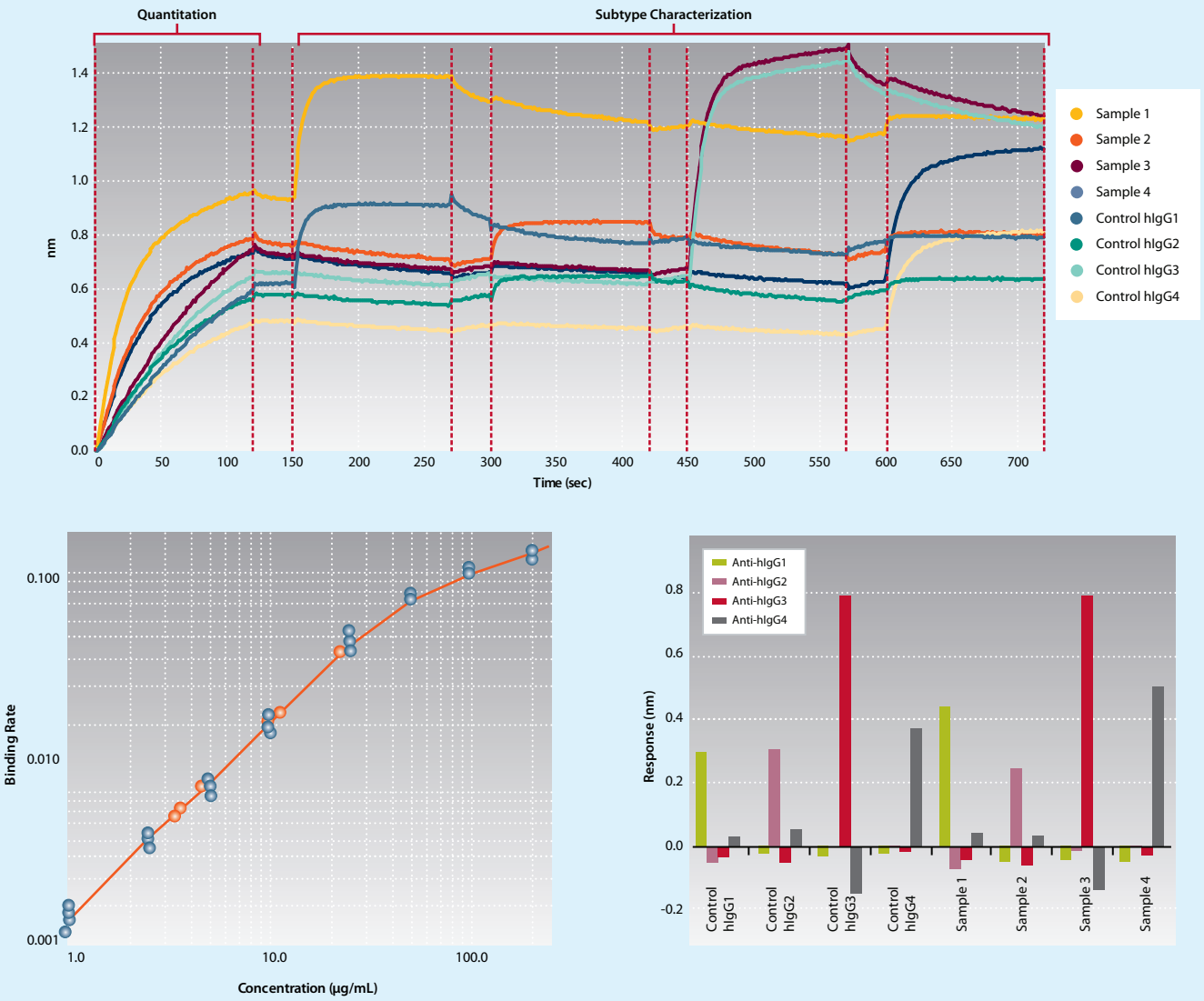

FIGURE 7: Human IgG quantitation and subtype characterization.

Human IgG Quantitation and Subtype Characterization

The data in Figure 7 was generated using ForteBio anti-Human IgG Fc biosensors and an Octet RED 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. The data shown here was generated by Jun Zhang of Medarex (a subsidiary of Bristol-Myers Squibb) and is used with permission.

DISCLAIMER

ForteBio reserves the right to change its products and services at any time to incorporate the latest technological developments. This technical note is subject to change without notice.

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P/N TN-3020 Rev A