

Octet® ARC Biosensors

For Kinetic and Quantitation Analysis of Rabbit IgG Antibodies



Technical Note

This Technical Note describes in detail kinetic and quantitation assay workflows using the Octet® ARC Biosensors for characterization of RbIgG proteins. It also provides guidelines on the best approaches for assay optimization using these biosensors.

Introduction

Rabbits have been used for decades as animal models in immunological investigations since rabbit antibodies possess features such as diverse immune response and sensitivity that make them attractive for many therapeutics applications. In addition, they are used in standard immunization procedures, with production and purification of monoclonal (mAbs) and polyclonal (pAbs) rabbit antibodies being well established and validated processes. Therefore, there is a need for easy and cost-effective methods to characterize these antibodies for various applications. The Octet® Anti-Rabbit Capture (ARC) Biosensors enable easy, high-throughput and label free kinetic and quantitation analysis of Fc-containing rabbit IgG (RbIgG) antibodies in both crude and purified cell culture samples during the development and manufacture of rabbit IgG-derived therapeutics.

The Octet® ARC Biosensors are highly specific towards the only isotype of RbIgG and do not cross-react with other species such as human, monkey, mouse, and rat IgG (Figure 1, (A)). Furthermore, these sensors show strong binding to rabbit Fc fragment with no binding to rabbit Fab fragment and minimal to no binding to specific isotypes of human IgG (Figure 1, (B)). These sensors offer high binding capacity, sensitivity and a wide dynamic range. They can be regenerated up to 20 times for kinetic and quantitation assays, while maintaining consistent and precise measurements.

This makes them a cost-effective option for a wide range of high-throughput applications, including lead identification and optimization, cell line development, process development, and QC in both crude and purified protein samples. This Technical Note describes in detail kinetic and quantitation assay workflows using the Octet® ARC Biosensors for characterization of RbIgG proteins. It also provides guidelines on the best approaches for assay optimization using these biosensors.

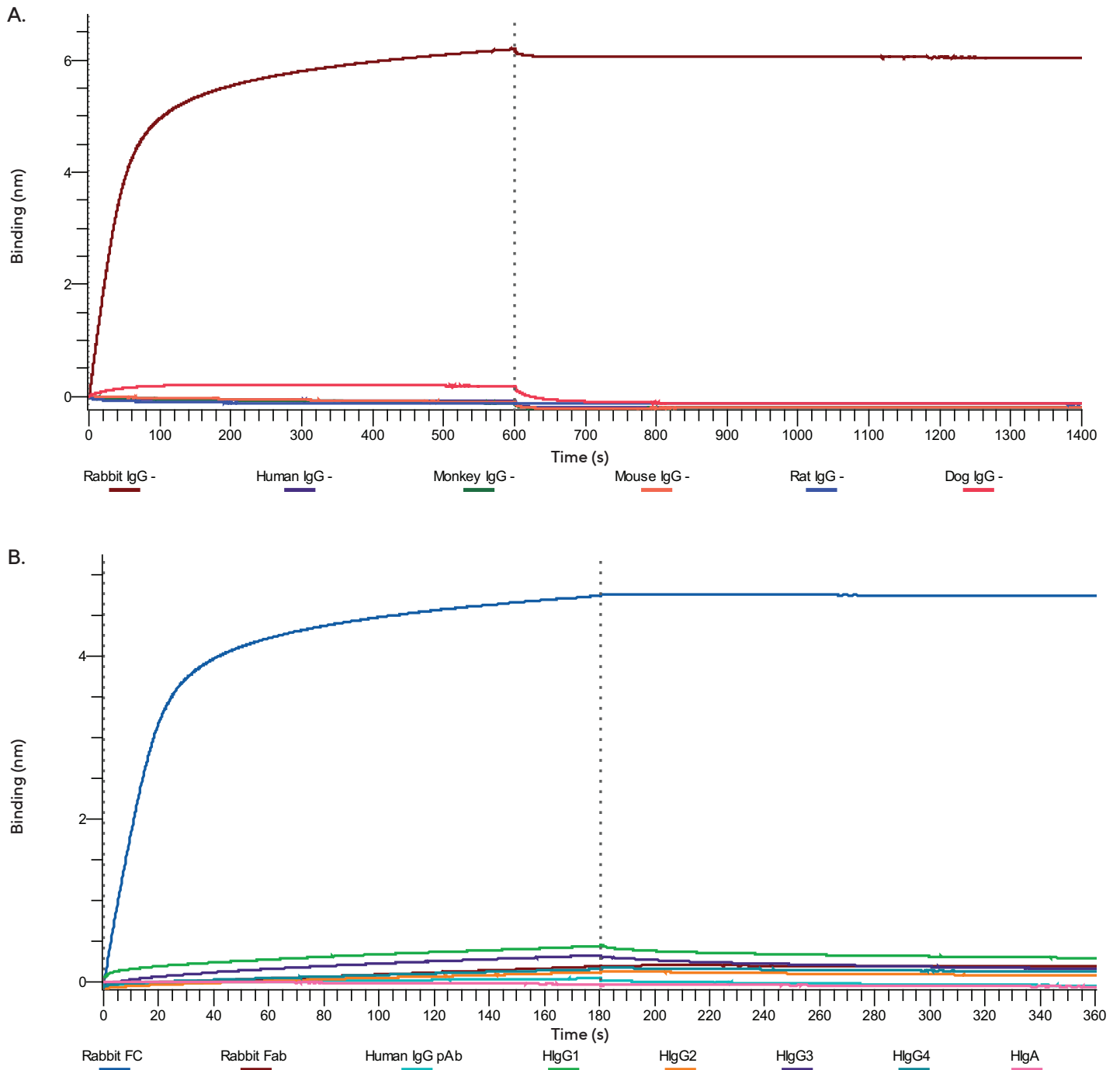


Figure 1: Specificity of the Octet® ARC Biosensors to the Rabbit IgG. (A) No appreciable binding is observed with IgGs from human, monkey, mouse, rat and dog. (B) High binding to rabbit Fc-fragment with zero or minimal binding to rabbit Fab-fragment, human IgG pAb, HlgG1, HlgG2, HlgG3, HlgG4 and HlgA.

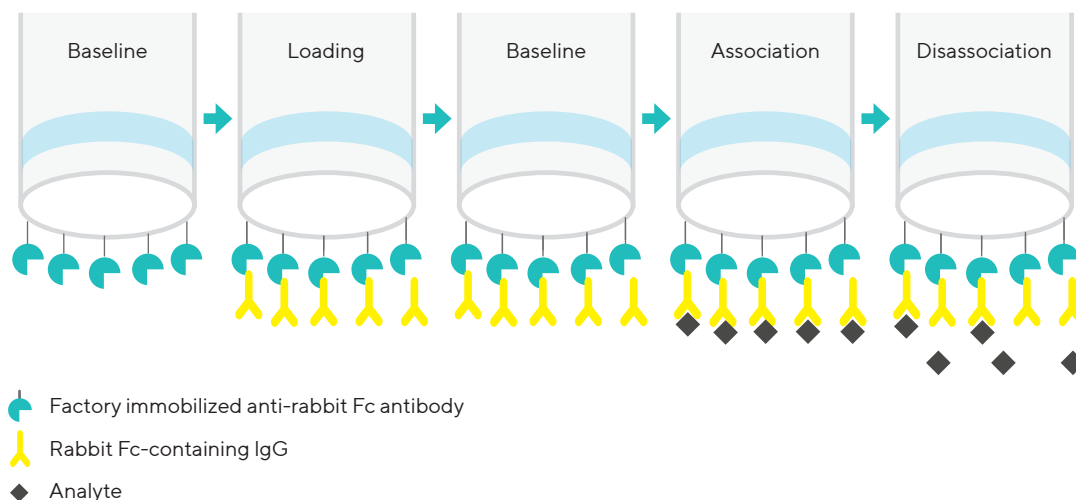


Figure 2: Kinetic assay workflow using the Octet® ARC Biosensors which typically include baseline (equilibration), loading (capture) of RbIgG, baseline, association and finally dissociation.

Kinetic Assay Workflow

The Octet® ARC Biosensors are pre-immobilized with anti-rabbit Fc-specific antibody, which enables the capture of RbIgG and rabbit Fc-containing proteins directly from crude or purified samples. These biosensors provide high binding capacity for RbIgG and Fc-containing proteins, making them particularly suitable for the analysis of proteins at low concentrations and for the characterization of small proteins. An example assay workflow utilizing the Octet® ARC Biosensors to characterize the interaction between an analyte and RbIgG is outlined in Figure 2.

Materials Required

- Octet® BLI system with Octet® BLI Discovery and Octet® Analysis Studio Software
- Octet® ARC Biosensors (Sartorius part no. 18-5168 (tray), 18-5169 (pack), 18-5170 (case))
- For all Octet® BLI systems: 96-well, black, flat bottom microplate (Greiner Bio-One part no. 655209)
- Optional for Octet® RH16 and RH96 BLI systems: Octet® 384-well, black, tilted bottom polypropylene microplate (Sartorius part no. 18-5166 (pack); 18-5167 (case)), 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209).
- RbIgG for immobilization. The RbIgG can be present in either a buffer or a complex mixture such as cell culture supernatant.
- Analyte protein that interacts with RbIgG. The analyte proteins can be dissolved in a buffer solution or a complex mixture such as a cell culture supernatant. The buffer matrix of the analyte should be identical to the baseline buffer immediately prior to the association step, where the concentrations of the bulk components of the baseline buffer and the analyte buffer are the same.

- Assay buffer: Octet® 1X Kinetics Buffer (1XKB) + 0.1% BSA is recommended for kinetic assays. This buffer can be prepared by diluting Octet® 10X Kinetics Buffer (Sartorius part no. 18-1105) with 1X PBS, pH 7.4 and adding 0.1% (w/v) BSA. As an example, to make 100 mL of the Octet® 1X Kinetic Buffer (1XKB) + 0.1% BSA (the assay buffer), add 10 mL Octet® 10X KB to 90ml 1X PBS, pH7.4, and add 100 mg BSA to this solution. Other buffers can also be used. The best results are obtained when all matrices are matched as closely as possible.

Assay Optimization Steps

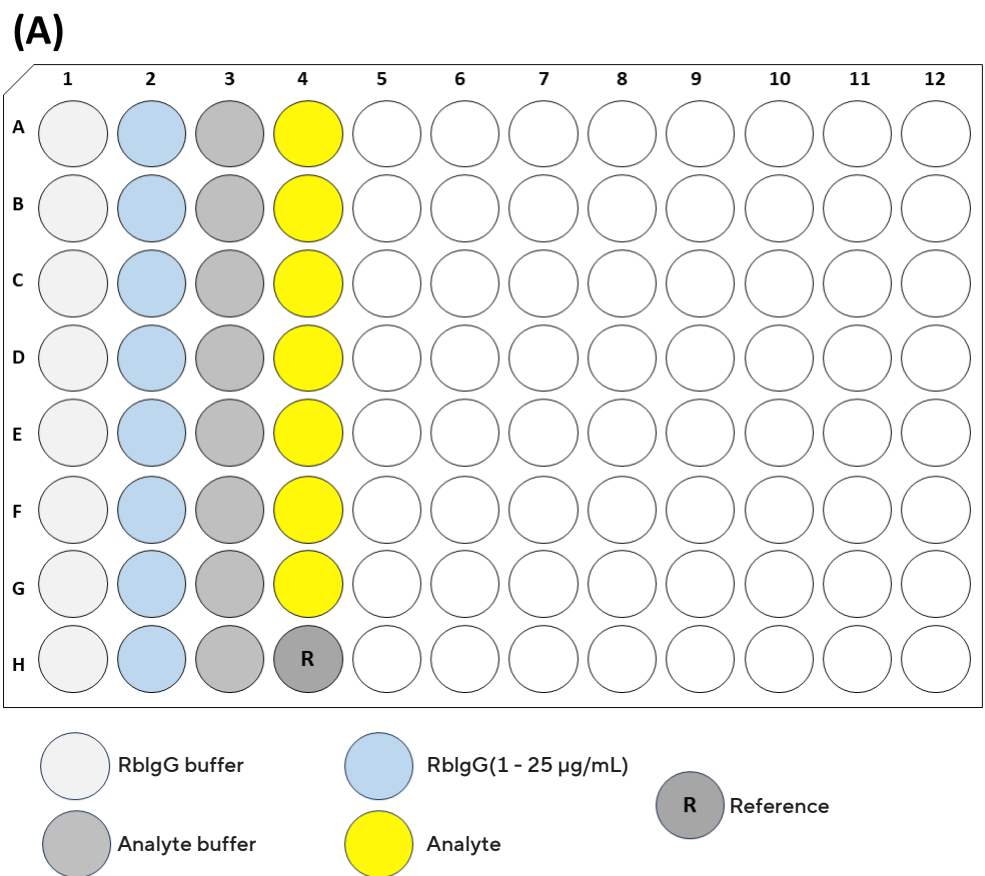
When using a capture-based biosensor, some background level of dissociation of the captured IgG ligand from the sensor will occur resulting in a baseline drift. Use a reference sample to correct for this baseline drift. The reference sample is a buffer-only negative control where the biosensor which has been coated with the ligand in the loading step, is exposed to buffer-only well in the association step. By subtracting these negative-control samples from the association and dissociation steps, this background dissociation, or assay drift can be accounted for. In addition, 1XKB + 0.1% BSA gives the lowest baseline drift and therefore is strongly recommended to be used for minimizing this background baseline drift.

The dissociation step and the baseline step right before the association should be performed in the same wells for each biosensor. This enables the inter-step correction feature to align the association and dissociation steps when processing data.

Assay Procedure

For details on setting up a kinetic assay in Octet® software, please refer to the Octet® BLI Discovery Software User Guide. Figure 3 shows an example microplate layout and assay design for a kinetic characterization assay using the Octet® ARC Biosensors.

For all steps, use a 200 µL sample volume for 96-well plates, 80 µL for standard 384-well plates and 40-80 µL for tilted 384-well plates.



(B)

| Step # | Column # | Description | Step Type | Time (s) | Shaking Speed (RPM) |
|--------|----------|----------------------------|-----------------|----------|---------------------|
| 1 | 1 | Equilibration Buffer | Custom/Baseline | 180 | 1000 |
| 2 | 2 | Loading of RblgG | Loading | 120-600 | 1000 |
| 3 | 3 | Baseline in Analyte Buffer | Baseline | 300-600 | 1000 |
| 4 | 4 | Association of Analyte | Association | 300-900 | 1000 |
| 5 | 3 | Dissociation of Analyte | Dissociation | 300-3600 | 1000 |

Figure 3: (A) Sample plate map and (B) assay steps with corresponding parameters for the ARC kinetic assay.

Before the Assay

Warm up all reagents and samples to room temperature. Pre-hydrate the ARC biosensors in 200 μ L per well of a similar matrix as RblgG to be captured. Pre-hydration is performed in a 96-well, black, flat-bottom plate for a minimum of 10 minutes. Set up the assay procedures according to the plate map and assay steps shown in Figure 3, or a custom procedure.

Assay Steps

Assay Step 1 – *Equilibration of the pre-hydrated ARC biosensors in 1XKB + 0.1% BSA or RblgG custom buffer*

Add buffer, media, or diluted lysate to column 1 of the sample plate according to the map in Figure 3. Note the equilibration buffer should match the buffer matrix of RblgG to be captured.

Assay Step 2 – *Capture of RblgG or Fc-containing protein (Loading/Immobilization)*

Dilute the RblgG or Fc-containing protein to the appropriate concentration in 1XKB + 0.1% BSA or the corresponding sample matrix and add the solution to the sample plate. The matrix or buffer used should typically match the one used for equilibration in assay step 1. The typical immobilization concentration is 1-25 μ g/mL and should be optimized for each interaction being studied. The concentration of ligand to be used will depend on its affinity for the associating analyte, as well as the size of both ligand and analyte.

For the best kinetic data and most accurate affinity constants, a loading optimization experiment should be performed to determine the optimal ligand loading concentration and time. Load only enough ligand so that the highest concentration of analyte used has adequate association signal at equilibrium and allows measurement of the dilution series. Loading more ligand than what is needed can cause artifacts such as non-specific binding, heterogeneity, or mass transport limitation. Loading optimization is recommended to define the optimal ligand density.

Assay Step 3 – *Baseline step in assay buffer (Baseline)*

Add 1XKB + 0.1% BSA or alternative buffer matching the analyte samples being analyzed to the sample plate according to Figure 3. It is important to match the baseline buffer matrix to that of the analyte samples, where the concentrations of the bulk components of the baseline buffer and the analyte buffer are the same. The baseline step should be run for a long enough time to allow for any change in baseline drift to stabilize. We recommend 300-600 seconds of baseline if a new buffer matrix is used in this step. If the buffer is identical to the RblgG ligand buffer, a baseline step of 120-300 seconds should be adequate.

Assay Step 4 – *Association to interacting analyte (Association)*

If detailed kinetic characterization is being performed, the analyte protein must be purified and of known concentration. It is recommended to run a titration series of at least four to five concentrations of the analyte protein and perform a global fitting of all concentrations to determine k_{on} , k_{off} , and K_D values. The highest analyte concentration should be 10 times greater than the expected K_D . For screening assays or qualitative interaction analysis, a single concentration of the interacting protein can be sufficient to characterize the binding. Analyte samples must be diluted in the same buffer used for the baseline and dissociation steps. Include a reference sample, consisting of assay buffer blank with no analyte present in this step, to enable subtraction of background baseline drift.

Assay Step 5 – *Dissociation of interacting analyte (Dissociation)*

The dissociation step is performed in the same buffer well(s) used for the baseline step (step3). Using the same wells for baseline and dissociation enables the inter-step correction feature to be used in data analysis for more accurate curve fitting.

Process and Analyze Data

1. Load data into the Octet® Analysis Studio Software.
2. Process the data by specifying methods for reference subtraction, y-axis alignment by baseline, inter-step correction by dissociation and check the Savitzky-Golay filtering.
3. Analyze the data by specifying steps for analysis, fitting method (1:1 binding, global fit) and time window of interest.
4. To export the analyzed data, click "Save Report" to generate an Excel report.

Representative Data

Figure 4 shows ARC biosensors loaded with rabbit IgG (Thermo Fisher Scientific, Cat.no. 02-6102) at 5 $\mu\text{g}/\text{mL}$, followed by kinetic analysis of an analyte AffiniPure Fab fragment goat anti-rabbit IgG (H+L) (50 kDa) (Jackson Immuno Research, Code:111-007-003). The kinetic analysis results are summarized in Table 1.

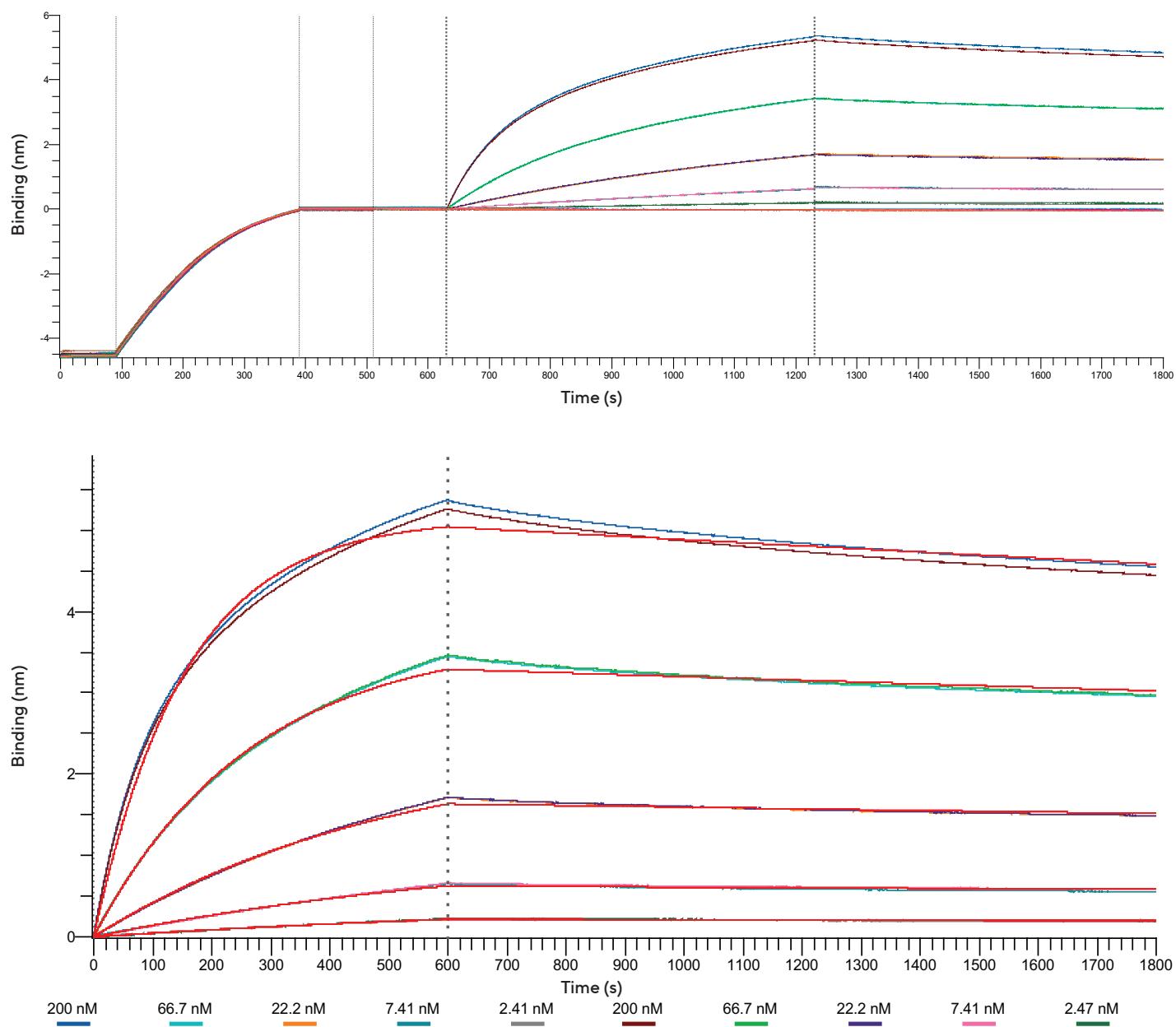


Figure 4: Binding kinetics of RbIgG (150 kDa) and an analyte fab fragment goat anti-RbIgG (H+L) (50 kDa), on the Octet® BLI platform. (A) The raw data for a full assay. 1XKB +0.1% BSA was used as a buffer matrix throughout the assay. (B) The association and dissociation traces after data processing (including reference subtraction using the 0 nM trace and fitting using a 1:1 binding model).

| k_s (1/Ms) | k_d (1/s) | K_b (M) |
|--------------|-------------|-----------|
| 2.82E04 | 1.63E-04 | 5.78E-09 |

Table 1: Binding kinetics of RbIgG (150 kDa) and an analyte fab fragment goat anti-RbIgG (H+L) (50 kDa), on the Octet® BLI platform.

Quantitation Assay Workflow

The Octet® ARC Biosensors can be used to quantitate both crude and purified samples with concentrations in the range of 0.05–4,000 µg/mL depending on the assay conditions. For RbIgG samples with concentration range of 0.5–4,000 µg/mL, it is recommended to use a shaking speed of 400 RPM with a 2-min assay read time. However, if RbIgG concentration is in the range of 0.05–0.5 µg/mL, it is typically recommended to use 96-well plate as the sample plate, shaking speed of 1,000 RPM and a longer 10-min assay time for improved sensitivity.

Materials Required

- Octet® BLI systems with Octet® BLI Discovery and Analysis Studio Software.
- Octet® ARC Biosensors (Sartorius part no. 18-5168 (tray), 18-5169 (pack), 18-5170 (case)).
- For all Octet® instruments: 96-well, black, flat bottom microplate (Greiner Bio-One part no. 655209) -Optional for Octet® RH16 and RH96 BLI systems: Octet® 384-well, black, tilted bottom, polypropylene micro-plate (Sartorius part no. 18-5166 (pack); 18-5167 (case)) 384-well, black, flat bottom, polypropylene microplate (Greiner Bio-One part no. 781209).
- Purified standard protein (that is of the same molecule as the unknown samples) to be used as a calibration standard.
- Octet® Sample Diluent (Sartorius part no. 18-1104) for dilution of all samples. If undiluted crude samples are to be quantified, a blank buffer (that is free of the molecules of interest) with the same matrix is required.

Best Practices

- The following practices are recommended each time when the quantitation assay involves a new matrix or new RbIgG protein to get the best results:
- The calibration standard should be identical to the molecule present in the unknown sample for best results.
- Concentrations of the calibration standards should cover the range of concentrations in the unknown samples.
- Match the matrix of the samples, standards, references, and pre-hydration solution as closely as possible.
- Use a blank negative control in a matching matrix for background signal subtraction. This is especially important when optimizing accuracy and detecting low-concentration analytes.
- Determine the minimal dilution factor required to achieve the targeted assay performance.
- Perform a spike/recovery study to determine the assay dynamic range.
- Qualify the reagents and buffers used in the assays routinely and use best laboratory practices to aliquot and store reagents and samples.

- Establish data analysis parameters in Octet® Analysis Studio Software.
- Apply the finalized protocol and data analysis parameters in routine assays.

Dilution Factor Determination for Sample Matrix

Components in complex matrices such as cell culture media can potentially interfere with assay performance. Diluting the sample matrix using the Octet® Sample Diluent (SD) is an effective means of minimizing matrix effects. Dilution factor guidelines for various sample types are described in Table 2. However, before running a quantitation assay it should be empirically determined whether dilution of samples is needed.

| Sample Type | Minimum recommended dilution using the Octet® Sample Diluent |
|-------------------------------------|--|
| RbIgG in Octet® Sample Diluent (SD) | Neat |
| RbIgG in CHO | Neat |
| RbIgG in DMEM | 2-fold |
| RbIgG in 50% DMEM.SD | Neat |
| RbIgG in 10% FBS+DMEM | Neat |
| RbIgG in PBS | Neat |
| RbIgG in 50% PBS.SD | Neat |

Table 2: Recommended minimum dilution for common sample types. In all cases the matrix for the diluted samples, the standards and the biosensor hydration solution should be matched as closely as possible.

1. Prepare 1 mL of each sample matrix (without target protein) diluted both 2-fold and 10-fold in the Octet® Sample Diluent buffer.
2. Add target protein to the neat matrix, each of the matrix dilutions, and to sample diluent as a control. The final concentration of the target protein in each of the four samples should be in the middle of the desired quantitation range.
3. Transfer each sample to a 96- or 384-well sample plate in duplicate (eight wells total).
4. Pre-hydrate biosensors in the sample matrix that matches each sample type (e.g., biosensors to be used in wells with a 10-fold diluted matrix should be hydrated in the 10-fold diluted matrix).
5. Set up a basic quantitation assay according to the Octet® BLI Discovery Software User Guide.
6. Run the assay.
7. Data will be displayed in real-time during the assay. Data and method files will be saved automatically to a location specified by the user.
8. Load data into Octet® Analysis Studio Software.
9. Visually inspect the real-time binding traces and determine the dilution required to:
 - a. Minimize non-specific binding of matrix components.
 - b. Show equivalent binding in the matrix spiked sample and the sample diluent control.
10. Use this dilution factor for routine assays.

Assay Precision and Accuracy

To determine the quantitation range in any matrix, a precision and accuracy study should be carried out as follows:

1. Prepare a series of protein standards in the appropriate matrix diluent using the dilution factor determined in the dilution factor determination for sample matrix experiment. The dilution series should span the entire range of the assay based upon user experimental goal, such as 0.5–4,000 µg/mL for assays run at 400 RPM.
 2. Using the same matrix diluent as in Step 1, prepare two protein samples of known concentration for recovery measurement. The concentration of these samples should be within the range of the standard curve being generated, preferably one at the low end and one at the high end. These will be defined as unknown samples in the assay for calculating recovery.
 3. Transfer triplicates of the prepared standards and the samples to a sample plate. It is recommended to organize samples in columns, from A–H. Fill at least one well with blank diluted matrix for reference subtraction during data analysis. An example plate map is shown in Figure 5.
 4. Hydrate biosensors for 10 minutes in matching matrix diluent.
 5. Set up a basic quantitation assay using the same assay parameters that were used in the dilution factor determination for matrix experiment. Define sample replicate groups to calculate replicate averages and %CVs.
6. Run the experiment. Data will be displayed in real time during the assay. Data files, method files and assay pictures will be saved automatically.
 7. Load the data into Octet® Analysis Studio Software.
 8. If a blank matrix was included as a reference, use the reference subtraction option to correct the data as appropriate.
 9. Calculate the binding rate. The results table will populate with calculated concentrations and data statistics.
 10. Define assay dynamic range by selecting acceptable %CV values for the lower and upper concentration limits in the standard curve.
 11. Exclude data points for the standard curve that lie outside the defined dynamic range if necessary.
 12. Select the appropriate equation to fit the standard curve. Linear point to point or 5PL (weighted Y^2) are recommended for the Octet® ARC Biosensors. 5PL standard curve equation is used for an asymmetric sigmoidal concentration, therefore it incorporates different dilution series and weighted Y^2 fits the lower end concentrations better.
 13. Evaluate the accuracy and precision of the assay using calculated concentration value of the unknowns to determine % recovery and %CV.

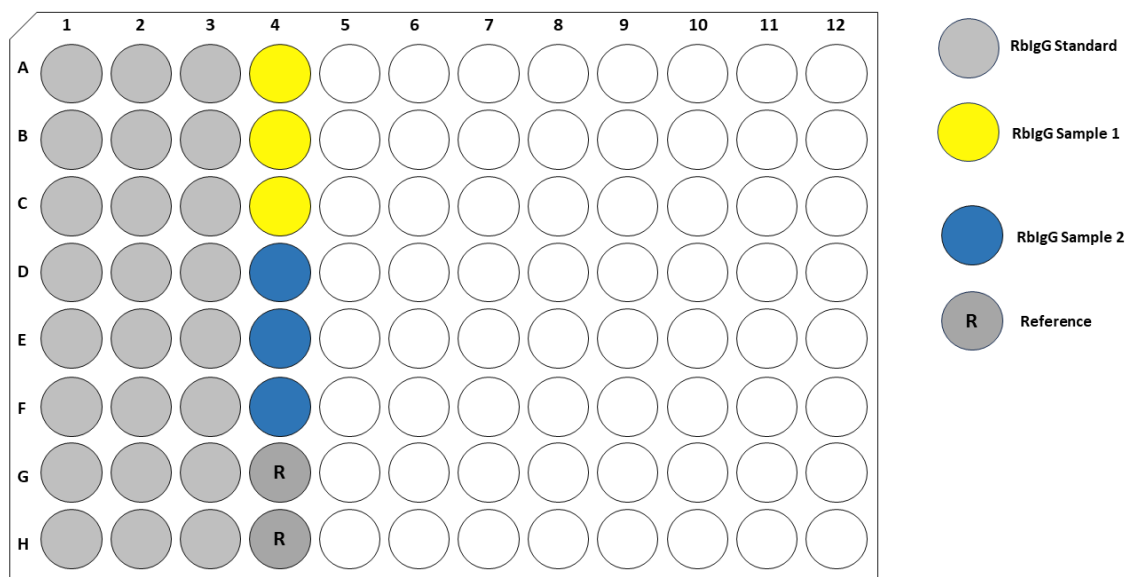


Figure 5: Example plate layout for a spike recovery assay.

Running the Assay to Quantify the Protein of Interest

1. Prepare samples, calibration standards and hydration solution according to the conditions determined in optimization steps in the prior sections.
2. Set up a basic quantitation assay using the parameters described previously in the optimization experiments. See Figure 6 for example assay set up.
3. Run the assay.
4. Load data into Octet® Analysis Studio Software. Analyze as in previous optimization steps to determine concentration of samples and data statistics.
5. To export the analyzed data, click "Save Report" and select desired format, Excel or PDF.

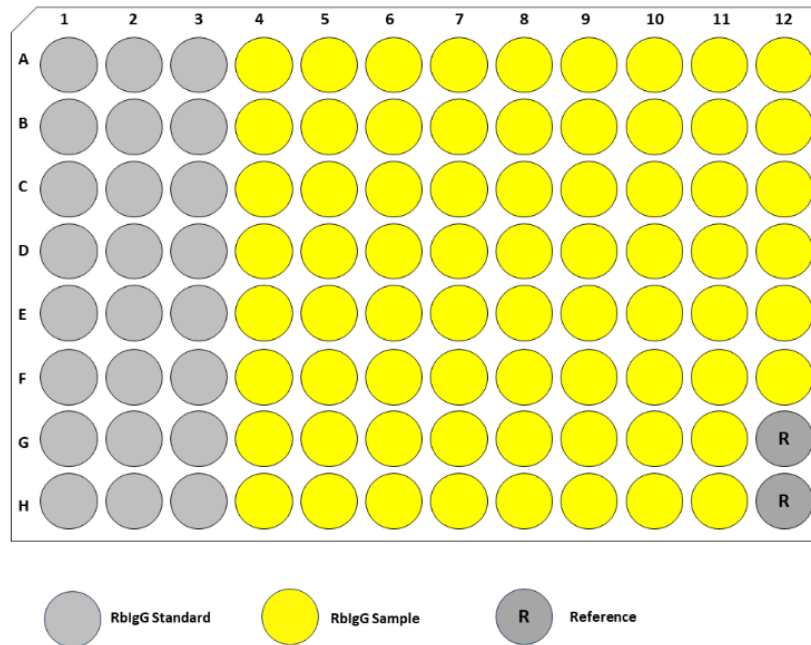


Figure 6: Example plate layout for a routine quantitation assay run in a 96-Well microplate.

Representative Data

Figure 7 and 8 show the detection of RbIgG using the ARC biosensors on the Octet® RH16 system. Two standard curves with two different assay settings were run to demonstrate quantitation dynamic range (0.05 - 4,000 µg/mL). Tables 3 and 4 show the calculated concentration, %CV within the replicates and the recovery for the concentration.

It is important to point out that in order to calculate accurate data for the highest and the lowest ends of the dynamic range, one more level of concentration is recommended to be added to the both ends. For example, to acquire accurate data for 4,000 µg/mL and 0.05 µg/mL, it is recommended to incorporate 8,000 µg/mL and 0.025 µg/mL at both ends of the dilution and standard curve.

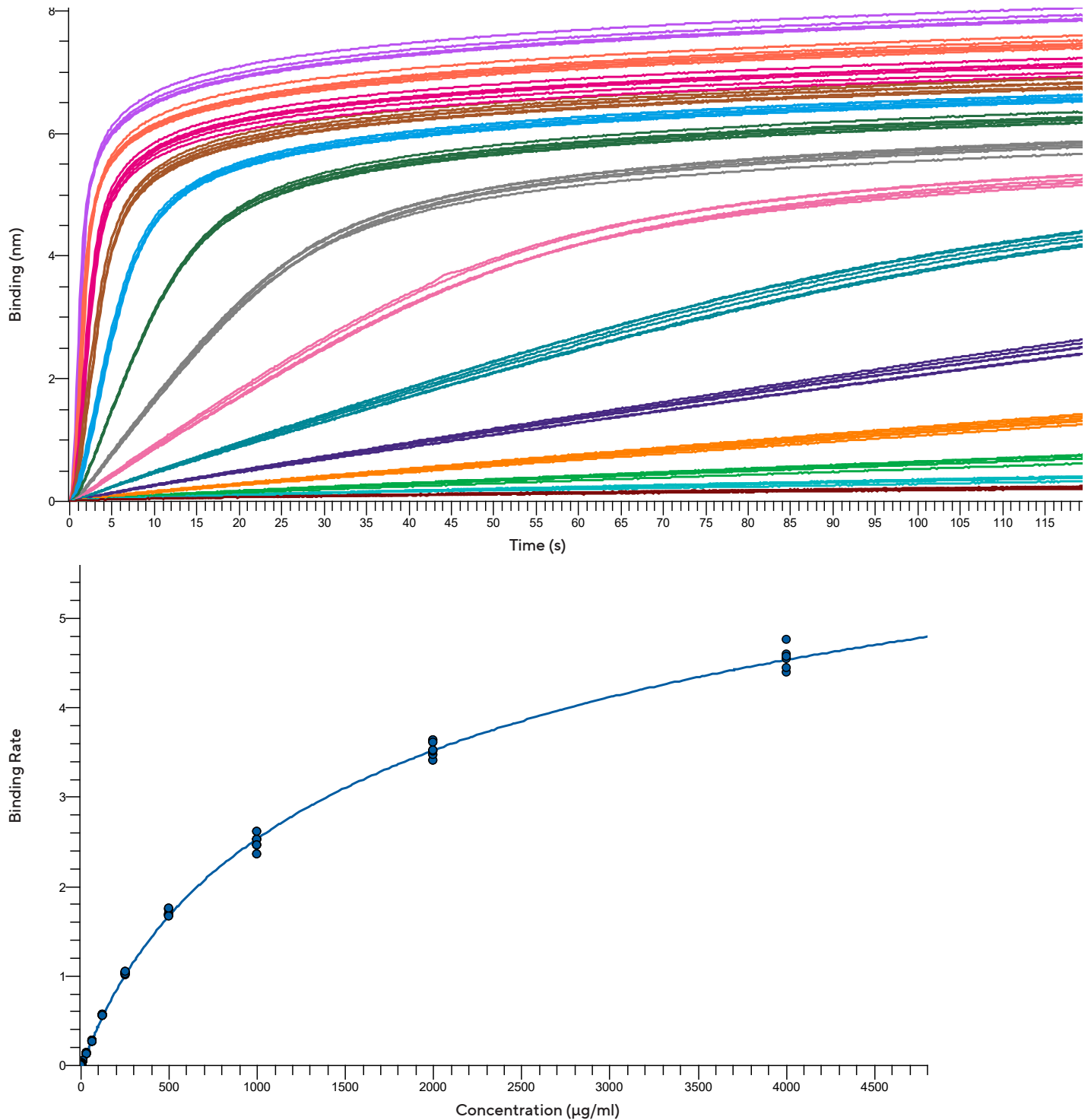


Figure 7: Quantitation of RbIgG using the Octet® ARC Biosensors. (A) RbIgG dose response for concentrations within the dynamic range of 0.5 - 400 µg/mL with six replicates on the Octet® RH16 instrument with assay parameters: 400 rpm, 2 min. Colors show concentrations in µg/mL (B) RbIgG standard calibration curve generated from six replicates and calculated using 5PL (weighted Y²) fitting model. The Octet® Sample Diluent Buffer was used as a matrix for all samples.

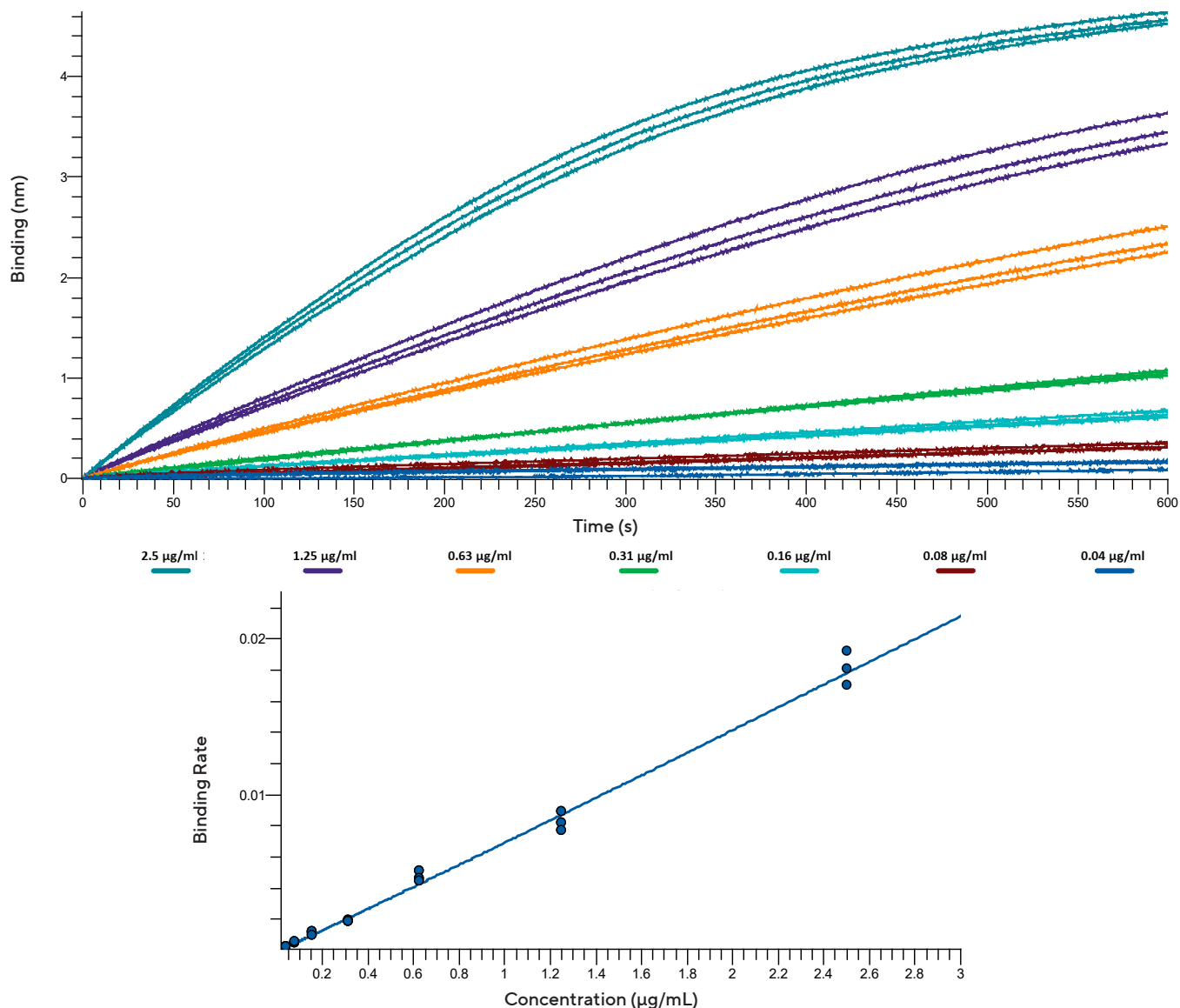


Figure 8: RblgG dose response for concentrations within the dynamic range of 0.04 - 2.5 µg/mL in triplicates on the Octet® RH96 instrument with assay parameters: 1,000 rpm, 10 min. (B) RblgG standard calibration curve generated from triplicates and calculated using 5PL (weighted Y) fitting model. The Octet® Sample Diluent Buffer was used as a matrix for all samples.

| Known Conc. (µg/mL) | Calculated Conc. (µg/mL) | %CV (n=6) | Recovery |
|---------------------|--------------------------|-----------|----------|
| 4000 | 3712.55 | 7% | 93% |
| 2000 | 1788.58 | 6% | 89% |
| 1000 | 897.45 | 6% | 90% |
| 500 | 503.23 | 3% | 101% |
| 250 | 267.30 | 1% | 107% |
| 125 | 137.61 | 1% | 110% |
| 62.5 | 66.42 | 2% | 106% |
| 31.25 | 33.81 | 4% | 108% |
| 15.63 | 14.37 | 5% | 92% |
| 7.81 | 6.82 | 3% | 87% |
| 3.91 | 3.80 | 3% | 97% |
| 1.95 | 2.18 | 8% | 112% |
| 0.98 | 1.01 | 10% | 103% |
| 0.49 | 0.58 | 7% | 119% |

Table 3: Calculated concentrations, %CV and %Recovery for RblgG (0.49-4,000 µg/mL) quantitation assay with six replicates.

| Known Conc. ($\mu\text{g/mL}$) | Calculated Conc. ($\mu\text{g/mL}$) | %CV (n=6) | Recovery |
|----------------------------------|---------------------------------------|-----------|----------|
| 2.50 | 2.68 | 6% | 107% |
| 1.25 | 1.21 | 8% | 97% |
| 0.63 | 0.69 | 7% | 110% |
| 0.31 | 0.28 | 4% | 90% |
| 0.16 | 0.17 | 10% | 107% |
| 0.08 | 0.08 | 4% | 105% |
| 0.04 | 0.04 | 20% | 100% |

Table 4: Calculated concentrations, %CV and %Recovery for RblgG (0.04-2.5 $\mu\text{g/mL}$) quantitation assay in triplicates.

Regeneration of Octet® ARC Biosensors

The Octet® ARC Biosensors can be cost-effectively regenerated and re-used up to 20 times in kinetic and quantitation assays to generate replicate data for ligand-analyte pairs, or to analyze large numbers of samples in sequence. Typically, the regeneration is performed by dipping the biosensors into a solution of 10 mM glycine pH 1.7 for 5 seconds, followed by a dip in the assay buffer for 5 seconds. These regeneration steps should be repeated 3–5 times in sequence to fully remove bound RblgG or the interaction complex. After regeneration, the biosensor can be immobilized with RblgG for a new analysis. For best results it is recommended to pre-condition biosensors by running the regeneration protocol prior to loading the ligand the first time.

Regeneration results will depend on the captured molecule and a small loss in binding capacity may occur after each regeneration cycle.

The exact number of possible regenerations should be determined experimentally and will depend on assay precision requirements. See example of kinetic and quantitation assays with 20 regeneration cycles in Figures 9 and 10, and Tables 5 and 6.

Regeneration tips

- Depending on the assay conditions or protein being captured, the regeneration buffer and/or conditions may require additional optimization.
- It is recommended to pre-condition the biosensors before the first assay cycle for most consistent results when incorporating regeneration. Biosensors are pre-conditioned by performing the regeneration procedure one time prior to the first loading step.
- It is important to ensure that the regeneration of biosensors for quantitation applications is complete. This is because the quantitation results are significantly dependent on surface capacity of the sensor. For example, a loss of 20% capacity over multiple regeneration cycles could affect precision of quantitation by 10–20%.

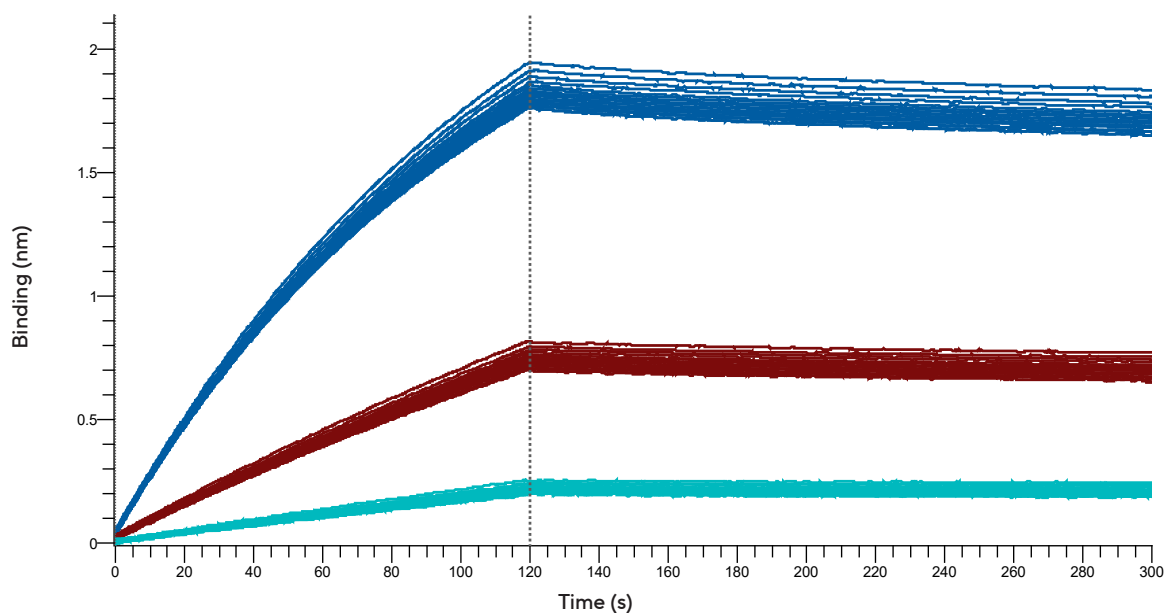


Figure 9: Overlay of association-dissociation curves for RblgG-Fab anti-RblgG kinetic assay after 20 regeneration cycles. The data traces overlap closely, and kinetic parameters show low variability from cycle to cycle for 20 regenerations.

| Kinetic Assay | K_d (M) | k_a (1/Ms) | k_d (1/s) |
|----------------------------|-----------|--------------|-------------|
| Regen 1 | 5.81E-09 | 4.76E+04 | 2.77E-04 |
| Regen 2 | 6.34E-09 | 4.74E+04 | 3.00E-04 |
| Regen 3 | 6.57E-09 | 4.74E+04 | 3.11E-04 |
| Regen 4 | 6.65E-09 | 4.75E+04 | 3.16E-04 |
| Regen 5 | 6.63E-09 | 4.70E+04 | 3.12E-04 |
| Regen 6 | 6.99E-09 | 4.72E+04 | 3.30E-04 |
| Regen 7 | 6.82E-09 | 4.79E+04 | 3.26E-04 |
| Regen 8 | 6.74E-09 | 4.94E+04 | 3.33E-04 |
| Regen 9 | 6.73E-09 | 4.97E+04 | 3.34E-04 |
| Regen 10 | 6.74E-09 | 4.92E+04 | 3.32E-04 |
| Regen 11 | 6.68E-09 | 5.00E+04 | 3.34E-04 |
| Regen 12 | 6.78E-09 | 5.06E+04 | 3.43E-04 |
| Regen 13 | 6.51E-09 | 5.10E+04 | 3.32E-04 |
| Regen 14 | 6.38E-09 | 5.01E+04 | 3.20E-04 |
| Regen 15 | 6.56E-09 | 5.05E+04 | 3.31E-04 |
| Regen 16 | 6.73E-09 | 5.06E+04 | 3.41E-04 |
| Regen 17 | 6.47E-09 | 5.10E+04 | 3.30E-04 |
| Regen 18 | 6.43E-09 | 5.23E+04 | 3.36E-04 |
| Regen 19 | 6.62E-09 | 5.10E+04 | 3.38E-04 |
| Regen 20 | 6.53E-09 | 5.13E+04 | 3.35E-04 |
| Average (20 regenerations) | 6.58E-09 | 4.94E+04 | 3.25E-04 |
| %CV (20 regenerations) | 4% | 3% | 5% |

Table 5: K_d , k_a , and k_d values and the corresponding %CVs for RblgG-Fab anti RblgG binding assay over 20 cycles of regeneration using 10 mM Glycine pH 1.7

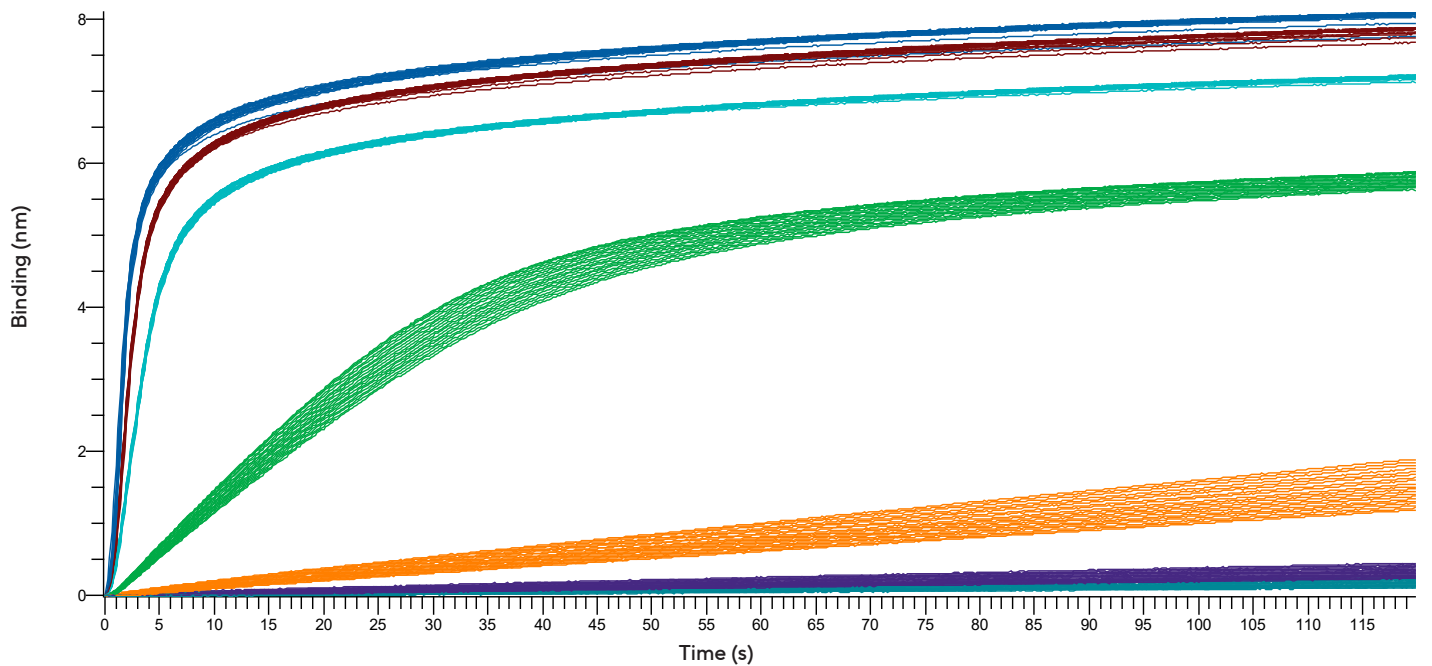


Figure 10: Overlay of binding curves for RblgG quantitation assay for concentration range of 0.5-2000 $\mu\text{g/mL}$ after 20 regeneration cycles.

| Known Well Concentration (ug/mL) | Average Calculated Concentration (20 Regenerations) | %CV (20 Regenerations) |
|----------------------------------|---|------------------------|
| 2000 | 2007.42 | 8% |
| 1000 | 1000.78 | 3% |
| 500 | 499.85 | 2% |
| 50 | 50.35 | 7% |
| 5 | 3.59 | 12% |
| 1 | 0.84 | 13% |
| 0.5 | 0.50 | 14% |

Table 6: Calculated concentrations after %CV for 20 cycles of regeneration for RblgG quantitation assay.

Summary

Octet® ARC Biosensors demonstrate high ligand binding capacity and high specificity that make them a reliable and versatile tool for kinetic and quantitation characterization of Fc-containing rabbit monoclonal and polyclonal antibodies in both purified and crude samples. Furthermore, they offer a wide dynamic range in quantitation applications making them suitable for titer applications in both upstream and downstream workflows. In addition, they can be regenerated for multiple cycles of re-use making them a cost-effective option for rabbit IgG samples detection, quantitation and kinetics based applications.

References

1. **Application Note: Biomolecular Binding Kinetic Assays on the Octet® BLI Platform**
2. Octet® BLI Discovery Software User Guide

Ordering Information

| Description | UOM | Cat. No. |
|--|------|----------|
| One tray of Octet® ARC Biosensors | Tray | 18-5168 |
| Five trays of Octet® ARC Biosensors | Pack | 18-5169 |
| Twenty trays of Octet® ARC Biosensors | Case | 18-5170 |
| Octet® Sample Diluent Buffer, 50 mL | Each | 18-1104 |
| Octet® Kinetics Buffer 10X (10X KB), 50 mL | Each | 18-1105 |

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