SARTORIUS

Octet® DYKDDDDK Biosensors

For Quantitation and Kinetic Characterization of FLAG®-Tagged Proteins

Technical Note

Scope: This Technical Note describes kinetic and quantitation assay workflows that use the Octet® DYKDDDDK Biosensors for characterization of FLAG®-tagged proteins.

Keywords or phrases: DYKDDDDK, FLAG, FLAG tag, Affinity Tag, Quantitation, Kinetics, Regeneration, Octet® BLI, Biosensors

Abstract

DYKDDDDK is a short peptide sequence (also known as FLAG® Tag) typically added to the N or C terminal ends of a protein of interest to facilitate easier purification and characterization. The Octet® DYKDDDDK Biosensors are designed to bind to the FLAG tag with high affinity and specificity, enabling easy, high-throughput, and label-free kinetic and quantitation analysis of FLAG-tagged proteins in both purified and crude cell culture samples.

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Introduction

Octet® DYKDDDDK Biosensors offer high binding capacity, sensitivity and a wide dynamic range. They can be regenerated up to five times for kinetic and 10 times for 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 that use the Octet® DYKDDDDK Biosensors for characterization of FLAG-tagged proteins and provides guidelines on the best approaches for assay optimization using these biosensors.

Kinetic Assay Workflow

The Octet® DYKDDDDK Biosensors are pre-immobilized with anti-FLAG tag-specific antibody, which enables the capture of FLAG tag containing proteins directly from crude or purified samples. These biosensors provide high binding capacity for FLAG tag proteins, making them particularly suitable for the analysis of proteins at low concentrations or proteins with lower molecular weights. An example assay workflow utilizing the Octet® DYKDDDDK Biosensors to characterize the interaction between a FLAG-tagged ligand and an untagged analyte is outlined in Figure 1.

Materials Required

- Octet® BLI system with Octet® BLI Discovery and Octet® Analysis Studio software
- Octet® DYKDDDDK Biosensors, Sartorius Part No. 18-5178 (Tray), 18-5179 (Pack), 18-5180 (Case)
- For all Octet® BLI systems: 96-Well, Black, Flat Bottom Microplate, Sartorius Part No. 18-5172 (Pack), 18-5173 (Case)
- Optional for Octet® R8e, 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)
- **FLAG-tagged protein for capture.** The FLAG-tagged protein can be present in either a buffer or a complex mixture such as cell culture supernatant.
- **Analyte protein that interacts with the FLAG-tagged protein.** 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 Kinetic Buffer (1X KB) 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. As an example, to make 100 mL of the 1X KB (the assay buffer), add 10 mL Octet® 10X KB to 90 mL 1X PBS, pH 7.4. Other buffers can also be used. The best results are obtained when all matrices are matched as closely as possible.
- **Optional regeneration buffer.** Octet® 10 mM Glycine pH 1.7 (Sartorius Part No. 18-1184).

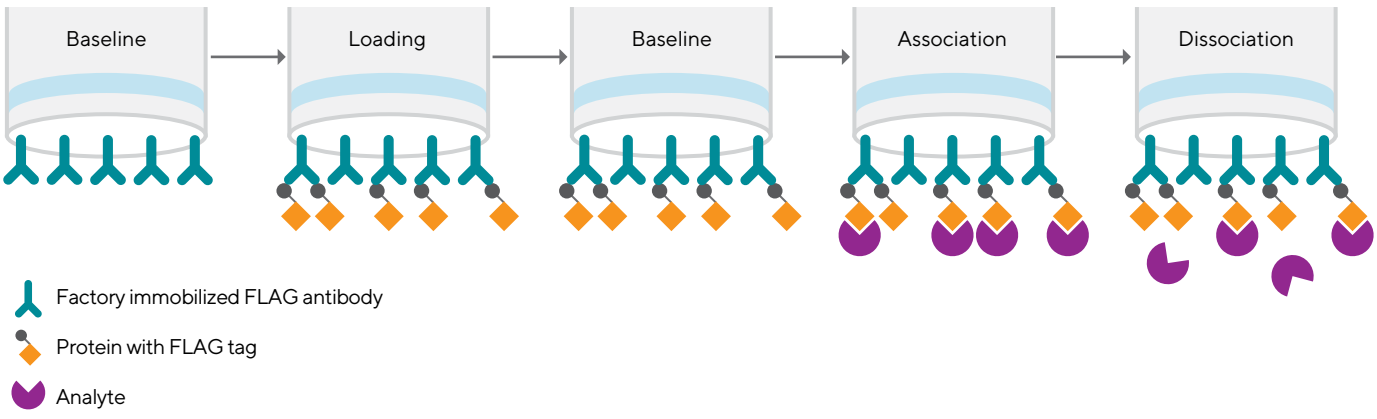


Figure 1. Kinetic assay workflow using the Octet® DYKDDDDK Biosensors which typically include Baselines (Equilibration), Loading (capture) of FLAG-tagged protein, Baseline, Association, and finally Dissociation.

Assay Optimization Steps

When using a capture-based biosensor such as the Octet® DYKDDDDK Biosensor, some background level of dissociation of the captured IgG ligand from the biosensor will occur resulting in a baseline drift. Please note that baseline drift will vary slightly between ligands due to ligand-specific effects of amino acids in spatial proximity to the FLAG tag. Use a reference sample to correct for this ligand-specific baseline drift. The reference sample is a buffer-only negative control where the biosensor which has been immobilized with the FLAG-tagged ligand in the loading step is exposed to a 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, 1X KB 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 step 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.

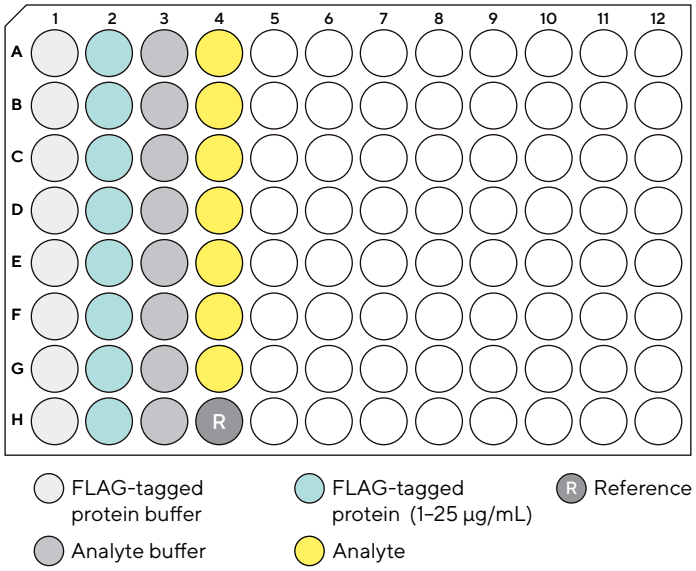
Assay Procedure

For details on setting up a kinetic assay in Octet® software, please refer to the Octet® BLI Discovery Software User Guide. Figure 2 shows an example microplate layout and assay design for a kinetic characterization assay using the Octet® DYKDDDDK 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.

Before the Assay

Warm up all reagents and samples to room temperature. Pre-hydrate the Octet® DYKDDDDK Biosensors in 200 µL per well of a similar matrix as FLAG-tagged protein 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 according to the plate map and assay steps shown in Figure 2, or a custom procedure.

A.



B.

Step	Column	Description	Step Type	Time (s)	Shaking Speed (rpm)
Step 1	1	Equilibration Buffer	Custom/ Baseline	180	1000
Step 2	2	FLAG-Tagged Protein Loading	Loading	120–600	1000
Step 3	3	Baseline in Analyte Buffer	Baseline	300–600	1000
Step 4	4	Association of Analyte	Association	300–900	1000
Step 5	3	Dissociation of Analyte	Dissociation	300–3600	1000

Figure 2. (A) Sample Plate map and (B) assay steps with corresponding parameters for the FLAG tag kinetic assay.

Assay Steps

Assay Step 1

Equilibration of the pre-hydrated biosensors in 1X KB or FLAG-tagged protein custom buffer. Add buffer, media, or diluted lysate to column 1 of the Sample Plate according to the map in Figure 2. Note the equilibration buffer should match the buffer matrix of the FLAG-tagged protein to be captured.

Assay Step 2

Capture of FLAG-tagged protein (Loading/ Immobilization). Dilute the FLAG-tagged protein to the appropriate concentration in 1X KB 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 capture concentration is 1–25 µg/mL (corresponds to 10–150 nM for most proteins) 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. Loading optimization is recommended to define the optimal ligand density. For more details refer to the Application Note "Biomolecular Binding Kinetic Assays on the Octet® BLI Platform".

Assay Step 3

Baseline step in assay buffer (Baseline). Add 1X KB or alternative buffer matching the analyte samples being analyzed to the Sample Plate according to Figure 2. 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 FLAG-tagged protein 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_a, k_d, and K_D values. The highest analyte concentration should be greater than 10 times 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 an 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 (Step 3). Using the same wells for the Baseline and Dissociation steps 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 3 shows Octet® DYKDDDDK Biosensors loaded with FLAG-tagged human TRAIL (Adipogen, Cat. No. AG-40B-0003-5010) at 1 µg/mL (~40 nM), followed by kinetic analysis of an analyte monoclonal mouse anti-human TRAIL antibody (150 kDa) (R&D systems, Cat. No. MAB3751). The result of the kinetic analysis of this assay is summarized in Table 1. Raw sensorgrams in figure 3A and 3B have been aligned to the start of the Association step.

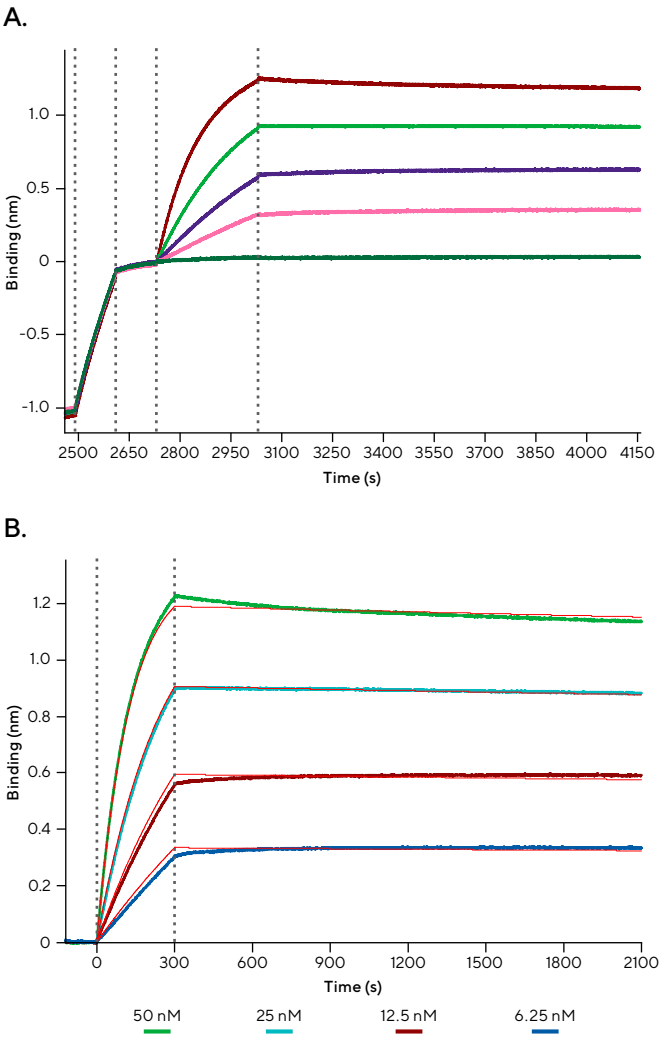


Figure 3. Binding kinetics of FLAG–tagged TRAIL protein (23 kDa) and an analyte mouse anti-TRAIL antibody (150 kDa), on the Octet® BLI platform. (A) The raw data for a full assay. 1X KB was used as a buffer matrix throughout the assay. (B) The association and dissociation traces after data processing (including reference subtraction using the 0 nM trace and fitting using a 1:1 binding model).

k_a (1/Ms)	K_D (1/s)	K_D (M)
1.607E-05	1.788E-05	1.112E-10

Table 1. Kinetic results for the interaction between the ligand FLAG-tagged TRAIL protein (23 kDa) and an analyte anti-TRAIL antibody (150 kDa), using the Octet® DYKDDDDK Biosensors for the data shown in Figure 3.

Quantitation Assay Workflow

The Octet® DYKDDDDK Biosensors can be used for quantitation in both crude and purified samples. For FLAG-tagged protein samples with a concentration range of 0.5–100 µg/mL, it is recommended to use a shaking speed of 1000 rpm with a 2-minute assay time. Optimal shake speed and concentration range of quantitation are dependent on sample molecular weight.

Materials Required

- Octet® BLI systems with Octet® BLI Discovery and Analysis Studio software
- Octet® DYKDDDDK Biosensors, Sartorius Part No. 18-5178 (Tray), 18-5179 (Pack), 18-5180 (Case)
- For all Octet® BLI systems: 96-Well, Black, Flat Bottom Microplate, Sartorius Part No. 18-5172 (Pack), 18-5173 (Case)
- Optional for Octet® R8e, 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)
- 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.
- Optional regeneration buffer. 100 mM glycine pH 3.5 (supplied by user)

Best Practices

The following practices are recommended each time the quantitation assay involves a new matrix or a new FLAG-tagged 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.
- If high concentration calibration standards show poor initial binding rate fitting, or poor separation of binding traces, consider reducing the shaking speed to 400 rpm (vs. 1000 rpm).
- 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 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.

DYKDDDDK (FLAG®)-Tagged Protein in Sample Matrix	Minimum Recommended Dilution in Sample Diluent Buffer
Sample Diluent (SD)	Neat
CHO	Neat
OptiCHO	Neat
293	Neat
293 conditioned	Neat
DMEM	2-fold
10% FBS+DMEM	Neat
RPMI	2-fold
10% FBS+RPMI	2-fold
Bacterial cell pellet lysate by sonication	10-fold
Bacterial cell pellet lysate by B-PER	20-fold

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. 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.
7. Load data into Octet® Analysis Studio Software.
8. 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.
9. 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 0.5–100 µg/mL for assays run at 1000 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 and standards 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 4.
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 Sample 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 sensorgram images will be saved automatically.
7. Load the data into Octet® BLI Analysis Studio software.
8. If a blank matrix is 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® DYKDDDDK Biosensors. The 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 the calculated concentration value of the unknowns to determine % recovery and %CV.

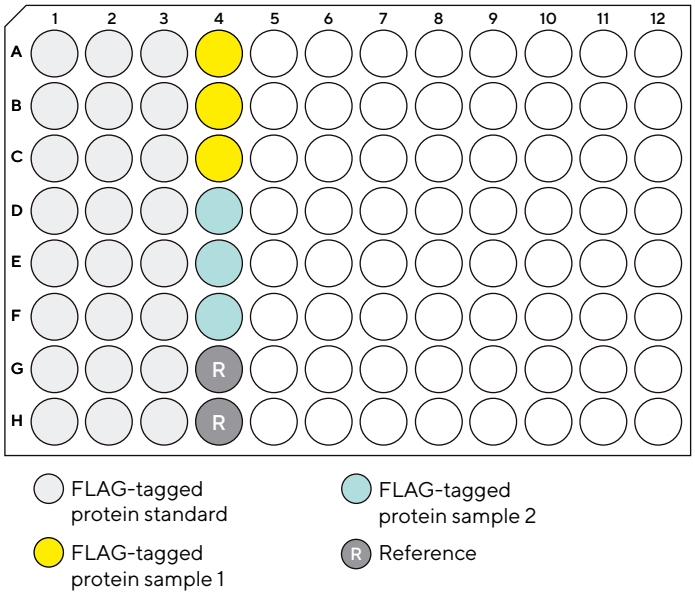


Figure 4. Example plate layout for a spike recovery assay.

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 5 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 the desired format, Excel or PDF.

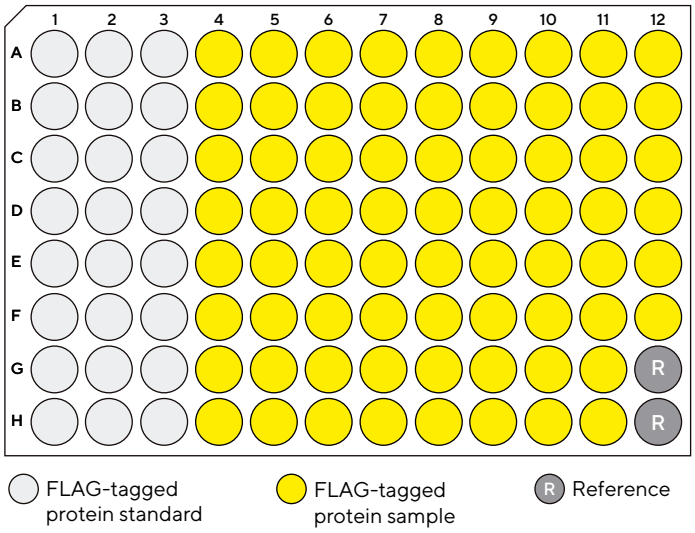


Figure 5. Example plate layout for a routine quantitation assay in a 96-well plate.

Representative Data

Figure 6 shows the detection of FLAG-tagged protein using the Octet® DYKDDDDK Biosensors on the Octet® RH16 system. A standard curve was run to demonstrate quantitation dynamic range (0.5–100 µg/mL). A 5PL (weighted Y^2) fitting model was used to fit the binding rate vs. known concentration. This model was chosen as there is a biphasic shape to this standard curve. Exact choice of fitting model will depending on the ligand in question and should be determined empirically by trying different fitting models and looking at quality of fit.

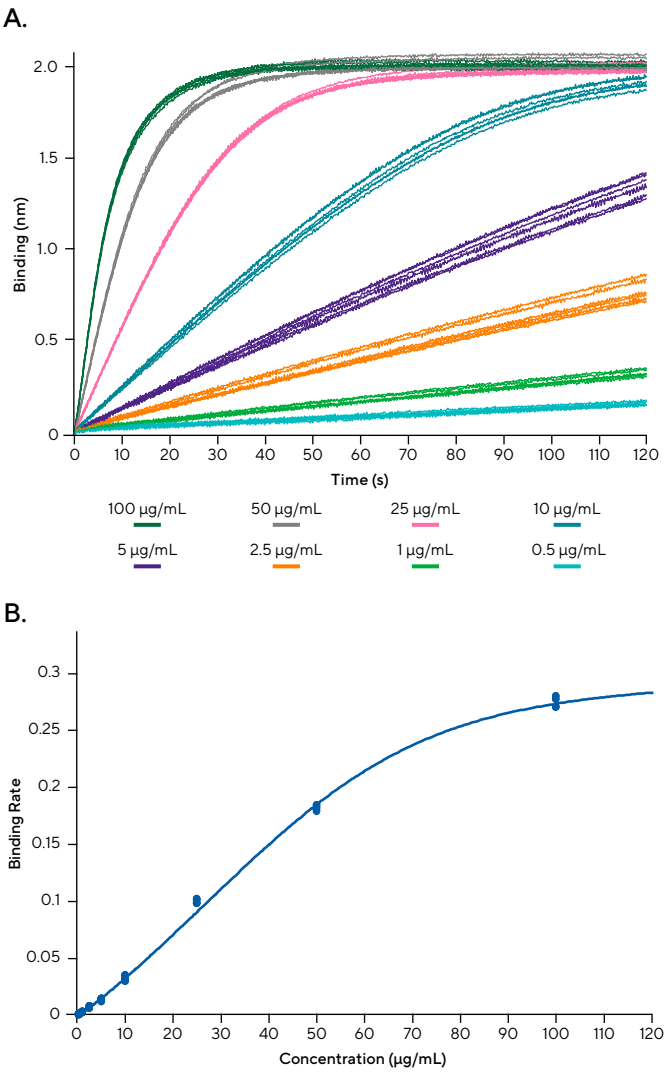


Figure 6. Quantitation of FLAG-tagged protein using the Octet® DYKDDDDK Biosensors. (A) FLAG-tagged protein dose response for concentrations within the dynamic range of 0.5–100 µg/mL with six replicates on the Octet® RH16 instrument with assay parameters: 1000 rpm, 2 min. Colors show concentrations in µg/mL. (B) FLAG-tagged protein standard calibration curve generated from six replicates and calculated using 5PL (weighted Y^2) fitting model. The Octet® Sample Diluent Buffer was used as a matrix for all samples.

Table 3 shows the calculated concentration, %CV within the replicates and the recovery for the concentration. It is important to point out that 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 both ends. For example, to acquire accurate data for 100 µg/mL and 0.5 µg/mL, it is recommended to incorporate 150 µg/mL and 0.25 µg/mL at both ends of the dilution and standard curve.

Known Conc. (µg/mL)	Calculated Conc. (µg/mL)	%CV (n=6)	Recovery
100	102.6	6.6	102.6
50	48.7	1.6	97.3
25	26.9	1.7	107.5
10	10.0	3.7	99.7
5	4.6	5.2	91.8
2.5	2.6	8.7	105.3
1	1.1	11.1	109.5
0.5	0.5	15.3	99.9

Table 3. Calculated concentrations, %CV and %Recovery for FLAG-tagged protein (0.5–100 µg/mL) quantitation assay with six replicates.

Regeneration of Octet® DYKDDDDK Biosensors

The Octet® DYKDDDDK Biosensors can be cost-effectively regenerated and reused up to five times for kinetic assays, and 10 times for quantitation assays for generating replicate data for ligand-analyte pairs, or for analyzing large numbers of samples in sequence. The regeneration is performed by dipping the biosensors into a solution of 100 mM glycine pH 3.5 (quantitation) for five seconds, OR 10 mM glycine pH 1.7 (kinetics) for 20 seconds, followed by a dip in the assay buffer for five or 20 seconds. These regeneration steps should be repeated 3–5 times in sequence to fully remove bound FLAG-tagged protein or the interaction complex. The pH 1.7 regeneration is harsher to the biosensor surface and it takes longer for the biosensor surface to stabilize following pH 1.7 regeneration. However it gives an enhanced baseline stability and complete surface regeneration which are important for kinetic assays. This makes pH 1.7 regeneration suitable for longer kinetics assays but not for quantitation assays. The pH 3.5 100 mM glycine can also be used for kinetics assays but there may be higher baseline drift. Users should determine the best regeneration solution empirically based on the FLAG-tagged protein behavior with binding partner.

After regeneration, the biosensor can be reloaded with FLAG-tagged protein for a new analysis. For best results it is recommended to precondition 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 examples of kinetic and quantitation assays with five regeneration cycles in Figures 8 and 9, and Tables 5 and 6.

Kinetic Assay	K_D (M)	k_a (1/Ms)	k_d (1/s)
Regen 1	3.62E-10	1.29E+05	4.65E-05
Regen 2	3.89E-10	1.33E+05	5.16E-05
Regen 3	4.10E-10	1.39E+05	5.70E-05
Regen 4	4.48E-10	1.44E+05	6.43E-05
Regen 5	4.65E-10	1.48E+05	6.89E-05
Average	4.15E-10	1.39E+05	5.77E-05
%CV for 5 Regenerations	9.4%	5.3%	14.7%

Table 5. K_D, k_a, and k_d values and the corresponding %CVs for FLAG-tagged TRAIL - anti-TRAIL mAb kinetic assay over 5 cycles of regeneration using 10 mM glycine pH 1.7.

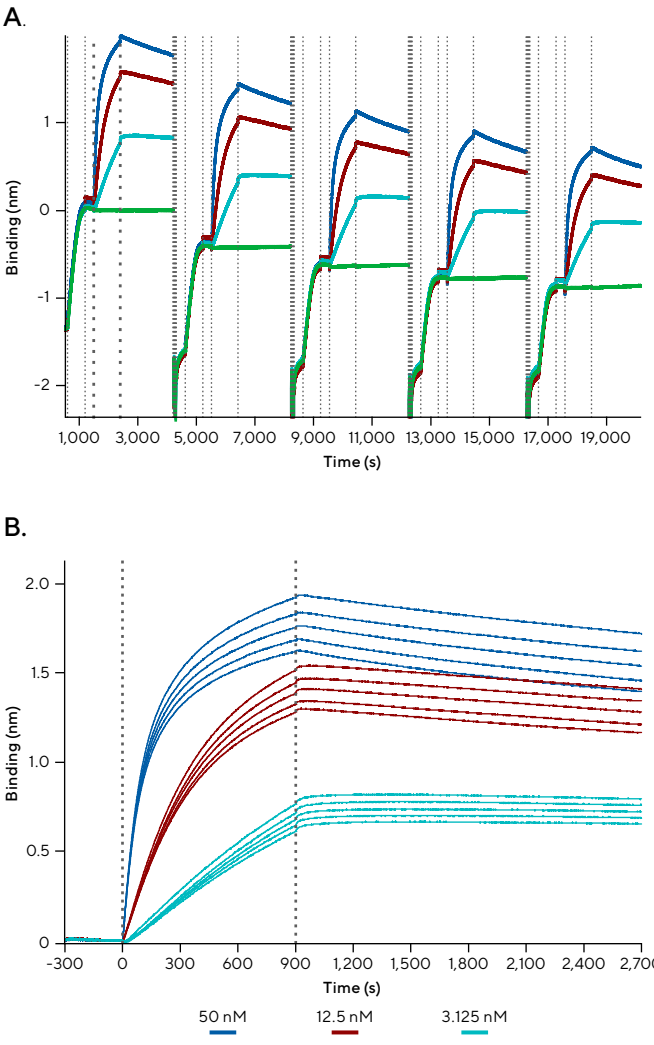


Figure 8. Overlay of association-dissociation curves for FLAG-tagged TRAIL - anti-TRAIL mAb kinetic assay for 5 regeneration cycles. A) Raw sensorgrams for 5 cycles are shown as sequential experiments within a single run. B) Analyte association and dissociation sensorgrams from 5 cycles are shown stacked in a single time frame. The association and dissociation traces overlap closely, and kinetic parameters show relatively low variability from cycle to cycle for 5 regenerations using 10 mM glycine pH 1.7.

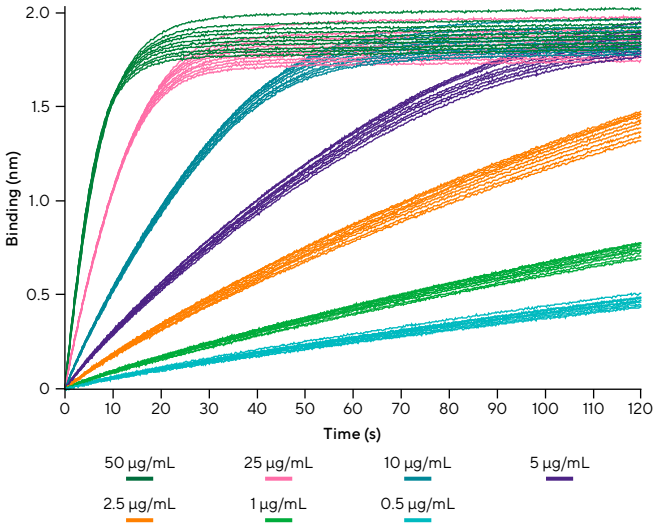


Figure 9. Overlay of binding curves for FLAG-tagged protein quantitation assay for concentration range of 0.5-50 µg/mL after 10 regeneration cycles using 100 mM glycine pH 3.5.

Known Well Concentration (µg/mL)	Average Calculated Concentration (10 Regenerations)	%CV (10 Regenerations)
50	50.0	5.27
25	25.1	2.89
10	10.0	1.15
5	4.98	2.91
2.5	2.49	4.50
1	1.00	5.46
0.5	0.51	5.57

Table 6. Calculated concentrations and %CV for 5 cycles of regeneration for FLAG-tagged protein quantitation assay.

Regeneration Tips

- Regenerate the Octet® DYKDDDDK Biosensor surface after a kinetic or quantitation assay by dipping the biosensors into 100 mM glycine pH 3.5 (quantitation) for five seconds OR 10 mM glycine pH 1.7 for 20 seconds (kinetics) followed by neutralization in assay buffer for five or 20 seconds, then repeating these regeneration steps three times.
- Depending on the assay conditions or protein being captured, the regeneration buffer and/or conditions may require additional optimization.
- It is recommended to precondition the biosensors before the first assay cycle for most consistent results when incorporating regeneration. Biosensors are preconditioned by performing the regeneration procedure once 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 biosensor. For example, a loss of 20% capacity over multiple regeneration cycles could affect precision of quantitation by 10–20%.

Summary

Octet® DYKDDDDK Biosensors demonstrate high ligand binding capacity and high specificity that make them a reliable and versatile tool for kinetic and quantitation characterization of FLAG-tagged proteins in both purified and crude samples. They offer a wide dynamic range of quantitation applications making them suitable for titer applications in both upstream and downstream workflows. In addition, they can be regenerated for multiple cycles of reuse making them a cost-effective option for FLAG-tagged protein sample detection, quantitation and kinetics applications.

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