SARTORIUS

Residual Protein A Detection Kit

Ready BLI Kit for simple, sensitive, and accurate quantitation of leached Protein A in bioprocess samples.

Technical Note

Scope

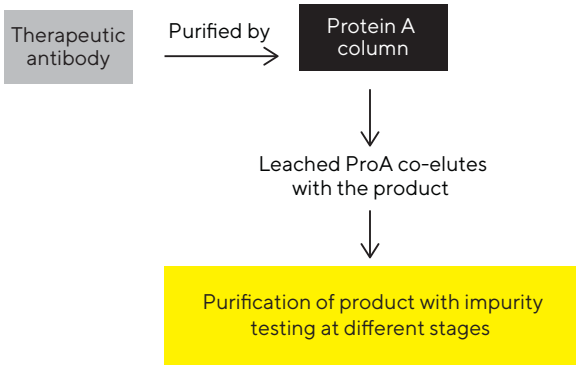
- What is BLI?
- Assay principle and workflow
- Important notes
- Assay protocol
- Data analysis
- Precision and accuracy

The Residual Protein A Detection Kit is intended for the detection and quantitation of recombinant Protein A or other Protein A constructs such as MabSelect SuRe™. It has been developed as a simpler, faster alternative to ELISA method with reduced hands-on time for customers who require a sensitive and robust assay for measuring small amounts of leached Protein A in antibody or Fc-fusion protein samples. This kit is for research and manufacturing use only and is not intended for diagnostic use in humans or animals.

Introduction

Detection of residual Protein A (RPA) in product purified from Protein A purification columns is a critical quality control step in manufacturing of antibody therapeutics. During the purification process, Protein A can leach off of the chromatography matrix and co-elute with the drug substance. To prevent potentially dangerous adverse reactions in patients, Protein A contamination must be detected and minimized. Testing for leached Protein A is performed in several stages of biologic development and production including purification process development, manufacturing, and finished product release testing.

The Residual Protein A Detection Kit provides sensitivity to accurately measure down to 100 pg/mL of contaminating Protein A in samples containing up to 5 mg/mL of antibody. It is designed for use with both native and unnatural Protein A constructs, including MabSelect SuRe™. The kit is compatible with all Octet® systems with the exception of the Octet® R2 system.



What is BLI?

Bio-Layer Interferometry (BLI) is a technology based on measuring interference patterns of white light applied to the tip of a disposable fiber-optic biosensor in order to detect molecular binding events (Figure 1). The family of BLI instruments provides an extremely versatile and easy-to-use platform that can replace or complement a variety of analytical functions – from rapid quantitation and titer assays to determining affinity constants and binding activity of antibodies or receptors to their targets. Systems are widely utilized in the biopharmaceutical industry from early discovery and characterization through bioprocess development and quality control. With BLI, measurements that ordinarily require high sample volumes, complex setup, or excessive time and experimenter intervention can be run efficiently and with minimal effort to achieve fast, accurate results.

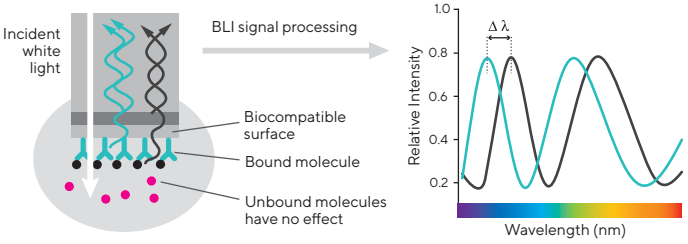


Figure 1: BLI measurement using Dip and Read biosensors. BLI is an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces. Changes in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that is measured in real time.

Assay Principle and Workflow

Assay Optimization

The Residual Protein A Detection Kit applies the principles of BLI to enable sensitive and accurate measurement of leached Protein A in bioprocess samples while maintaining a simple workflow. The kit utilizes a validated sample treatment method for dissociating Protein A from antibodies without boiling, neutralization, or centrifugation steps. After sample treatment, Dip and Read RPA biosensors with pre-immobilized chicken anti-Protein A antibody are sequentially dipped into treated samples, buffers, and detection antibody arrayed in 96-well plates to create an immunoassay on the biosensor tip. Signal detection occurs in real time at the secondary antibody step without need for conjugates or enzymatic reactions (Figure 2).

Depending on the instrument used, either all or part of the RPA assay is automated. On the Octet® RH16 instrument, the initial capture step is performed on a Octet® AS shaker and the biosensors are transferred to the Octet® instrument for automated detection steps. Using the Octet® RH96 instrument, all steps are automated after sample treatment with no further intervention required. Table 1 lists performance and workflow information for each instrument format, as compared to a typical Protein A ELISA assay. Comparison to ELISA requirements demonstrates significant time savings and less hands-on intervention with BLI detection.

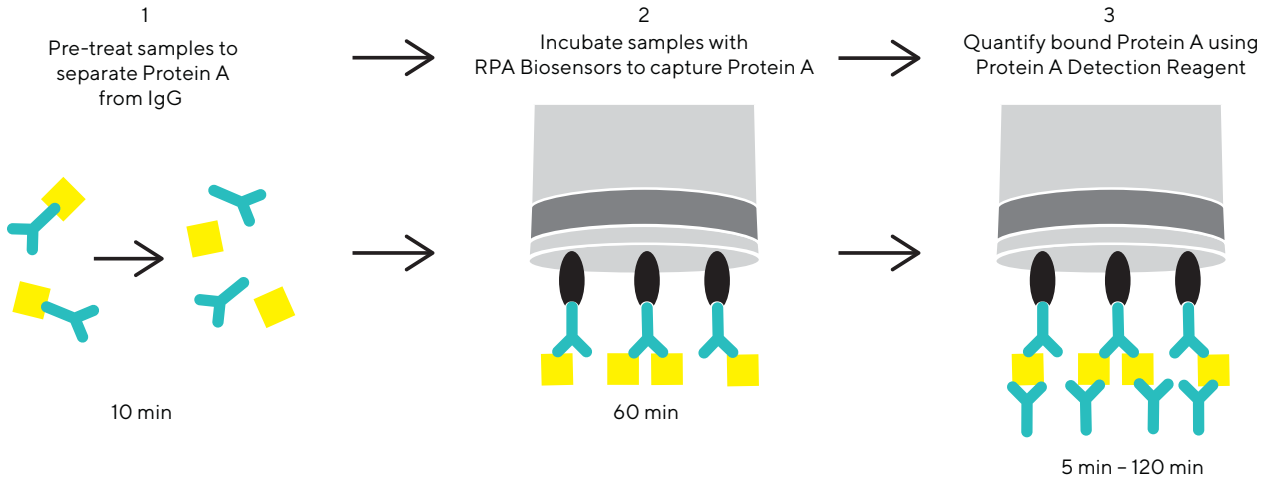


Figure 2: Illustration of RPA detection with BLI.

Table 1: Overview of workflow and requirements for Residual Protein A Detection Kit using different Octet® systems.

Octet® system | assay format	Quantitation range (ng/mL)		Minimum sample required	Assay time per plate			Workflow | hands-on steps	Throughput per day (plates)
	Protein A Standard	MabSelect SuRe™		Hands-on	Octet® instrument	Total assay time		
Octet® RH96 (96-channel detection)	0.1 – 25	0.25 – 25	60 µL	30 min	1 h 11 min	< 2 h	Sample treatment ↓ Place on instrument	7
Octet® RH96 (16-channel detection)	0.1 – 25	0.1 – 25	60 µL	30 min	2 hs 3 min	2.5 h	Sample treatment ↓ Place on instrument	5
Octet® RH16 + Octet® AS	0.1 – 25	0.1 – 25	60 µL	1.5 h (with intermission)	36 min	2.25 h	Sample treatment ↓ Octet® AS incubation (1h) ↓ Octet® AS wash ↓ Place on instrument	4
Octet® R8 + Octet® AS	0.1 – 25	0.1 – 25	130 µL	1.5 h (with intermission)	1 h 12 min	< 3 h	Sample treatment ↓ Octet® AS incubation (1h) ↓ Octet® AS wash ↓ Place on instrument	2-3
ELISA	Varies by manufacturer	Varies by manufacturer	50 µL	5 h (with intermission)	n/a	5 h + O/N incubation	Sample treatment ↓ Incubation of samples (1h) ↓ 4 manual washes ↓ Incubate with antibody (1h) ↓ 4 manual washes ↓ Incubate with conjugate (30 min) ↓ 4 manual washes ↓ Add substrate/ Read plate	3

Materials Included

- 1 tray of 96 Residual Protein A (RPA) biosensors
  Pre-immobilized with chicken antibody and preserved
- Sample Dilution Buffer (2 x 50 mL)
  Phosphate buffered saline with detergent, BSA and preservative
- Denaturing Buffer (1 x 20 mL)
  Citrate buffer with detergent and preservative
  (Caution: Acidic)
- Detection Diluent – Protein A (1 x 50 mL)
  Phosphate buffered saline with PEG, detergent, BSA and preservative
- Protein A Detection Reagent (1 mg/mL, 1 x 450 µL)
  Chicken antibody in Phosphate buffer with preservative
- Protein A Standard (1 mg/mL, 1 x 100 µL)
  Recombinant Protein A in phosphate buffered saline

(Components are not available separately for purchase)

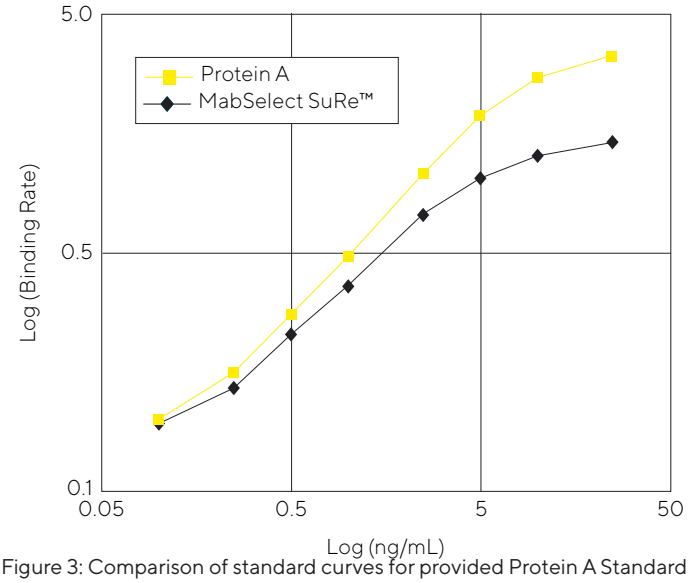


Figure 3: Comparison of standard curves for provided Protein A Standard and MabSelect SuRe.™

Additional Materials Required

- Octet® system with Octet® BLI Discovery Software and Octet® Analysis Studio Software software version 9.0 or later
- Octet® AS shaker (not required with Octet® RH96 system)
- Black polypropylene 96-well, flat bottom microplates (Greiner Bio-One # 655209)

Optional: Black polypropylene 384-well microplates (Greiner Bio-One # 781209)
Optional: Black polypropylene 384-well Tilted Bottom microplates (# 18-5080)
Optional: MabSelect SuRe™ protein (GE Healthcare Life Sciences # 28-4018-60)

Storage and Stability

- Protein A Standard can be stored at 2°C to 8°C for up to 1 month. For longer-term storage, place at -20°C.
- All other reagents should be stored at 2°C to 8°C.
- RPA biosensors should be stored at room temperature in the provided re-sealable bag with desiccant packet away from direct sunlight. Do not refrigerate or freeze biosensors.
- The kit is stable under recommended storage conditions until the date printed on the kit and biosensor package.

Important Procedural Notes for Optimal Performance

1. **Selection of Protein A Standard:** The Protein A standard provided in the kit is unconjugated Pierce™ Recombinant Protein A (Thermo Scientific #21184). It functions similarly to native Protein A and can be used to accurately quantitate similar recombinant Protein A constructs. An important exception is MabSelect SuRe™ from GE Healthcare. MabSelect SuRe™ has significantly modified structure compared to more natural recombinant Protein A constructs, and is recognized differently by Protein A antibodies. These differences can result in quantitation inaccuracies against the included Protein A Standard (Figure 3).

   For accurate quantitation of Protein A in samples purified with MabSelect SuRe™, we recommend using unconjugated MabSelect SuRe™ ligand to generate the standard curve. MabSelect SuRe™ can be ordered directly from GE Healthcare Life Sciences, # 28-4018-60.

2. **Assay Interference:** Samples with concentrations of antibody of up to 5 mg/mL can be quantitated accurately using this assay. However, some antibodies can interfere with Protein A quantitation and lead to inaccuracies. To minimize interference, we recommend diluting samples to antibody concentration of 1 mg/mL or below, keeping Protein A within the quantitation range of the assay. Samples should only be diluted in the provided Sample Dilution Buffer.

Some antibodies or other proteins can interfere with the assay even at low concentrations. Certain sample matrices can cause interference as well. It is important to test for assay interference as part of end user validation by one of two methods:

- Quantitate several 2-fold dilutions of antibody sample that fall within the Protein A quantitation range of the assay and assess dilution linearity. Interference will cause non-linearity in quantitation of sample dilutions.
- Use Protein A Standard or MabSelect SuRe™ to perform a spike-recovery experiment in antibody sample that contains very low to undetectable levels of Protein A. Recoveries that are not within 20% of expected values indicate assay interference.

In most cases protein and/or matrix interference can be eliminated by further dilution of the sample in Sample Dilution Buffer. For assistance with quantitation in problematic matrices, please contact Technical Support.

3. **Octet® AS Shaker:** Use of the Octet® AS shaker is recommended for this assay as it decreases total assay time and ensures precise measurements. The Octet® AS is designed to mimic sensor incubation in the Octet® system at precise shaking speed and temperature. Since the Protein A quantitation assay has been validated on the Octet® AS shaker we cannot guarantee assay precision, accuracy or robustness with other sample incubation methods. Note that only 96-well plates can be used on the shaker.

   A Octet® AS shaker is not required when running this assay on the Octet® RH96 system, since all steps can be performed on the instrument.

4. **Re-use of detection reagent:** Assay plate wells containing Detection Reagent at working concentration can be reused in a single experiment for multiple biosensor dips in a 96-well plate. For example, on the Octet® R8 a single 8-well column of Detection Reagent is used to measure all 96 samples – resulting in 12 biosensor dips per well. Over-dipping in Detection Reagent wells can cause data artifacts or trending. To maintain accuracy:
   - Detection reagent in a 96-well plate (200 µL volume) should not be dipped into more than 12 times.
   - **Detection reagent in a 384-well plate (80 µL volume) should be dipped into only once.**

5. **Run a standard curve for each assay.** Standards and samples should be assayed at least in duplicate.

6. **Overall assay signal will vary from lot to lot.** This variation does not impact results or assay quantitation range. The Binding Rate Separation value on the enclosed Certificate of Analysis will indicate approximate assay signal range to expect for the associated lot.

7. **Do not mix and match kits and biosensor trays.** Kit reagents are paired with a specific lot of biosensors to perform optimally. Lot numbers of individual components of a kit are printed on the Certificate of Analysis.

8. **Minimum volume required** in microplate well for biosensor dipping is 195 µL in a 96-well microplate or 80 µL in a 384-well microplate. These minimum volumes apply for treated samples, buffers and reagents.

9. Using 384-well Tilted Bottom microplates (# 18-5080) instead of standard 384-well microplates can reduce noise and increase sensitivity when running the assay on the Octet® QK384 system or the Octet® RH96 system in 96-Channel Detection mode. Minimum volume required in these plates is 40 µL.

Assay protocol

Sample pre-treatment

- Equilibrate all samples and reagents to room temperature.
- Be sure to process all samples identically.
- Ensure that the Octet® instrument is turned on and allowed to warm up for at least 1 hour before starting the assay.
- Before starting, open the appropriate Residual Protein A advanced quantitation template in the Octet® BLI Discovery software software. In the Plate Definition tab, define the well location, Sample ID, concentration, dilution factor and replicate group information for each of your standards and samples. This will serve as a map or work list for sample plate preparation.

1. Prepare dilutions of standards and samples
- If MabSelect SuRe™ resin was used for purification, use MabSelect SuRe™ protein as the standard to ensure accurate results.
- All samples and standards should be diluted in Sample Dilution Buffer.
- To cover the entire assay range, suggested concentrations for Protein A Standard curve are 25, 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, zero ng/mL. Alternatively, prepare initial dilution of 20 ng/mL Protein A and perform 2-fold serial dilutions down to 78 pg/mL. Include a zero ng/mL standard as a negative control.
- To minimize interference, we recommend diluting samples so that antibody concentration is 1 mg/mL or lower, and Protein A falls within the assay quantitation range.

2. Transfer 130 µL diluted standards and samples to designated wells of 96-well black, flat-bottom microplate (Sample Plate). If using 384-well microplate on the Octet® RH96 instrument, transfer 60 µL of standards and samples.

3. Pre-treat standards and samples in the 96-well Sample Plate by adding 65 µL Denaturing Buffer to each well and mix thoroughly by pipetting up and down gently 15–20 times. For 384-well microplate add only 30 µL Denaturing Buffer.

4. Incubate on the benchtop for 10 minutes

Assay protocol for Octet® R8 instrument

1. Capture Protein A onto Residual Protein A biosensors using the Octet® AS shaker
   - Place the Sample Plate onto the shaker.
   - Lower the green tray of RPA biosensors onto the Sample Plate so that the tips of the biosensors are soaking in the samples.
   - Lock the plate and shake for 1 hour at 30°C with shaking at 1000 rpm.

2. Prepare working-strength Protein A Detection Reagent
   - Dilute Protein A Detection Reagent 20-fold by adding 90 µL to 1710 µL of Detection Diluent (This volume will fill one column of a 96-well plate, which is adequate for measuring 96 samples)

3. Prepare Wash Plate and Detection Plate
   - Wash plate: Add 200 µL Detection Diluent into each well of a black, flat-bottom 96-well microplate corresponding to biosensors in the incubating microplate.
   - Detection plate: Add 200 µL Detection Diluent into Column 1, wells A through H, of a black, flat-bottom 96-well microplate. Add 200 µL of the diluted Protein A Detection Reagent into Column 2, wells A through H, of the same microplate (Figure 4A).

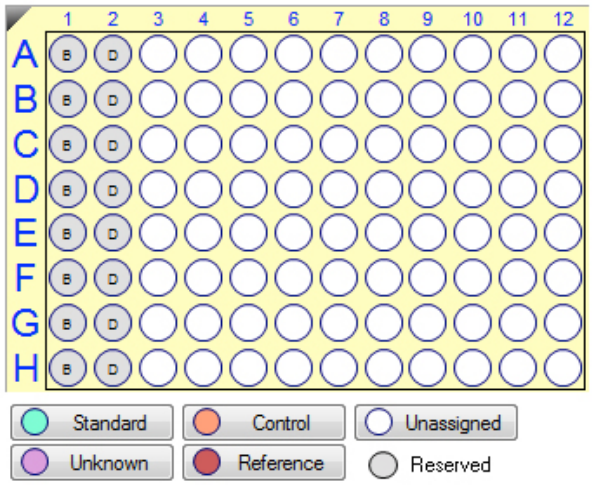
4. Wash the RPA biosensors
   - When Sample Plate incubation is complete, remove the tray of RPA biosensors and the Sample Plate from the shaker.
   - Place the Wash plate on the shaker.
   - Lower the tray of RPA biosensors onto the Wash Plate so that the tips of the biosensors are soaking in the Detection Diluent. Shake for 5 minutes at 30°C at 1000rpm.

5. Run Detection steps on Octet® R8 instrument
   - When the wash is complete, remove the tray of RPA biosensors and the Wash Plate from the shaker.
   - Place the Wash Plate into the blue biosensor tray holder for the RPA biosensor tray, and re-assemble the tray apparatus by placing the RPA biosensor tray on top so that the biosensors tips are soaking in the Detection Diluent.
   - Place the Detection Plate and the RPA biosensor tray assembly in the Octet® instrument in designated positions.
   - In Octet® BLI Discovery Software, open the advanced quantitation template **ResidualProteinA_8CH_96W.fmf.**
   - In the Plate Definition tab, define well location, Sample ID, concentration, dilution factor and replicate group information for each of the standards and samples if this was not done previously.
   - Ensure the 'Delay Start Time' box is checked in the Run Experiment tab. The 300 second delay is required to enable the Detection Plate to equilibrate to 30°C.
   - Start the run. A full 96-well plate of samples will take about 75 minutes to complete.

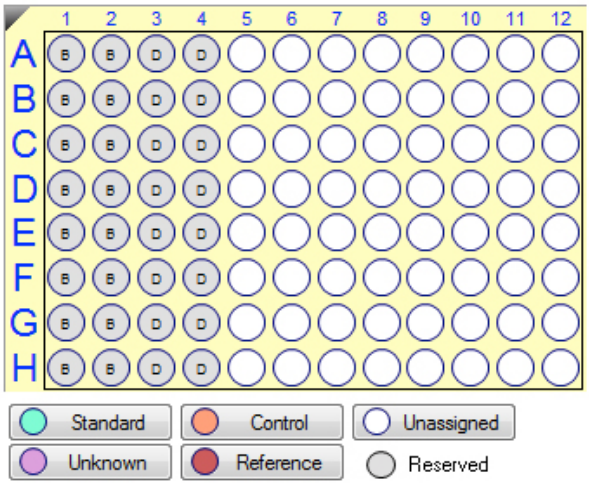
Assay protocol for Octet® RH16 instrument

1. Capture Protein A onto Residual Protein A biosensors using the Octet® AS shaker
   - Place the Sample Plate onto the shaker.
   - Lower the green tray of RPA biosensors onto the Sample Plate so that the tips of the biosensors are soaking in the samples.
   - Lock the plate and shake for 1 hour at 30°C with shaking at 1000 rpm.
2. Prepare working-strength Protein A Detection Reagent
   - Dilute Protein A Detection Reagent 20-fold by adding 180 µL to 3420 µL of Detection Diluent. (This volume will fill two columns of a 96-well plate, which is adequate for measuring 96 sample wells)
3. Prepare Wash Plate and Detection Plate
   - Wash plate: Add 200 µL Detection Diluent into each well of a black, flat-bottom 96-well microplate corresponding to biosensors in the incubating microplate.
   - Detection plate: Add 200 µL Detection Diluent into Columns 1 and 2, wells A through H, of a black, flat-bottom 96-well microplate. Add 200 µL of diluted Protein A Detection Reagent into Columns 3 and 4, wells A through H, of the same microplate (Figure 4B).
4. Wash the RPA biosensors
   - When Sample Plate incubation is complete, remove the tray of RPA biosensors and the Sample Plate from the shaker.
   - Place the Wash Plate on the shaker.
   - Lower the tray of RPA biosensors onto the Wash Plate so that the tips of the biosensors are soaking in the Detection Diluent. Shake for 5 minutes at 30°C at 1000 rpm.
5. Run Detection steps on the Octet® RH16 instrument
   - After wash is complete, remove the RPA biosensors and Wash Plate from the shaker.
   - Place the Wash Plate in the Octet® instrument in the biosensor tray position. Position the green RPA Biosensor tray on top of the plate so that the biosensors are soaking in the Detection Diluent.
   - Place the Detection Plate in the instrument in the **Reagent Plate** position. Do not place in the other plate position!
   - In Octet® BLI Discovery Software, open the advanced quantitation template **ResidualProteinA_16CH_96W.fmf.**
   - In the Plate Definition tab, define well location, Sample ID, concentration, dilution factor and replicate group information for each of the standards and samples if this was not done previously.
   - Ensure the 'Delay Start Time' box is checked in the Run Experiment tab. The 300 second delay is required to enable the Detection Plate to equilibrate to 30°C.
   - Start the run. A full 96-well plate of samples will take about 36 minutes to complete.

A.

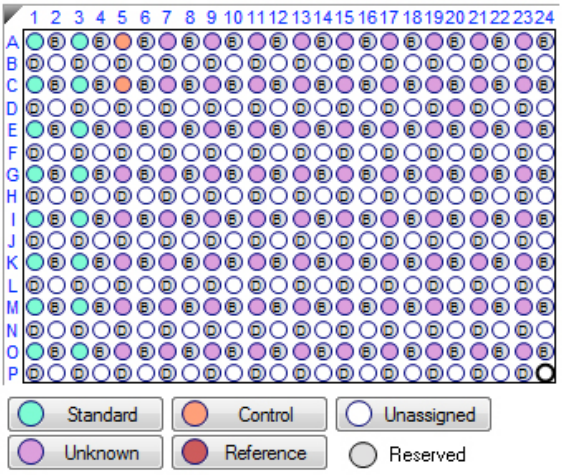


B.



B = Buffer (Detection Diluent)
D = Detection (Protein A Detection Reagent, working strength)

Figure 4: Detection plate loading guide for Octet® RH16



B = Buffer (Detection Diluent)
D = Detection (Protein A Detection Reagent, working strength)

Figure 5: Plate loading guide for Octet® RH96 96-Channel Detection format. All reagents are loaded into a single 384-well plate.

Assay protocol for Octet® RH96 instrument

1. Select assay format for the Octet® RH96 system. Three possible assay configurations can be run depending on throughput and sensitivity needs:

   a. 96-Channel Detection (Higher Throughput)
      - All Protein A standards, samples, and detection reagents are combined in a single 384-well microplate (Figure 5).
      - Each step of the assay is performed on 96 biosensors simultaneously, providing the fastest and simplest quantitation assay.
      - Note: Using 96-channel mode for detection results in noisier signal than the alternative 16-channel mode. Expect slightly lowered precision and reduced sensitivity at the bottom of the assay quantitation range when using this format.

   b. 16-Channel Detection (Higher Sensitivity)
      - Standards and samples are prepared in one 96-well plate (Sample Plate), while Detection reagents are added to a second 96-well plate (Detection Plate) (Figure 6A, 6C).
      - Sample incubation is performed on 96 biosensors simultaneously. Detection steps are performed on 16 biosensors at a time for greater sensitivity.This assay configuration takes more time but provides more precision at the low end of the quantitation range than 96-Channel Detection format.

   c. 16-Channel Detection, low sample volume
      - This configuration is identical to 16-Channel Detection, however a 384-well microplate is used for the Sample Plate in order to conserve sample volume. (Figure 6B, 6C)

2. Perform sample pre-treatment. After selecting your Octet® RH96 assay format, prepare Sample Plate and perform sample pre-treatment steps as described in the “Sample pre-treatment” section.

3. Pre-hydrate RPA biosensors
   - Add 200 µL Sample Dilution Buffer into each well of a black, flat-bottom 96-well microplate corresponding to biosensors in the incubating microplate (Hydration Plate).
   - Place the Hydration Plate in the instrument in the biosensor tray position. Position the green RPA Biosensor tray on top of the plate so that the biosensors are soaking in the buffer.
   - Pre-hydrate biosensors for a minimum of 10 minutes.

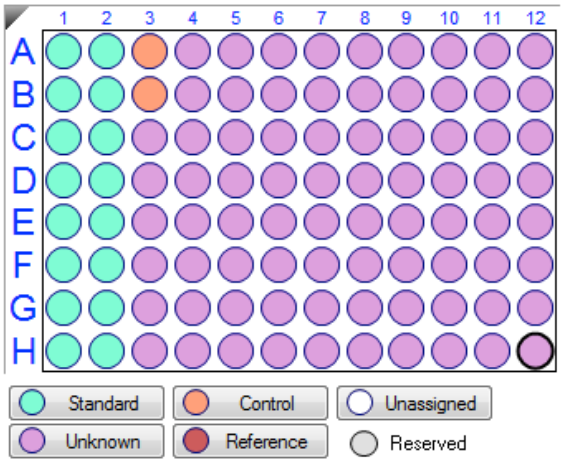
4. Prepare working-strength Protein A Detection Reagent
   - For 96-Channel Detection: Dilute Protein A Detection Reagent 20-fold by adding 400 µL to 7.6 mL Detection Diluent. This volume will fill 96 wells of a 384-well plate.
   - For 16-Channel Detection: Dilute Protein A Detection Reagent 20-fold by adding 180 µL to 3420 µL of Detection Diluent. This volume will fill two columns of a 96-well plate, which is adequate for measuring 96 sample wells.

5. Prepare Detection Plate
   - For 96-Channel Detection: Add 80 µL treated samples/standards, Detection Diluent, and working strength Protein A Detection Reagent into wells of a single 384-well plate (Figure 4).
   - For 16-Channel Detection: Add 200 µL Detection Diluent into Column 1, wells A through H, of a black, flat-bottom 96-well microplate. Add 200 µL of the diluted Protein A Detection Reagent into Column 2, wells A through H, of the same microplate (Figure 5).

6. Place assay plate(s) in the instrument
   - For 96-Channel Detection: Place the combined Sample/Detection Plate in the instrument in the **Plate 1** position.
   - For 16-Channel Detection: Place the Sample Plate in the Plate 1 position. Place the Detection Plate in the **Plate 2** position.

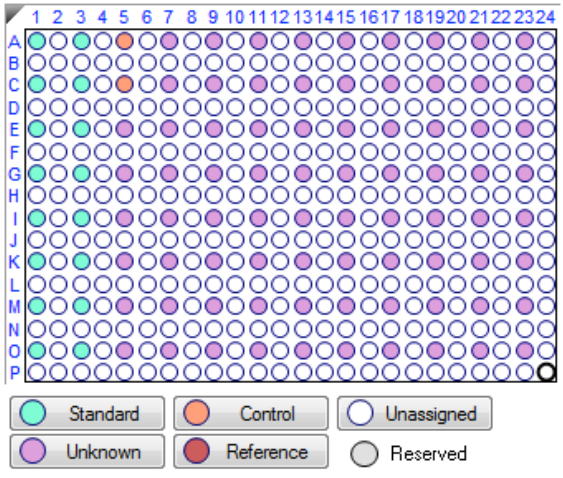
Sample plate

A.



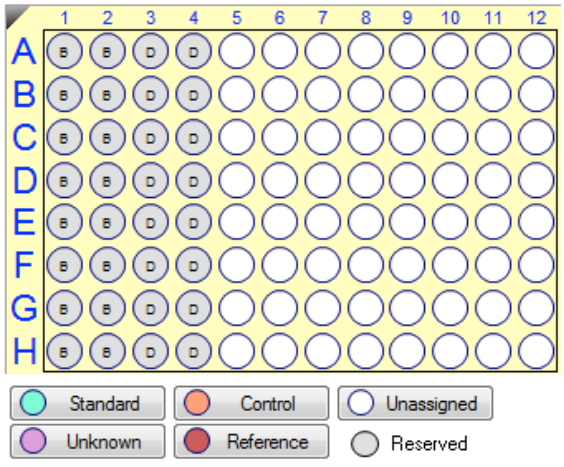
Sample plate – low sample volume

B.



Detection Plate

C.



B = Buffer (Detection Diluent)
D = Detection (Protein A Detection Reagent, working strength)

Figure 6: Plate loading guide for Octet® RH96 16-Channel Detection format. A) Sample Plate (suggested layout) using 96-well plate, B) Sample Plate (suggested layout) using low sample volume option in 384-well plate, C) Detection Plate layout for both formats

7. In Octet® BLI Discovery Software, open the appropriate advanced quantitation template.
    - In the Plate Definition tab, define well location, Sample ID, concentration, dilution factor and replicate group information for each of the standards and samples if this was not done previously.
    - Ensure the 'Delay Start Time' box is checked in the Run Experiment tab. The 300 second delay is required to enable the Sample/Detection Plate(s) to equilibrate to 30°C.
    - Start the run.
        - A full plate of 96 samples will take 1 hour 11 minutes to complete.
        - For 16-Channel Detection: A full plate of samples will take 2 hours 3 minutes to complete.

Data analysis

1. After the run is complete, open the Octet® BLI Analysis Software. In the Data Selection tab, load the experimental folder to be analyzed. Multiple data sets can be selected at once for analysis. All selected data sets will be analyzed as a group.

2. In the Results tab, select the appropriate Standard Curve Equation.

3. Important: Select 'R Equilibrium' as the binding rate equation.

4. Click the **Calculate Binding Rate! Button**. This will determine binding rate equilibrium signal for each data trace. Binding rates and calculated results are presented in the data table.

5. If desired, utilize the Octet® BLI Analysis Software features to group data or data sets, display individual graphs, set sample threshold alerts, and view data statistics.

6. Click the **Save Report** button to generate a complete Microsoft® Excel formatted report.

Example data and performance qualification

The Residual Protein A Detection Kit has been qualified for performance using standard criteria described below. We recommend the user perform their own specific qualification and validation to assess kit performance with internal samples and specific Protein A construct.
The performance data shown demonstrate the kit's ability to detect Protein A in the presence of up to 0.5 mg/ml hIgG in Sample Dilution buffer and also determination of precision, accuracy, linearity, and sensitivity.

Example data

Standard curve was generated using Protein A Standard provided in the kit. Samples were run on the Octet® RH96 system using 16-Channel Detection. mode. Curve fitting was performed using weighted 4 parameter logistic model. Figure 7 shows sample raw data traces and plotted standard curve.

Protein A Standard curve (n=8)

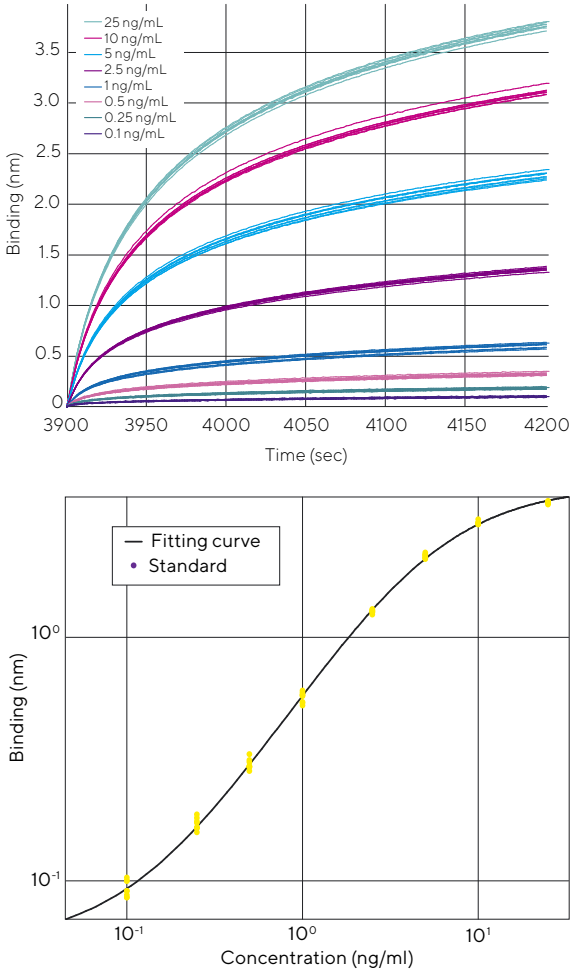


Figure 7: Standard curve made with Protein A Standard included in the kit.

Precision and accuracy

Precision (%CV where %CV = Standard Deviation X 100 / Average) and Accuracy (% Recovery where % Recovery = Calculated Conc X 100 / Expected Conc) were calculated for data generated on the Octet® RH96 system using 16-channel detection mode. Table 2 shows results for standard curves generated with Protein A Standard included in the kit and with MabSelect SuRe™.

Inter-assay precision was determined using 3 different preparations of sample in 3 independent assays. Protein A Standard or MabSelect SuRe™ were spiked into Sample Dilution Buffer containing 0.5 mg/mL human IgG. Data shown in Table 3.

Table 2: Intra-assay precision and accuracy for Protein A Standard and MabSelect SuRe™.

Protein A Standard (included) n = 8			MabSelect SuRe™ n = 8		
Target (ng/mL)	**% CV**	**Recovery**	**Target (ng/mL)**	**% CV**	**Recovery**
10	3.6%	105%	10	3.7%	99%
2.5	1.6%	98%	2.5	3.5%	105%
0.5	4.4%	101%	0.5	2.5%	92%
0.1	12.5%	100%	0.1	11.7%	100%

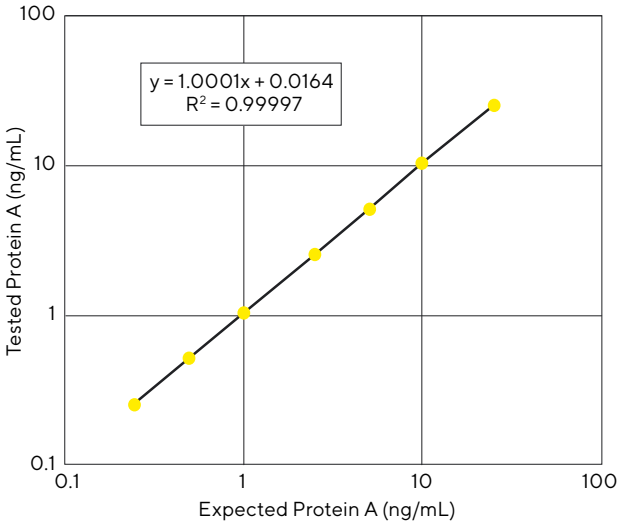
Table 3: Inter Assay precision data for samples containing 0.5 mg/mL human IgG.

Protein A Standard (included)			MabSelect SuRe™		
No. tests (n=3)	**Target (ng/mL)**	**% CV**	**No. tests (n=3)**	**Target (ng/mL)**	**% CV**
3	5	6.4%	3	5	7.3%
3	2.5	6.6%	3	2.5	7.8%
3	0.5	4.8%	3	0.5	7.0%

Dilution linearity

Linearity of dilution was established by comparing expected concentration to calculated concentration. The chart shown in Figure 8 shows excellent correlation of calculated concentrations in the presence of human IgG throughout the range of the assay.

Dilution linearity



Sensitivity

Limit of detection (LOD) is defined as the minimum concentration at which an analyte's presence can be detected by a given assay, whereas Lower Limit of Quantitation (LLOQ) is defined as the minimum concentration at which an analyte can be reliably quantified. Here LOD was determined for various assay formats as the concentration corresponding to binding rate signal three standard deviations above the mean of the zero standard. LLOQ was determined based on the lowest concentration where recovery is within 20% of the expected/theoretical value and precision (%CV) is below 20%. LOD and LLOQ values for both Protein A Standard and MabSelect SuRe™ can be found in Table 4.

	LOD (ng/mL)		LLOQ (ng/mL)	
Octet® instrument	**Protein A Standard**	**MabSelect SuRe™**	**Protein A Standard**	**MabSelect SuRe™**
Octet® RH96 (96-channel detection)	0.07	0.08	0.1	0.25
Octet® RH96 (16-channel detection)	0.03	0.06	0.1	0.1
Octet® RH16	0.03	0.06	0.1	0.1
Octet® R4	0.07	0.08	0.1	0.25

Table 4: LOD and LLOQ values for Protein A Standard and MabSelect SuRe™ for samples containing 0.5 mg/mL human IgG.

Ordering information and technical support

Part no.	UOM	Description
18-5128	Kit	Residual Protein A Detection Kit. Contains 1 tray of 96 Residual Protein A biosensors and reagents for analysis of 96 samples.

MabSelect SuRe™ is a trademark of GE Healthcare.

Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 888 OCTET 75
Or +1 650 322 1360

For further information, visit
www.sartorius.com/octet-support

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