SARTORIUS

CHO Host Cell Protein Detection

Technical Note

Keywords and phrases
Host Cell Proteins, CHO Cells, CHO HCP detection, HCP contaminants, Impurities, Octet Biosensors, Octet® Anti-CHO HCP Kit

Abstract

The Octet® Anti-CHO HCP Kit has been designed for a rapid high-throughput quantitation of residual CHO Host Cell Proteins (HCPs) in bioprocessing samples during production and development of biopharmaceuticals. The kit utilizes the industry-standard Cygnus 3G Anti-CHO HCP antibody and enables assays with broad HCP recognition, high precision (5-10 %CV) and high sensitivity (detection limit as low as 0.5 ng/mL). These features, combined the Octet® platform's real-time, label-free high-throughput analysis capability, make the Octet® Anti-CHO HCP kit a superior alternative to ELISA for HCP detection. The Octet® RH96 system allows for a completely hands-off, walk-away HCP analysis of 96 samples in less than one hour, which is three times faster than the manual and labor-intensive ELISA platform.

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Overview

Host cell proteins (HCPs) are contaminants found in biopharmaceuticals expressed in bacterial, yeast or mammalian production cell lines. Among protein expression cell lines, Chinese hamster ovary (CHO) cells are the most commonly used mammalian hosts for industrial production of recombinant protein therapeutics. However, manufacturing and production processes of biopharmaceuticals often leave behind contaminating HCPs from CHO cells. Such residual HCPs carry substantial risk of decreasing efficacy of the drug and causing adverse immunogenic reactions in patients. Hence, the detection of residual host cell protein contaminants and methods that reduce them to the lowest acceptable levels have become critical aspects of drug safety and qualification.

HCP analyses can be broadly classified into two categories, *generic* assays and process-specific assays. Commercially available generic assays are intended to detect HCPs that might contaminate a product independent of downstream purification processes. These assays normally use HCPs obtained from far upstream (e.g., conditioned media) or after some minimally selective purification step (e.g., clarification or filtration) as an antigen to generate broadly-reactive polyclonal antibodies for detection. They are very useful when most HCPs are conserved among related strains and processes. On the other hand, process-specific assays are those derived from an immunogen which is specific to a defined purification process. These assays are tailor-made for an established process and thus have the potential to be more specific to the panel of HCPs produced within such processes. However, high specificity for typical HCPs occurring within the process carries the risk that any atypical HCPs arising due to unintended and undetected deviations in a purification process may go undetected — potentially leading to substantial setbacks in the drug development timeline.

The vast majority of process-specific assays are developed only after a process is set and defined, which typically occurs at the later stages of drug development. Detection antibodies for process-specific assays also require substantial time and cost to develop. Since the FDA generally does not require process-specific HCP analyses until phase III trials, generic assays are typically employed for overall HCP detection until a drug candidate survives the initial phases of clinical trials. Even after a process-specific assay is developed and implemented, generic assays can still be used routinely to complement process-specific testing to maximize detection of any potential atypical HCPs. Because of all these factors, generic assays will always remain the most easily assessable and highly useful tools in any drug development program.

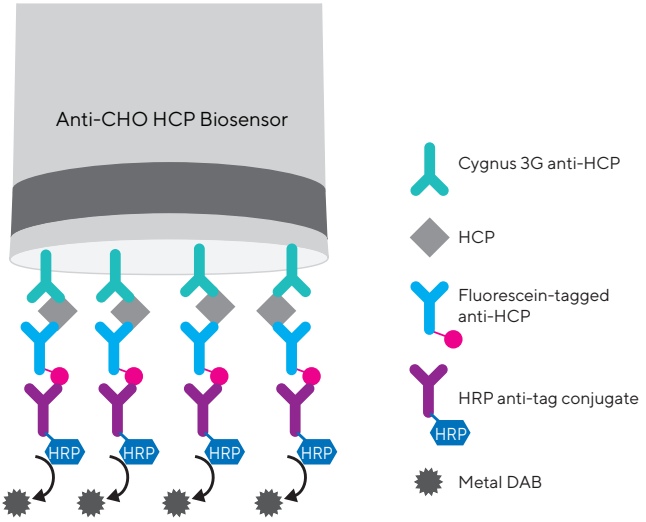


Figure 1: Biosensor-based assay format for the detection of CHO HCP.

Principle

Among existing HCP analytical methods, ELISA is perhaps the more commonly used analytical method. Western blots and SDS-PAGE are also used, but are limited by their qualitative nature and lack of quantitation sensitivity.

There are several inherent problems with ELISA, stemming from its reliance on highly manual processing steps that introduce variability in measurement, multiple time-consuming incubation steps, and reliance on colorimetric or fluorescent probes that can yield false positive signals. Sartorius' Octet® Platform provides a superior alternative to ELISA with improved precision in measurements, better or equivalent sensitivity and dynamic range, low user intervention, rapid assay development enabled by real-time monitoring, and much faster time-to-results. The platform is used for generic CHO HCP assays in early phases of clinical product development, and process-specific CHO HCP assays can also be constructed using the same assay format if a process-specific antibody has been developed.

This Technical Note outlines a protocol for using the Octet® Anti-CHO HCP Kit in developing and routine running of process-independent assays on the Octet® platform. The measurement involves a sandwich-type assay on an Anti-CHO HCP Biosensor that is pre-coated with the gold-standard 3G Anti-CHO HCP antibody from Cygnus Technologies (Figure 1). A completely hands-off, walk-away HCP assay analyzing 96 samples can be set up to run automatically on an Octet® RH96 instrument with results obtained in one hour. The assay can also be run on other 8-and 16-channel Octet® instruments together with the Octet® AS Station with time-to-results of 75 and 90 minutes, respectively.

Assay Accuracy and Specificity

In certain cases, the drug products themselves or components in the formulation buffer may interfere with the assay's ability to detect HCPs. Also, different lots of the same anti-CHO HCP antibody might give results that are 2-4X different, whereas different generations of the same antibody might produce results that are 10-20X different. The expected trend of results in accordance with the process stage (decreasing HCP readings with further purification) is considered more important than the absolute HCP values. Factors such as extremes in pH, detergents, organic solvents, high protein concentration, and high buffer salt concentrations are all potential interference factors. Also, different lots of the same anti-CHO HCP antibody might give results that are 2-4X different, whereas different generations of the same antibody might produce results that are 10-20X different. The expected trend of results in accordance with the process stage (decreasing HCP readings with further purification) is considered more important than the absolute HCP values. It is therefore necessary to validate by established experimental procedures (*i.e.* ICH and FDA guidelines) that the assay results will be accurate. We recommend users perform two critical experiments in establishing assay accuracy and specificity: spike recovery and dilutional linearity (please also refer to "Assay Matrix" on page 4). If it is determined that there is significant product or matrix interference in the assay, further dilution or buffer exchange of the product to render it into a more assay compatible buffer might be necessary. The same diluent used to prepare the kit standards is ideally the preferred material for dilution or buffer exchange of your samples. For each sample type to be tested, users should demonstrate that the assay can recover added HCP or other contaminants spiked into that sample matrix. This can be performed by spiking the highest standard provided with the kit into your sample types and then testing in the assay.

Materials Required

- Octet® RH16, RH96 or R8 instrument with Octet® BLI Discovery and Analysis Studio Software version 8.1 or later.
- Octet® AS Station for high throughput assays if using Octet® RH16 or R8 instrument (not needed with the Octet® RH96 instrument).
- Black polypropylene 96-well or 384-well microplates (Greiner Bio-One part no. 655209 or 781209).
- Volume of samples to be analyzed (including positive and negative controls): 80 µL (384-well microplate) or 200 µL (96-well microplate).
- Octet® Anti-CHO HCP Kit (PN 18-5141), containing the following:
  - Part 1: Biosensors for Host Cell Protein Detection
  - Part 2: Reagents A for Host Cell Protein Detection
  - Octet® CHO Standard, 48 µL, 20 µg/mL
  - Octet® Fluorescein anti-CHO, 240 µL, 100X concentrate
  - Octet® Anti-FITC HRP, 480 µL, 50X concentrate
  - Octet® Sample Dilution Buffer, 3 x 50 mL
  - Part 3: Reagents B for Host Cell Protein Detection
  - Octet® DAB/Metal Concentrate, 2.4 mL, 10X concentrate
  - Octet® Peroxide Substrate Buffer (1X), 46 mL

Storage and Stability

- Anti-CHO HCP Reagents A should be stored at 4°C. The Octet® CHO Standard can be stored short-term up to one month at 4°C and long-term at -20°C. More than three freeze-thaw cycles should be avoided.
- Anti-CHO HCP Reagents B - Upon arrival, Octet® Peroxide Substrate Buffer should be stored at 4°C and Octet® DAB/Metal Concentrate should be stored at -20°C.
- Anti-CHO HCP biosensors should be stored at room temperature.

Assay Matrix

Differences between matrices can potentially influence assay performance. Diluting the sample matrix using Octet® Sample Dilution Buffer with Kathon is an effective way to minimize matrix effects. Prior to running the assay, we recommend an optimization step where samples are diluted with varying amounts of Sample Diluent in order to determine the minimum dilution factor required for optimal assay performance. It is also important to test the dilution linearity of the sample to ensure no interference from matrix.

Octet® RH96 System HCP Assay Protocol: A Completely Walk-Away Assay

The entire CHO HCP assay can be set up on the Octet® RH96 system to enable high throughput, walk-away assays. This automated assay format eliminates any potential user-related data variation that can be caused by manual processing and helps achieve the most streamlined and efficient laboratory workflow. A full assay can be performed and data obtained in about one hour with excellent assay precision and run-to-run consistency.

Prepare Samples and Detection Plates

Notes:

All buffers and diluents used in this assay should be azide-free. The presence of azide can inhibit the activity of the HRP enzyme.

Protect Octet® Fluorescein anti-CHO, Anti-FITC HRP, and Metal Enhanced DAB reagents from light.

Please refer to the materials safety data sheet (MSDS) for safety information on the Metal Enhanced DAB concentrate. Dispose of unused and used reagent in accordance with all local, state, and federal guidelines. Proper personal safety measures should also be taken when handling hazardous materials.

1. Equilibrate the samples, reagents and buffers to room temperature and mix thoroughly prior to use. Metal Enhanced DAB concentrate should remain at -20°C until immediately before use.
2. Prepare each concentration of HCP calibration standard in Octet® Sample Dilution Buffer. The calibration standards selected should cover the HCP assay range from 0.5–200 ng/mL, and the recommended calibrator concentrations are 0.5, 1, 2, 8, 25, 75, and 200 ng/mL. It is also recommended to run buffer-only controls as references. A calibration curve should be included in each run.

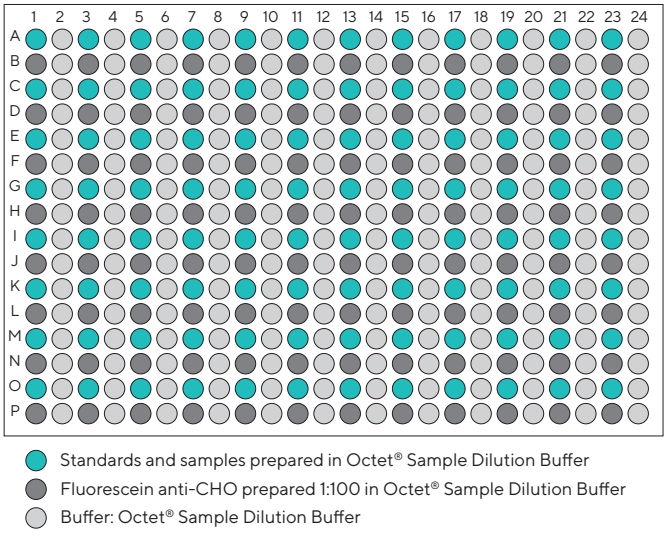


Figure 2: Sample Plate layout for 96 biosensor mode in 384-well plate for Octet® RH96 system.

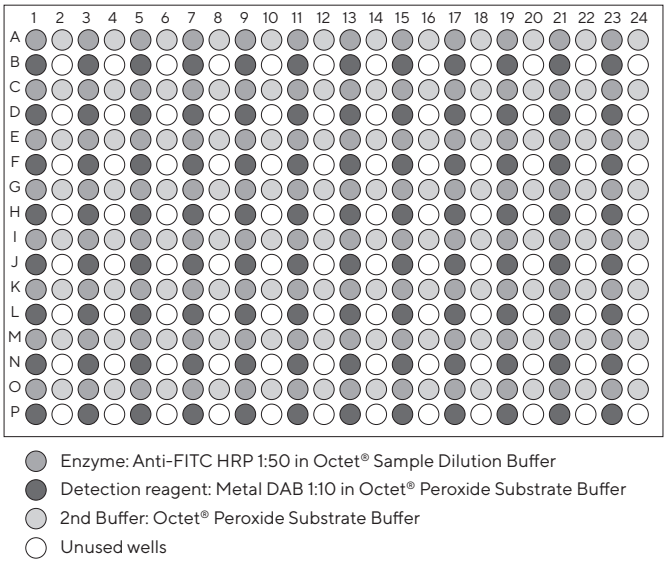


Figure 3: Detection Plate layout for 96 biosensor mode for Octet® RH96 system.

3. Prepare the Sample Plate (example plate layout shown in Figure 2):
   a. Pipette 80 µL of standard and unknown samples prepared in Octet® Sample Dilution Buffer into the wells of a 384-well microplate.
   b. Pipette 80 µL of Fluorescein anti-CHO prepared at 1:100 dilution in Octet® Sample Dilution Buffer into the wells of the 384-well microplate.
   c. Pipette 80 µL of Octet® Sample Dilution Buffer into the wells of the 384-well microplate.
4. Prepare the Detection Plate (example plate layout shown in Figure 3):
   a. Pipette 80 µL of Anti-FITC HRP prepared at 1:50 dilution in Octet® Sample Dilution Buffer into the wells of a 384-well microplate.
   b. Pipette 80 µL of Octet® Peroxide Substrate Buffer into the wells of the 384-well microplate.
   c. Pipette 80 µL of Metal Enhanced DAB prepared at 1:10 dilution in Octet® Peroxide Substrate Buffer into the wells of the 384-well microplate.
5. Prepare the Biosensor Tray. Biosensor locations should correspond to filled wells in the Sample Plate (one biosensor for each filled well in the Sample Plate).
6. Prepare a Hydration Plate by pipetting 200 µL of Octet® Sample Dilution Buffer into each well of a 96-well polypropylene plate. Well locations filled with Sample Diluent in the Hydration Plate should correspond to biosensor locations in Biosensor Tray.

Run the Assay

1. Place the Sample and Detection Plates in the Octet® RH96 instrument on the defined plate stations.
2. Launch Octet® BLI Discoveryand choose the **Advanced Quantitation** option in the Experiment Wizard. In the Assay Settings window, click **Modify** to set up the assay parameters as shown in Figure 4. The 96 biosensor mode settings are shown in the left and right panels. Note that the long sample incubation and Fluorescein anti-CHO incubation steps (run in

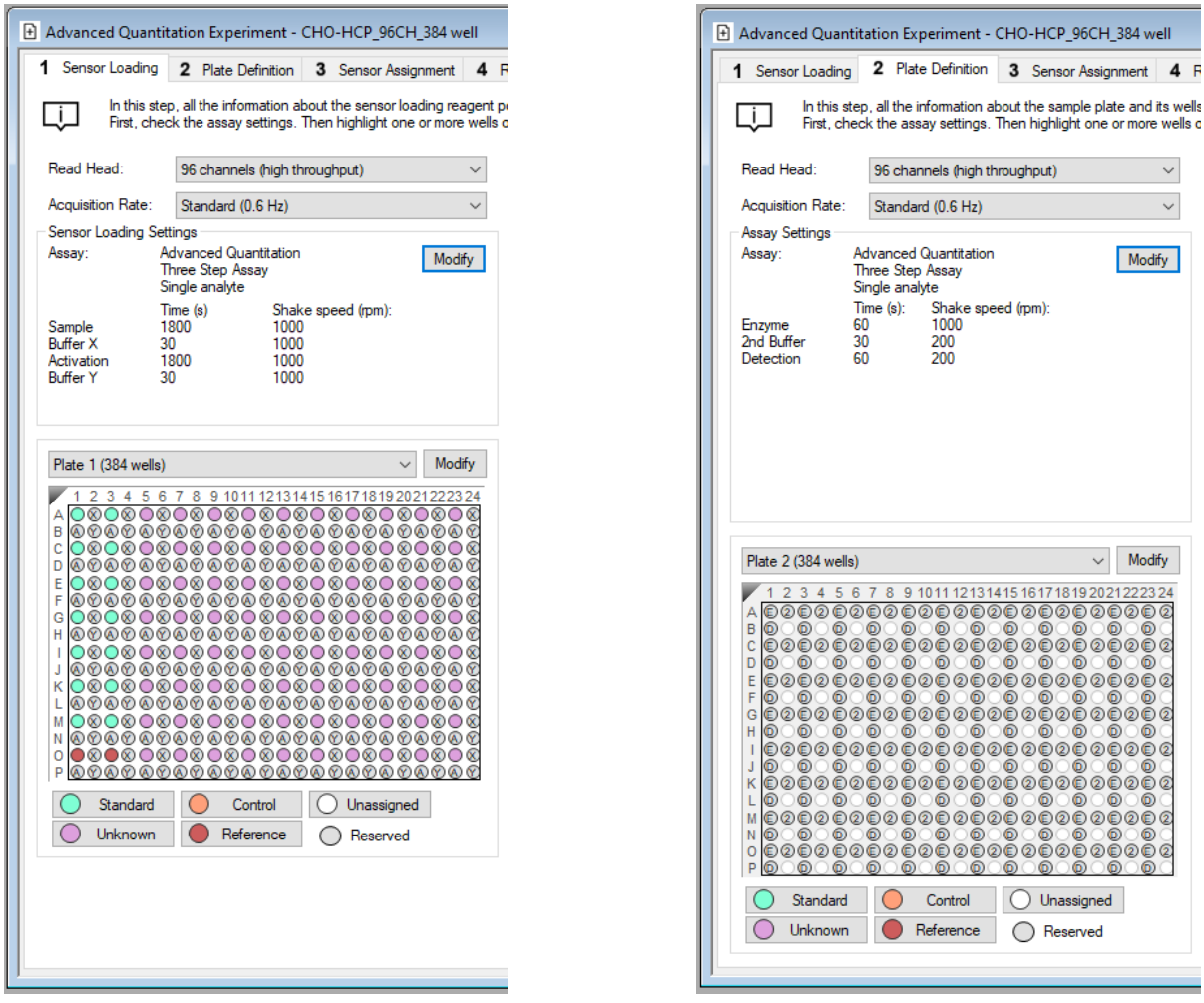


Figure 4: Settings for Advanced Quantitation assays for HCP detection on the Octet® RH96 system. The left panel shows the Sample Plate configuration and assay settings in 96 biosensor mode which contains a sample incubation step, an Fluorescein anti-CHO incubation step, and two wash steps with Sample Diluent. The right panel shows the Detection Plate configuration and assay settings for detection using 96 biosensor mode.

96 biosensor mode) are defined in the Sensor Loading tab, and the remaining steps are defined in the Plate Definition tab. Alternatively, users with Octet® BLI Discovery Software can open pre-set method files by choosing **Experiment** from the main menu, then **Templates**, then **Quantitation**, then **Advanced Quantitation**, and choose the appropriate method file.

3. Click **OK** when assay parameters have been defined.
4. Define the Sample Plate layout to correspond to the layout in Figure 2.
5. Define the Detection Plate layout to correspond to the layout in Figure 3.
6. Enter Sample and Sensor information in the Plate Definition tab and the Sensor Assignment tab as desired.
7. In the Run Experiment tab, enter a delay time of **600** seconds in order to give the plates at least 10 minutes inside the Octet® instrument to equilibrate to assay temperature.
8. Enter a location and file name for saving the data.
9. Click **GO** to run the assay.

Analyze Data

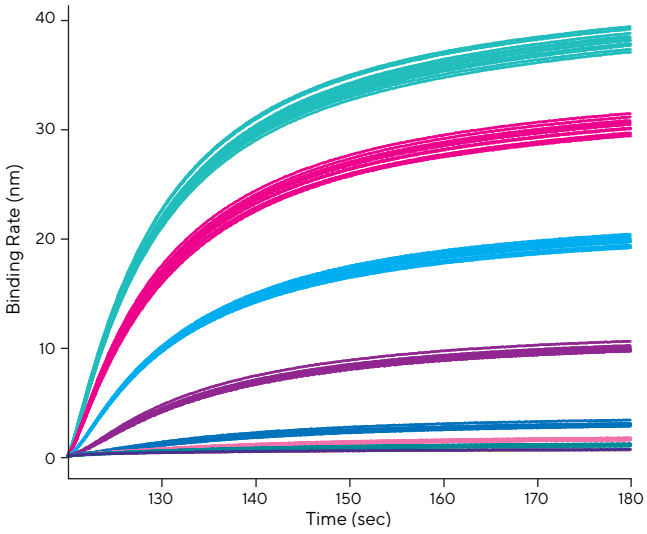
The analysis of the data obtained on the Octet® RH96 system is identical to the analysis of data obtained from other instruments. To analyze the data:

1. In Octet® BLI Analysis Software, load the data folder to be analyzed.
2. Select the reference well and perform reference subtraction if needed.
3. Group and Concentration information can be modified in the table if needed.
4. In the Results tab:
   a. Select **R equilibrium** as the binding rate equation. This equation will fit the binding curve generated during the experiment and calculate a response at equilibrium as the output signal.
   b. Click **Calculate Binding Rate**. Results will be displayed automatically in the table.
   c. Click **Save Report** or select **File > Save Report** to generate a Microsoft® Excel® report file.

Octet® RH16 and R8 System HCP Assay Protocol with Octet® AS Station

The HCP assay for 8- and 16-channel Octet® instruments is performed according to the steps shown in Figure 6. When using these systems, the initial incubation steps are processed using the Octet® AS Station. After biosensors are incubated with HCP samples and Fluorescein anti-CHO antibody, the remaining steps are performed on the Octet® instrument.

Example Data from a Routine Assay



Expected conc. (ng/mL)	Calculated conc. (ng/mL)	Recovery	%CV
200.0	200.0	100%	4.2%
75.0	76.3	102%	5.0%
25.0	25.3	101%	4.4%
8.0	8.08	101%	6.2%
2.0	2.01	100%	4.2%
1.0	1.00	100%	5.0%
0.5	0.52	104%	9.8%

Figure 5: Example data from a CHO HCP assay on the Octet® RH96 system. The data shows a dose response for the calibration standards (N=12). The calculated concentrations and %CV values resulting from the analysis of the data are shown in the accompanying table.

Process samples on the Octet® AS Station

Notes:

All buffers and diluents used in this assay should be azide-free. The presence of azide can inhibit the activity of the HRP enzyme.

Protect Octet® Fluorescein anti-CHO, Anti-FITC HRP, and Metal Enhanced DAB reagents from light.

Please refer to the safety data sheet for safety information on the Metal Enhanced DAB concentrate. Dispose of unused and used reagent in accordance with all local, state, and federal guidelines. Proper personal safety measures should also be taken when handling hazardous materials.

1. Equilibrate the samples, reagents and buffers to room temperature and mix thoroughly prior to use. Metal Enhanced DAB concentrate should remain at -20°C until immediately before use.
2. Prepare each concentration of HCP calibration standard in Octet® Sample Dilution Buffer. The calibration standards selected should cover the HCP assay range from 0.5–200 ng/mL, and the recommended calibrator concentrations are 0.5, 1, 2, 8, 25, 75, and 200 ng/mL. It is also recommended to run buffer-only controls as references. A calibration curve should be included in each run.
3. Prepare the Sample Plate (example plate layout shown in Figure 7):
   a. Pipette 200 µL of each standard into the wells of a 96-well microplate.
   b. Pipette 200 µL of each unknown sample into the wells in the remainder of the microplate.
4. Prepare the Biosensor Tray. Biosensor locations should correspond to filled wells in the Sample Plate (one biosensor for each filled well in the Sample Plate).
5. Prepare one Hydration Plate and two additional Wash Plates by pipetting 200 µL of Octet® Sample Dilution Buffer into each well of three polypropylene 96-well plates. Well locations filled with Sample Diluent in the Hydration Plate should correspond to biosensor locations in Biosensor Tray.
6. Using the Octet® AS Station, hydrate biosensors in the Hydration Plate at 1000 rpm and 30°C for 1 minute.
7. Incubate hydrated biosensors in the Sample Plate on the Octet® AS Station at 1000 rpm and 30°C for 30 minutes.
8. During this incubation, prepare the Octet® Fluorescein anti-CHO Antibody Plate:
   a. Prepare a 1:100 dilution of Octet® Fluorescein anti-CHO antibody in Octet® Sample Dilution Buffer.
   b. Pipette 200 µL into each well of a new 96-well microplate. Filled wells should correspond to biosensor location and number in the Biosensor Tray.
9. After 30 minutes of sample incubation, replace the Sample Plate with the first Wash Plate on the Octet® AS Station and incubate at 1000 rpm and 30°C for 30 seconds.
10. Replace the Wash Plate with the Octet® Fluorescein anti-CHO Antibody Plate. Incubate on the Octet® AS Station at 1000 rpm and 30°C for 30 minutes.
11. During this incubation, prepare the Detection Plate. Either a 96-well or a 384-well microplate can be used depending on the Octet® instrument being used. The fill volumes for 96- and 384-well microplates are 200 µL and 80 µL, respectively.
    a. Prepare an appropriate volume for the plate type of:
       - A 1:50 dilution of the Octet® Anti-FITC HRP antibody in Octet® Sample Dilution Buffer
       - A 1:10 dilution of Metal Enhanced DAB in Octet® Peroxide Substrate Buffer
    b. In a black, flat-bottomed 96- or 384-well microplate, pipette 200 µL or 80 µL respectively of each reagent specified into the wells of the microplate using the plate layout shown in Figure 8). Each reagent needs to be filled into all wells of one column (for 8-channel assays) or two columns (for 16-channel assays) in the Detection Plate.
12. After 30 minutes of Fluorescein anti-CHO incubation, replace the Sample Plate with second Wash Plate on the Octet® AS Station and incubate at 1000 rpm and 30°C for 30 seconds.

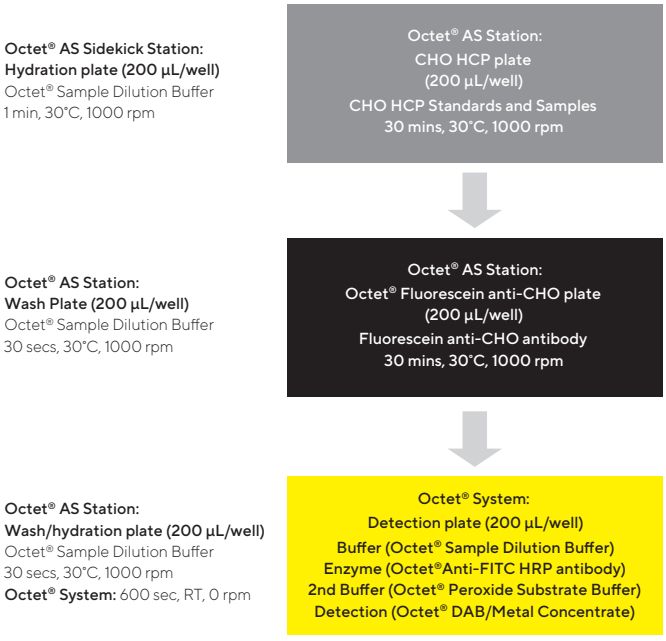


Figure 6: Flow chart of HCP assay steps on 8- and 16-channel Octet® systems with the Octet® AS Offline Immobilization Station.

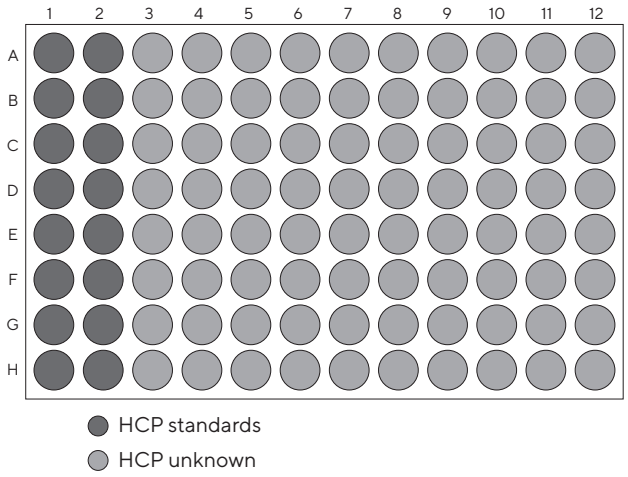


Figure 7: Sample Plate layout for incubation with biosensors on the Octet® AS Station.

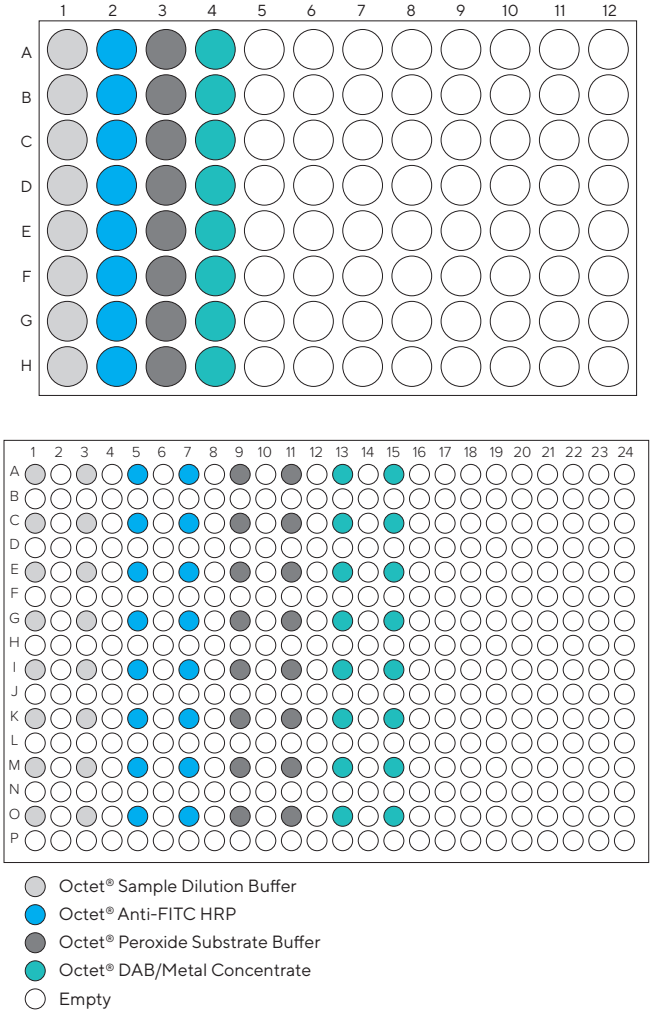


Figure 8: Detection Plate layout for 8-channel assays on Octet® R8 system (top) and for 16-channel assays on Octet® RH16 system (bottom).

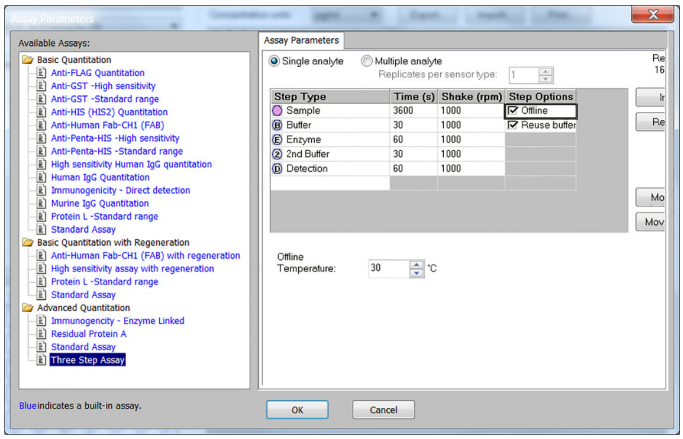


Figure 9: Settings for Advanced Quantitation assays for HCP detection on 8- and 16-channel Octet systems.

Run the Assay

1. After the second wash step on the Octet® AS Station is complete, place the Biosensor Tray over the second Wash Plate (the same plate used on the Octet® AS Station for the last wash) into the Octet® instrument.
2. Place the Detection Plate into the Octet® Instrument. For 8-channel Octet® systems, place the Detection Plate in the single plate station (Plate 1) in the instrument. For 16-channel systems, place the Detection Plate in the reagent plate station (Plate 2).
3. Launch Octet® BLI Discovery Software and choose the **Advanced Quantitation** option in the Experiment wizard. In the Assay Settings window, click **Modify** to set up the assay parameters as shown in Figure 9. Note that the sample step is specified to have been carried out offline.
4. Click **OK** once the assay parameters have been defined.
5. Define the Sample and Detection Plate layouts:

   For 16-channel Octet® systems:

   a. Define the Sample Plate layout to correspond to the layout in Figure 7. The Sample Plate Layout needs to be defined in the Octet® BLI Discovery Software even though the Sample step has been performed offline as this enables the software to assign the correct Sample IDs to the biosensors during analysis.

   b. Define the Detection Plate layout to correspond to the appropriate layout in Figure 9:
      - Octet® Sample Dilution Buffer = **B** (Buffer)
      - Octet® Anti-FITC HRP antibody = **E** (Enzyme)
      - Octet® Peroxide Substrate Buffer = **2** (2nd Buffer)
      - Octet® DAB/Metal Concentrate = **D** (Detection)

For 8-channel Octet® systems:

a. Specifying the Sample step to be carried out offline enables two plates to be opened in the software (even though there is only one plate position in the instrument). The first plate (Plate 1) has the same layout as the Sample Plate layout used in the Octet® AS Station incubation.
b. The layout of the second plate (Plate 2) is defined to correspond to the appropriate Detection Plate layout in Figure 9 where:
   - Octet® Sample Dilution Buffer = **B** (Buffer)
   - Octet® Anti-FITC HRP antibody = **E** (Enzyme)
   - Octet® Peroxide Substrate Buffer = **2** (2nd Buffer)
   - Octet® DAB/Metal Concentrate = **D** (Detection)

6. Enter Sample and Sensor information in the Plate Definition tab and the Sensor Assignment tab as desired.
7. In the Run Experiment tab, enter a delay time of **600** seconds in order to give the plates at least 10 minutes inside the Octet® instrument to equilibrate to assay temperature.
8. Enter a location and file name for saving the data.
9. Click **GO** to run the assay.

Analyze Data

1. In Octet® BLI Discovery Software, load the data folder to be analyzed.
2. Select the reference well and perform reference subtraction if needed.
3. Group and Concentration information can be modified in the table if needed.
4. In the Results tab:
   a. Select **R equilibrium** as the binding rate equation. This equation will fit the binding curve generated during the experiment and calculate a response at equilibrium as the output signal.
   b. Click **Calculate Binding Rate**. Results will be displayed automatically in the table.
   c. Click **Save Report** or select **File** > **Save Report** to generate a Microsoft® Excel® report file.

Example Data from a Routine Assay

The data shown was generated on the Octet® RH16 system using the protocol outlined in this technical note. CHO-HCP standards at 200, 75, 25, 8, 2, 1, and 0.5 ng/mL in Octet® Sample Dilution Buffer were prepared and run in triplicate. Three unknowns (Samples 1, 2 and 3) were run in 8 replicates to assess assay precision.

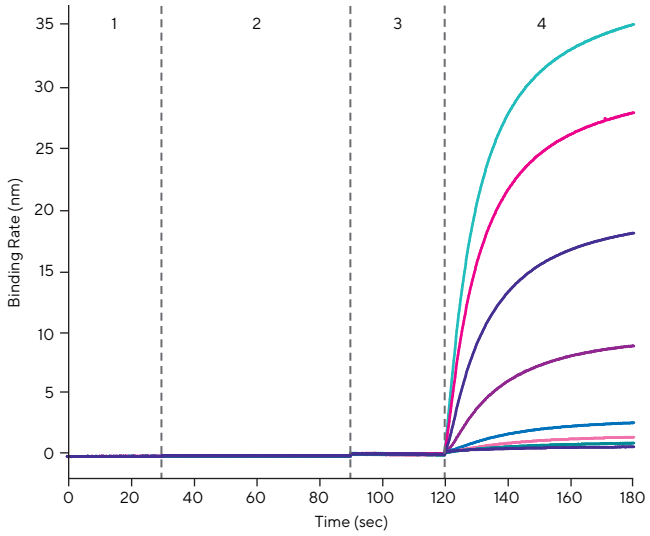
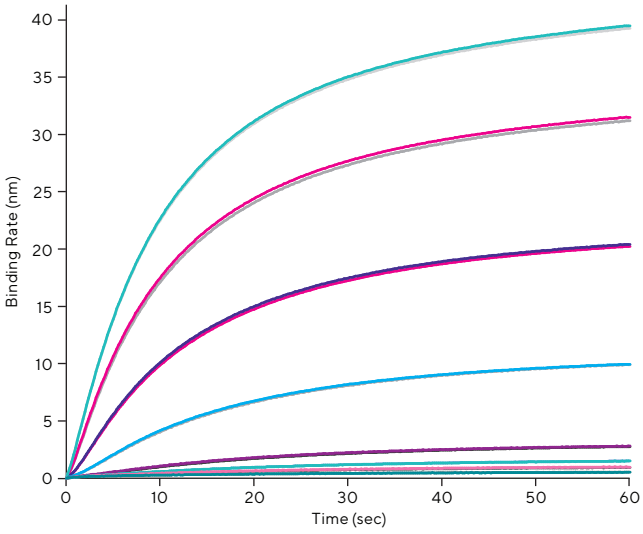
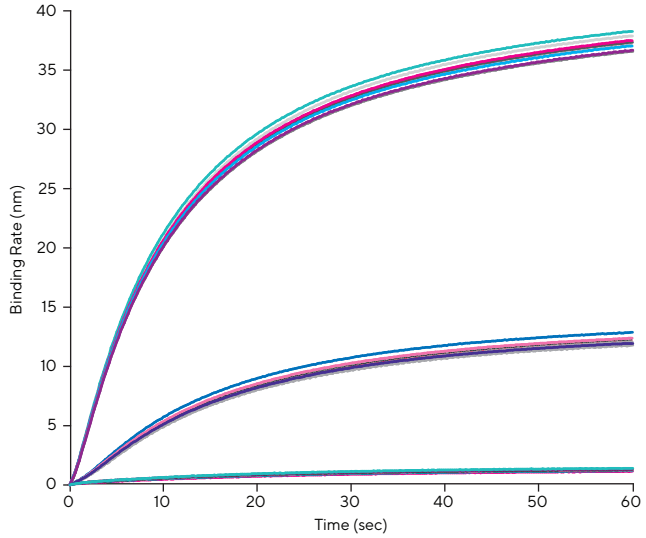


Figure 10: Real time data from detection steps measured on the Octet® RH16 system with the CHO HCP assay. Processing of the biosensors with samples and the binding of the Octet®™ Fluorescein anti-CHO antibody were performed using the Octet® AS Station. The steps shown include 1) baseline in Sample Diluent, 2) binding of Octet® Anti-FITC HRP antibody, 3) Octet® Peroxide Substrate Buffer equilibration and 4) detection of signal using Octet® DAB/Metal Concentrate. The assay was run according to the procedure outlined in this technical note.



Expected conc. (ng/mL)	Calc. conc. (ng/mL)	Recovery	%CV
200.0	201.0	101%	2.1%
75.0	73.9	99%	0.7%
25.0	25.5	102%	2.3%
8.0	8.08	101%	2.8%
2.0	2.01	101%	1.8%
1.0	1.00	100%	1.7%
0.5	0.50	100%	3.3%

Figure 11: The data shows a dose response for the calibration standards in triplicate. The calculated concentrations and %CV values resulting from the analysis of the data are shown in the accompanying table.



Sample	Calc. con. (ng/mL)	%CV
Sample 1	137.5	4.1%
Sample 2	10.6	4.0%
Sample 3	0.43	5.1%

Figure 12: Graph showing data for three unknown samples, each having eight replicates. The calculated concentrations and %CV are shown in the accompanying table. The concentrations were calculated using the calibration data shown in Figure 11.

Assay Troubleshooting Guide

Issue	Possible reasons	Suggested solutions
No signal from HCP standards	Peroxide Substrate Buffer and Sample Diluent switched.	Re-run assay taking care to dilute the Metal Enhanced DAB concentrate in Octet® Peroxide Substrate Buffer and samples and standards in Octet® Sample Dilution Buffer.
	Peroxide Substrate Buffer or Metal Enhanced DAB is no longer active.	Check for activity by combining Octet® Peroxide Substrate Buffer and Metal Enhanced DAB with 1 µL of HRP anti-FITC antibody. A dark precipitate should appear if all are active.
	Reagents no longer active.	Confirm that the Metal Enhanced DAB has been stored at -20°C, the Octet® Peroxide Substrate Buffer at either -20°C or 4°C (after opening), the biosensors at room temperature and the Octet® CHO standards, Fluorescein anti-CHO and HRP anti-FITC at 4°C.
No sample signal	HCP concentration is below the LOD.	Perform a dilution series of the sample in order to determine what dilution is required to bring the sample signal within the dynamic range of the assay.
	Azide present in samples.	Dialyze sample to remove azide.
Sample signal is too high	HCP concentration is above the concentration of the highest standard.	Perform a dilution series of the sample in order to determine what dilution is required to bring the sample signal within the dynamic range of the assay.
High variability between runs	Inconsistency in incubation times.	For best results, assay step times should be consistent across standards and samples. For incubation steps involving manual intervention, use a lab timer to ensure all samples and assays are treated similarly.
	Octet® Fluorescein anti-CHO, anti-FITC HRP and Metal Enhanced DAB not protected from light.	Protect Octet® Fluorescein anti-CHO, anti-FITC HRP and Metal Enhanced DAB from light by storing in a dark, dry place when not in use. Additionally, dilutions of these materials should be stored in a dark place until they are pipetted into micro plates and placed either into the instrument or onto the Octet® AS Station.

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