Pall ForteBio LLC-Cygnus Anti-CHO HCP Detection Kit for Fast and Easy Detection of Residual CHO Host Cell Proteins on Octet Systems

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Abstract

Pall ForteBio LLC has teamed up with Cygnus Technologies to jointly develop an Anti-CHO HCP Detection Kit for quantitation of residual host cell proteins. While Pall ForteBio LLC Octet® systems are known industry-wide for easy and rapid high-throughput protein analysis, Cygnus 3G HCP ELISA kits are known for their broad HCP recognition and excellent sensitivity. The Pall ForteBio LLC-Cygnus Anti-CHO HCP assay kits bring scientists the best of both worlds. Assay performance using sample data sets are described as evidence of assay robustness, sensitivity, and precision.

Introduction

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 ways to reduce them to the lowest possible levels have become critical aspects of drug safety and qualification.

Bio-Layer Interferometry (BLI) Technology

Bio-Layer Interferometry is an analytical technique that monitors the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer (Figure 1). Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern. This change, measured in nanometers (nm), is reported in real time.

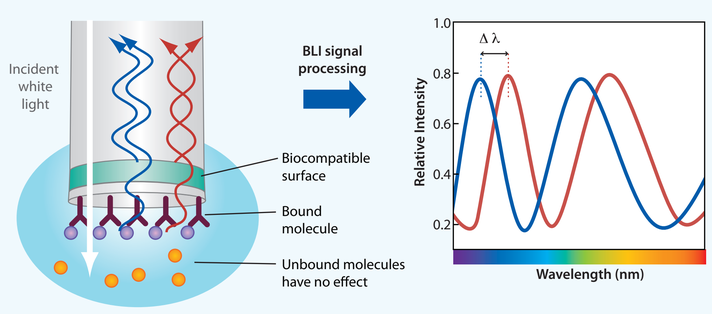


FIGURE 1: Bio-Layer Interferometry (BLI) technology principle.

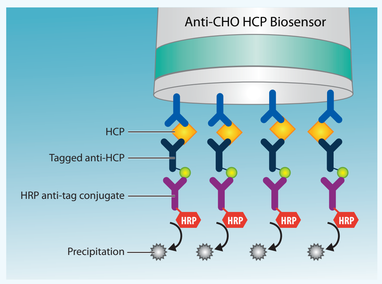
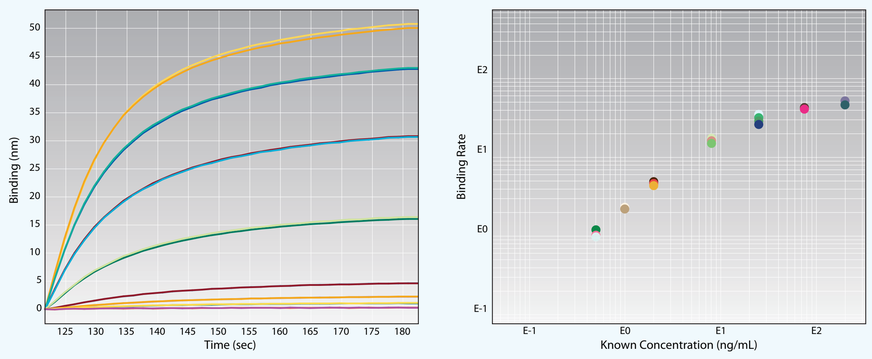


FIGURE 2: Step-wise illustration of anti-CHO HCP assay protocol on biosensor tip.

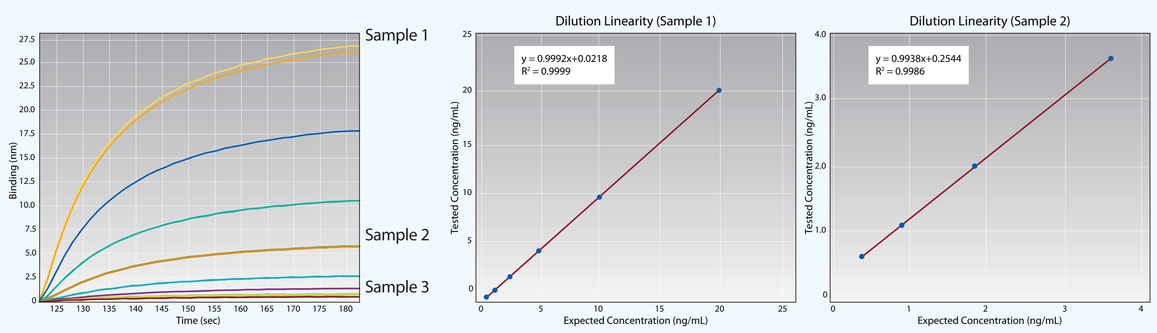
CHO HCP Quantitation Assay

The anti-CHO HCP detection assay performed on Pall ForteBio LLC's Octet platform is a superior alternative to ELISA, providing improved precision in measurements, enhanced sensitivity and dynamic range, low user intervention, and much faster time-to-results. The quantitation assay is performed on actual in-process samples, and assay results are shown below.



Expected Concentration (ng/mL)	Calculated Concentration (ng/mL)	% Recovery	%CV
200.0	200.00	100%	4.9%
75.0	75.55	101%	1.8%
25.0	25.10	100%	1.1%
8.0	8.06	101%	2.5%
2.0	2.01	100%	0.4%
1.0	1.00	100%	0.0%
0.5	0.50	100%	2.4%

FIGURE 3: Example data from the duplicate analysis of CHO HCP standard using Anti-CHO HCP Biosensors on the Octet QK[e] instrument. Real time binding curves for standards (top left) and resulting dose response curve (top right) are shown. The calculated concentrations and %CV values resulting from the analysis of the data are shown in the accompanying table. 100% recoveries were obtained with %CVs that ranged from 0.0–4.9%.



	Well Conc. (ng/mL)	Dilution Factor	Calc. Conc. (ng/mL)	%CV	Avg. HCP (ng/mL)	Drug Substance (mg/mL)	HCP (ppm)
Sample 1	19.95	1,000	2,0347	2.5%	19,967	0.02	998,367
	9.96	2,000	19,977	0.4%			
	5.08	4,000	20,327	0.2%			
	2.59	8,000	20,803	0.5%			
	1.18	16,000	18,839	0.3%			
	0.63	32,000	20,175	0.9%			
Sample 2	3.63	20	72	4.2%	81	0.32	253
	2.02	40	81	2.2%			
	1.12	80	89	0.9%			
	0.63	160	101	1.2%			
Sample 3	0.32	20	6.4	2.9%	6	10	0.6

FIGURE 4: Example data from the duplicate analysis of three unknown samples run in serial dilution (top left). Calculated concentrations and %CV values are shown in the accompanying table (bottom). Concentrations were calculated using the calibration data shown in Figure 3. The dilution linearity graphs for Sample 1 and Sample 2 are shown (top right). %CVs were <2.9% for samples under 1 ng/mL.

Materials and Methods

Anti-CHO HCP biosensors, part of the Anti-CHO HCP Detection Kit (Pall ForteBio LLC, part no. 18-5081), were hydrated in Sample Diluent (Pall ForteBio LLC, Part no. 18-1067) for a minimum of 10 minutes prior to use. The CHO HCP antigen concentrate, 3G protein (Cygnus, part no. F553H) and three unknown samples were analyzed. Typical analysis with standard was performed by diluting analyte into Sample Diluent at specified concentrations as shown in Figure 3. Three unknown samples were analyzed in serial dilutions as appropriate (Figure 4). Each sample was analyzed in duplicate on the Octet QK[e] instrument. Octet Data Analysis v8.0 Software was used to calculate well concentrations. Coefficients of variation (%CV) were calculated based on duplicate assays. Dynamic range was defined as a <15% CV and a separation of at least three times the standard deviation from neighboring concentrations.

Intra- and Inter-assay Precision

CHO HCP Concentration	Intra-assay %CV	Inter-assay %CV
1 ng/mL	7.3%	8.2%
8 ng/mL	1.6%	2.6%
75 ng/mL	2.6%	3.4%

TABLE 1: Both the intra- and inter-assay precision were determined on three samples prepared at 1 ng/mL, 8 ng/mL and 75 ng/mL. %CVs were calculated based on 16 replicates (N=16). %CVs ranged from 8.2% (1 ng/mL) to 2.6% (75 ng/mL).

Spike Recovery of CHO HCPs from Actual *In-Process* Samples

A spike recovery study of the purified CHO HCPs in actual in-process samples was performed and shows ±10% of expected concentrations.

	Endogenous Value (ng/mL)	Spike in Amt. (ng/mL)	Expected (ng/mL)	Tested (ng/mL)	% Recovery
Sample 1	14.3	50	64.3	70.7	110%
Sample 2	12.9	25	37.9	38.4	101%
Sample 3	16.3	10	26.3	26.6	101%

TABLE 2: Spike recovery of CHO HCPs in real samples using Anti-CHO HCP Biosensors on the Octet HTX instrument.

Materials and Methods

The CHO HCP antigen concentrate, 3G protein (Cygnus, part no. F553H) was spiked into three real in-process CHO samples at an appropriate amount (Table 2). The % recovery was calculated based on the tested value of the spiked sample over its expected value (endogenous value + spike value). Each sample was analyzed in duplicate on the Octet HTX instrument.

Performance on Multiple Instrument Platforms

The Anti-CHO HCP Detection Kit can be used on all current Pall ForteBio LLC systems with comparable performance. An HCP assay analyzing 96 samples can be set up to run automatically on the Octet HTX instrument in a completely hands-off walk-away manner, with results obtained in one hour. The assay can also be run on other 8– and 16–channel Octet instruments together with the Sidekick Station with time-to-results of 75 and 90 minutes respectively.

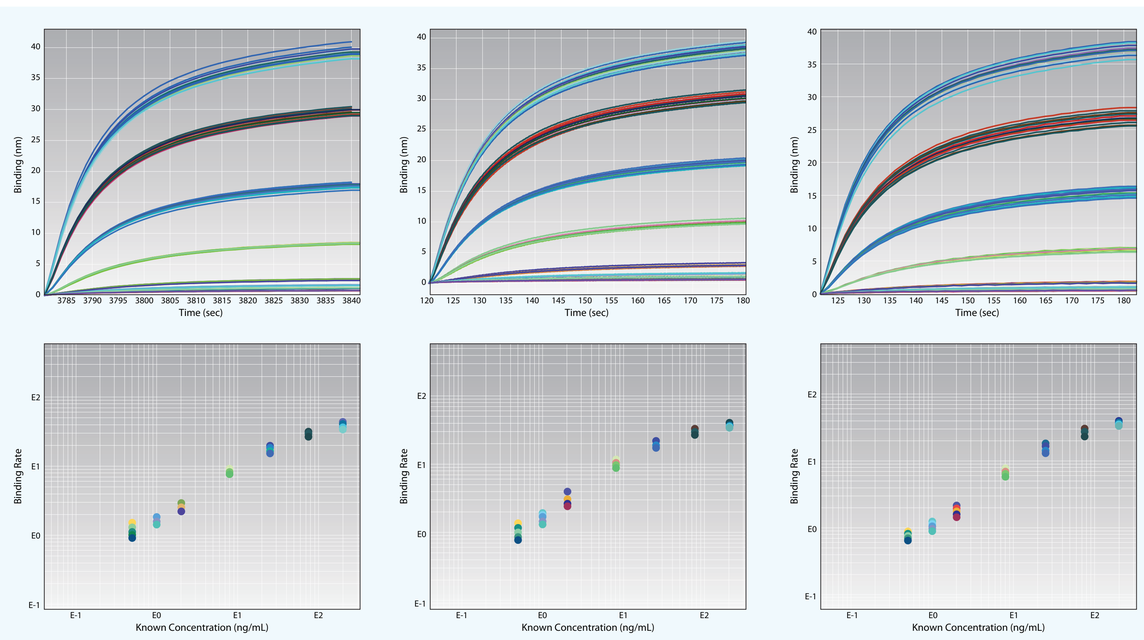


FIGURE 5: Replicate analysis (N=12) of CHO HCP protein using Anti-CHO HCP Biosensors on the following instruments: Octet HTX, Octet RED384, and Octet QKe systems. Analysis was performed in Sample Diluent at the concentrations specified in Table 3. Real time binding curves (top) and the resulting dose response curve (bottom) are shown.

	Instrument	Octet HTX		Octet RED384		Octet QK[e]	
	Concentration	BR (nm/s)	%CV	BR (nm/s)	%CV	BR (nm/s)	%CV
Dynamic Range	200 ng/mL	38.29	2.0%	37.35	1.9%	36.49	2.3%
	75 ng/mL	28.99	1.8%	29.67	2.2%	26.39	3.2%
	25 ng/mL	17.58	2.0%	19.54	2.0%	15.46	3.9%
	8 ng/mL	8.43	1.0%	9.91	2.5%	6.71	3.7%
	2 ng/mL	2.57	2.8%	2.94	4.7%	1.79	4.7%
	1 ng/mL	1.63	2.8%	1.57	3.9%	1.05	3.3%
	0.5 ng/mL	1.16	5.0%	1.03	5.8%	0.75	2.4%
	0 ng/mL	0.74	1.8%	0.56	8.3%	0.49	7.1%

TABLE 3: Dynamic range and precision data for 3G CHO HCP Antigen Concentrate detection with Anti-CHO HCP biosensors (N=12) on multiple instruments. A cross-platform dynamic range of 0.5–200 ng/mL with <8.3% CVs was achieved.

Materials and Methods

Biosensors from the Anti-CHO HCP Detection Kit (ForteBio part no. 18-5081) were hydrated in Sample Diluent (Pall ForteBio LLC, part no. 18-1067) for a minimum of 10 minutes prior to use. The CHO HCP antigen concentrate, 3G protein (Cygnus, part no. F553H) was analyzed on Anti-CHO HCP biosensors on the following instruments: Octet HTX, Octet RED384, and Octet QK[e] systems. Analysis was performed by diluting analyte into Sample Diluent at the specified concentrations as shown in Table 3. Each sample was analyzed in 12 replicates (N=12). Octet Data Analysis v8.0 Software was used to calculate the binding rates (BR). %CVs were calculated based on the N=12 replicate assays. Dynamic range was defined as a %CV of <15% and a separation of at least three times the standard deviation from neighboring concentrations.

Conclusion

- The Anti-CHO HCP Detection Kit can be used for specific detection and quantitation of residual CHO host cell proteins (HCPs) as an automated alternative method to ELISA that eliminates hands-on time, improves data quality and decreases time to analyzed results (one hour for 96 samples on the Octet HTX system).
- The results demonstrate the excellent specificity, accuracy, and precision of HCP assays performed using the Anti-CHO HCP Detection Kit on ForteBio's Octet systems. In the data shown in Figure 3, assay recovery ranged from 100-101% and assay precision ranged from 0.0–4.9%.
- Assay robustness is shown by excellent dilution linearity obtained on actual in-process samples. Assay data in Figure 4 shows R^2 values of 0.9986 and higher were obtained.
- The Anti-CHO HCP Detection Kit complements other pre-fabricated and custom biosensors for the Octet platform that provide a comprehensive set of tools for use across the entire bioprocessing workflow — from cell line development to process monitoring and quality control.

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