

Dip and Read™ Anti-CHO HCP Detection Kit

For CHO Host Cell Protein Detection

KEY FEATURES

- Completely hands-off, walk-away HCP analysis
- Fully analyzed HCP results for 96 samples in one hour
- High precision assays with 5-10% CVs
- Detection sensitivity as low as 0.5 ng/mL



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. These residual HCPs carry substantial risk of decreasing efficacy of the drug and causing adverse immunogenic reactions in patients. Hence, detecting residual HCP contaminants and methods to reduce them to the lowest acceptable levels are critical aspects of drug safety and qualification.

Pall ForteBio LLC and Cygnus Technologies have jointly developed the Anti-CHO HCP Detection Kit for quantitation of residual HCPs. The Octet® platform's rapid high-throughput protein analysis

combined with the broad HCP recognition and sensitivity of the industry-standard Cygnus 3G anti-CHO HCP antibody bring scientists the best of both worlds for HCP analysis.

WALK-AWAY ASSAYS

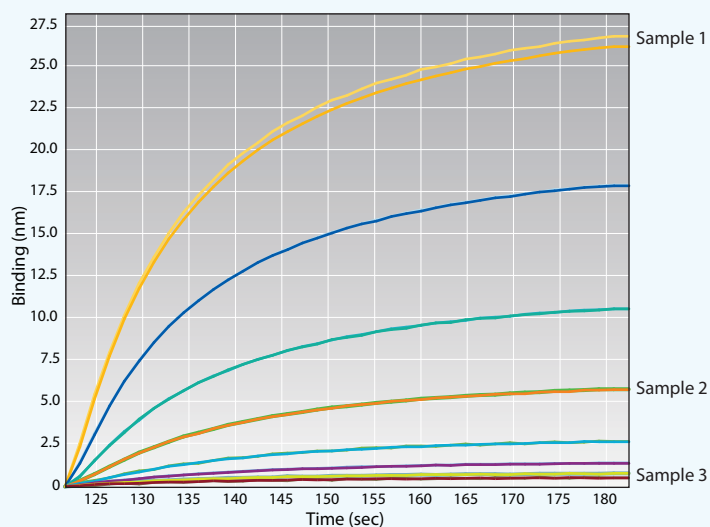
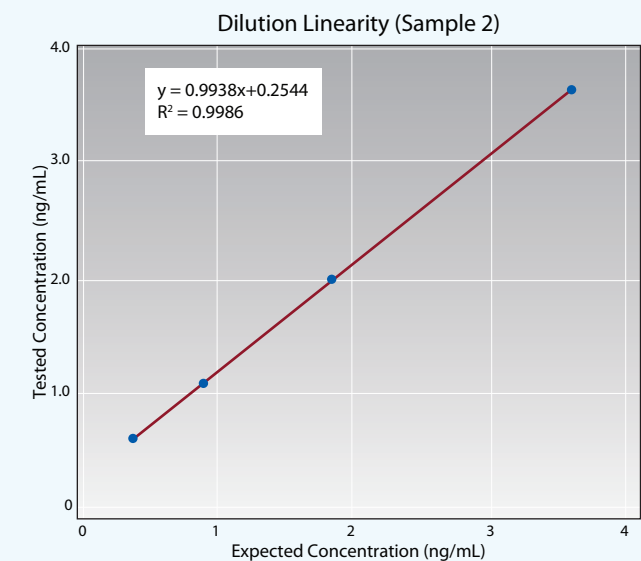
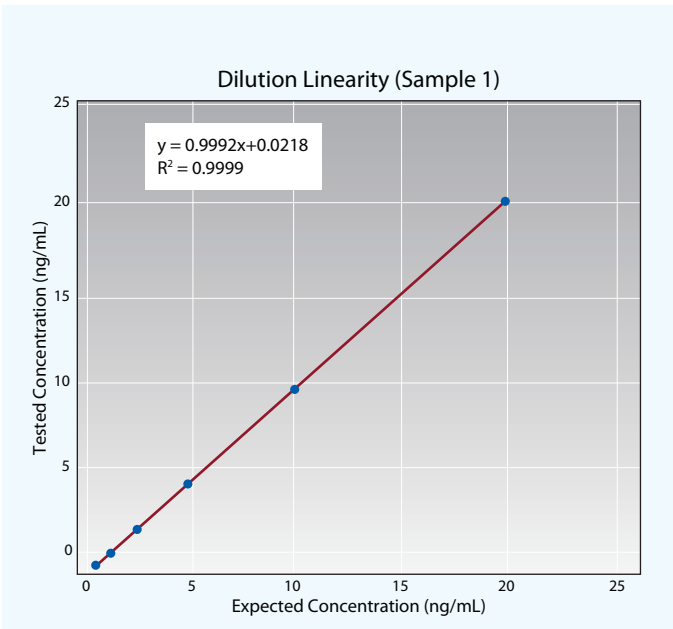
Among current HCP analytical methods, ELISA is the most commonly used. However, ELISA's highly manual processing steps introduce data variability and multiple time-consuming incubation steps are required. Octet systems automate the entire process to drastically improve assay precision and need minimal user intervention. A completely hands-off, walk-away HCP assay run on the Octet HTX system provides fully analyzed results for 96 samples in one hour, compared to three with ELISA. Assays run on other Octet systems with the Sidekick Station provide time to results of 75 and 90 minutes, respectively.

Assay Performance	Cygnus 3G ELISA Kit	Pall ForteBio-Cygnus Anti-CHO HCP Detection Kit
Time-to-Result	210 min	62 min on Octet HTX system
		75 min on Octet RED384 system with Sidekick Station
		90 min on Octet RED96 system with Sidekick Station
Dynamic Range	1–100 ng/mL	0.5–200 ng/mL
Precision	15–25%	5–10%

TABLE 1: Comparison of overall assay performance for HCP analysis on Octet systems and ELISA for 96 samples.

SAMPLE DATA

HCP assays involve greater technical complexity due to the sheer variety of proteins present in samples and unique HCP recognition pattern of the polyclonal anti-HCP antibody. It is therefore reasonable to expect HCP readings to differ with different polyclonal anti-HCP antibodies. Even different generations of the same anti-HCP antibody will give results that are 10-20X different. In the routine testing of HCP using several methods, a difference of 2-3X between results is normal and within expectations. The expected trend of results in accordance with process stage (decreasing HCP readings with further purification) is considered more important than the absolute HCP values. Furthermore, users should test and validate any HCP assay by performing spike recovery and dilutional linearity studies. The following are various examples of HCP detection assays and comparisons performed on various Octet systems at different pharmaceutical companies for in-process HCP samples.



	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

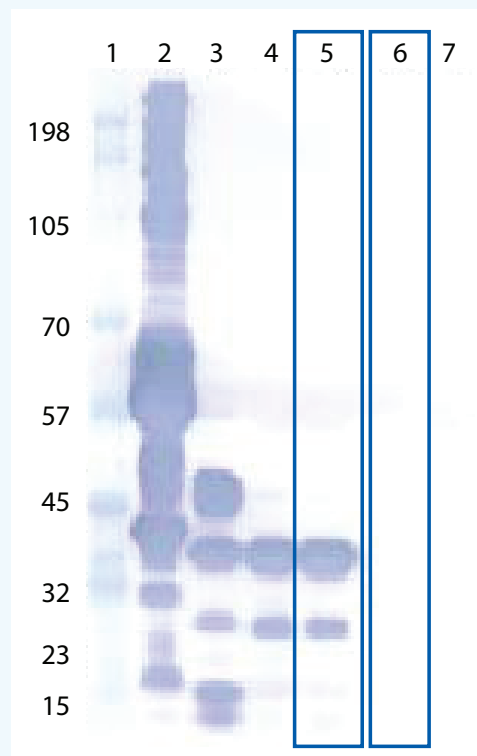
FIGURE 1: HCP quantitation on the Octet QK® system. Example data from the duplicate analysis of three unknown samples run in serial dilution (left). Calculated concentrations and %CV values are shown in the accompanying table (bottom). The dilution linearity graphs for Sample 1 and Sample 2 are shown (top and bottom right).

	Cygnus 1G ELISA	Cygnus 2G ELISA	Cygnus 3G ELISA	Pall ForteBio-Cygnus Kit
Sample 1	227	803	7712	7530
Sample 2	11	94	282	587
Sample 3	198	290	2491	3628

TABLE 2: Pall in-house bioprocess samples analyzed with different HCP assay kits. The Cygnus 3G ELISA and Pall ForteBio-Cygnus Anti-CHO HCP Detection Kit results show excellent agreement.

Sample Set	Drug Titer (mg/mL)	In-house HCP ELISA (ppm)	HCP Assay on Octet Platform (ppm)
Sample A	29.4	187	331
Sample B	30.7	301	336
Sample C	28.7	374	391
Sample D	30.0	310	375
Sample E	28.7	341	396
Sample F	28.9	353	363
Sample G	1.53	154	269
Sample H	1.52	247	467
Sample I	1.61	97	181
Sample Zero	0.95	127	279

TABLE 3: Sample data from a major US pharmaceutical company, comparing results from an HCP ELISA assay developed in-house to an HCP assay using the Anti-CHO HCP Detection Kit on the Octet platform. Results are in very good agreement for each sample between the two methods.



In-process Sample	Concentration (ng/mL)	ppm
Before Column	109791	105063
After Column	26	31

FIGURE 2: Sample data from a major US pharmaceutical company, in which the results from Anti-CHO HCP Kit confirmed prior sample data obtained from SDS-PAGE showing the near-complete removal of HCP from a process sample. Lane 5 data represents the sample before it was run through a purification column, lane 6 is the sample data post-purification.

Samples	Cygnus 3G ELISA			HCP Assay on Octet Platform			
	Dilution factor	Concentration (ng/mL)	CV	Dilution factor	Concentration (ng/mL)	CV	
In-process Sample 1	10000	253714	5.3%	2000	186870	4.2%	
	4000	254735	6.3%		4000	200190	5.8%
	20000	221625	5.6%				
In-process Sample 2	500	4577	4.0%	100	2695	3.5%	
				500	2577	1.1%	
In-process Sample 3	20	41	9.4%	10	121	2.3%	
				20	106	2.5%	

TABLE 4: Sample data from a major European pharmaceutical company, showing excellent agreement (1–2X) in concentration values between different in-process samples using the Cygnus 3G ELISA kit and the Pall ForteBio-Cygnus Anti-CHO HCP Detection Kit, with higher precision obtained using the Octet system.

ORDERING INFORMATION

Part No.	UOM	Description
18-5081	Tray	One Anti-CHO HCP Detection Kit. Contains all reagents, calibrators, buffers and biosensors to analyze 96 HCP samples.
18-5123	Pack	Five Anti-CHO HCP Detection Kits. Contains all reagents, calibrators, buffers and biosensors to analyze 480 HCP samples.




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