ProteinSEQ HCP quantitation — a qPCR-based approach for highly sensitive and rapid quantitation of host cell proteins

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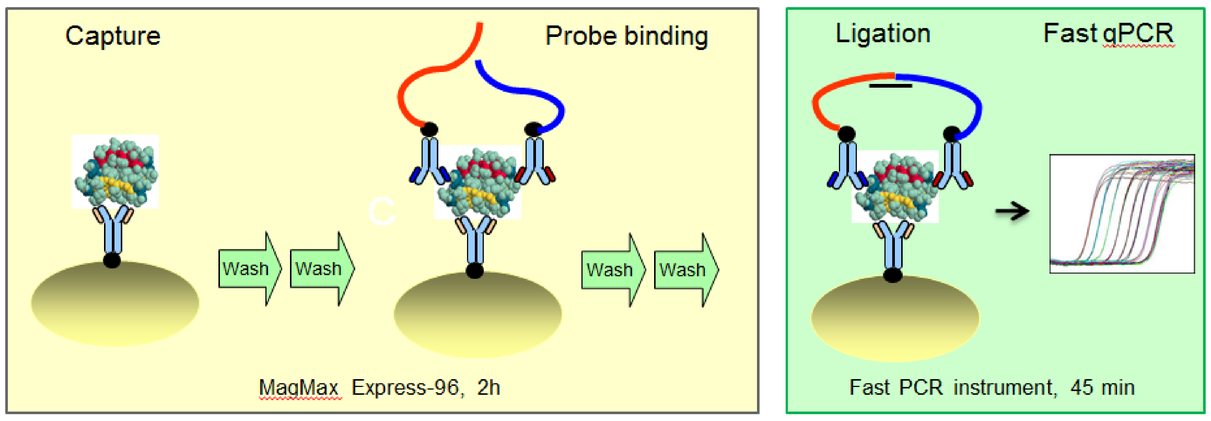
Abstract

Host cell proteins (HCP) are process-related impurities inherent to the manufacture of biopharmaceutical products. Regulatory concerns of HCP include direct or indirect clinical risks for the patients, including immunogenicity and product efficacy. HCP quantitation is a multi-hundred analyte assay that presents unique challenges due to the great number and diversity of the analytes.

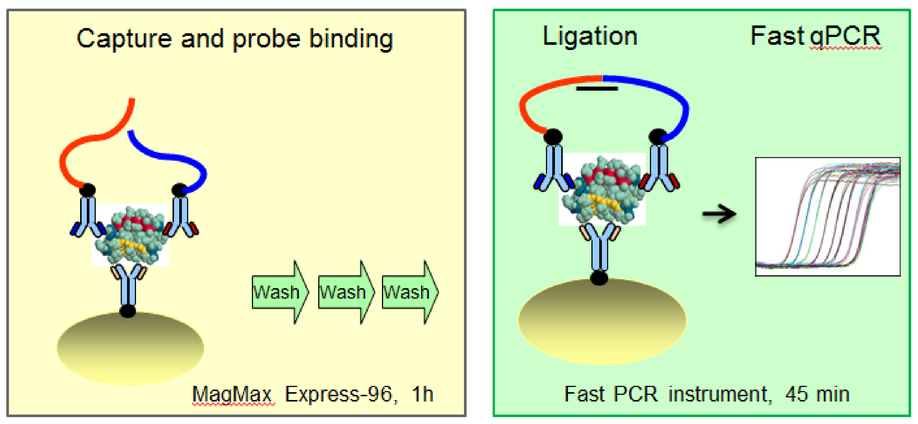
ProteinSEQ CHO HCP assay is based on the Solid-phase Proximity Ligation Assay (spPLA) technology, which combines the high specificity of antibody–protein binding with the high sensitivity of quantitative PCR (qPCR). ProteinSEQ CHO HCP assay (using Cygnus CHO HCP 3G antibody) demonstrated a wide and nearly linear dynamic detection range of 4 logs and is more sensitive than ELISA for HCP quantitation, with a limit of quantitation (LOQ) <1 ppm. Excellent robustness to challenging matrices is exhibited with a quantitation efficiency >70% in matrices containing high concentrations of salt, detergents and 10–100 mg/ml human IgG. These features position ProteinSEQ to be a more advanced immunoassay than ELISA for HCP quantitation in biological drugs.

Figure 1. ProteinSEQ work flow comprises sample prep, automated sample process on MagMax instrument and qPCR data acquisition.

Figure 1. Schematics of MagMax process (standard and short protocols) of the magnetic beads-based immunoassay.

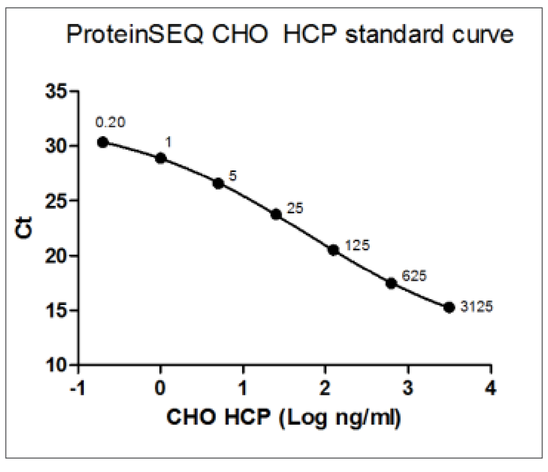


Short protocol



Results

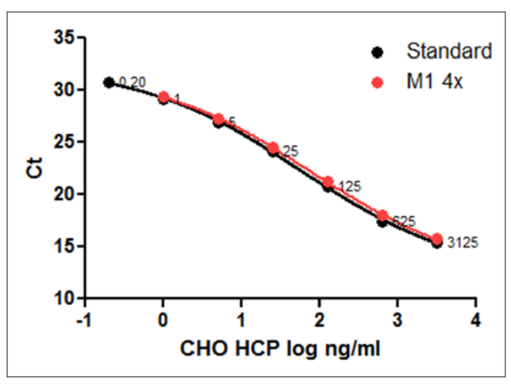
Figure 1. HCP quantitation with high sensitivity and broad dynamic range.



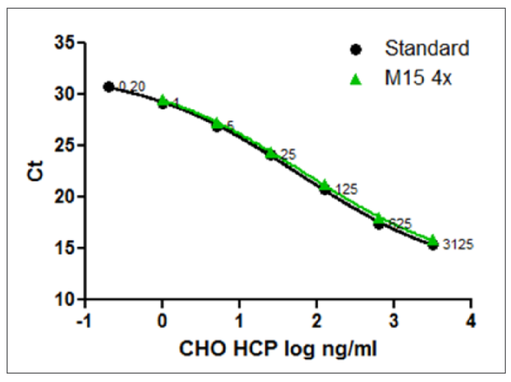
Input HCP ng/mL	Average Ct (n=3)	Back calculation		
		Average ng/ml	Recovery %	CV %
3125	15.3	3047.3	97.5	3.3
625	17.4	651.8	104.3	2
125	20.5	123	98.4	2
25	23.8	24.3	97.3	0.6
5	26.6	5.2	104.9	6.7
1	28.9	1	98	9.8
0.2	30.3	0.2	100.8	21.8
NPC	32			

Non-linear regression and back calculation were analyzed using AccuSEQ 2.1 software. NPC is “no protein control” for background Ct from the assay. Three replicates were run for each concentration. The results indicate a good back calculation recovery and small variation, with a dynamic range of 4 logs.

Figure 1: Quantitation efficiency and precision of HCP in the simulated elution buffer of ion-exchange chromatography.



Input ng/ml	Measured ng/ml	Efficiency %	CV %
3125	2241.9	71.7	1.3
625	504.0	80.7	1.1
125	92.7	74.2	5.4
25	19.7	78.6	4.0
5	4.3	86.1	5.5
1	0.9	87.5	16.8



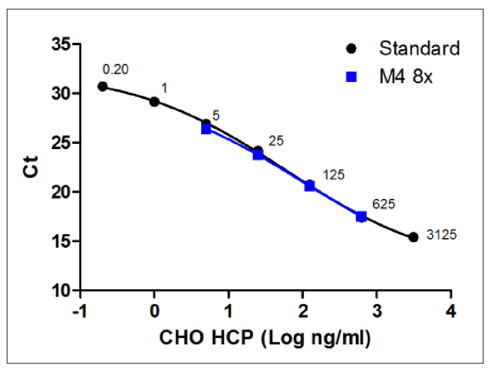
Input ng/ml	Measured ng/ml	Efficiency %	CV %
3125	2042.0	65.3	6.8
625	508.7	81.4	7.6
125	93.7	74.9	8.1
25	20.4	81.4	5.3
5	4.4	87.5	4.4
1	0.8	78.8	8.3

CHO HCP was spiked into Matrices M1 and M15. M1 and M15 simulate elution buffer of ion-exchange chromatography. The results indicated good quantitation efficiencies and small quantitation variations (CV% <20%).

Table 1: Comparison of solid-phase PLA and ELISA assays.

Method	Time	Quantitation limit	Quantity/reaction	Dynamic range
ELISA	3 h	1 ng/ml	50–500 ng	2 logs
ProteinSEQ	3–4 h	0.2 ng/ml	6–9x10⁴ ng	4 logs

Figure 1. Quantitation efficiency and precision of HCP in a simulated bulk drug substance.



Input ng/ml	Measured ng/ml	Efficiency %	CV %
625	659.0	105.4	6.5
125	128.8	103.0	4.0
25	28.4	113.4	1.6
5	7.2	143.4	2.1

CHO HCP was spiked into M4, which was 8-fold diluted with the assay diluent. M4 contains 100 mg/ml human IgG and simulates the bulk drug substance. The results indicated good quantitation efficiencies and small quantitation variations (CV% <20%).

Figure 1. Dilution linearity.

Input ng/ml	Measured ng/ml	Efficiency %	CV %
3125	2282.3	73.0	13.1
625	641.7	102.7	
125	129.0	103.3	
25	24.5	101.6	
5	5.2	102.6	
1	1.0	96.7	

Input ng/ml	Measured ng/ml	Efficiency %	CV %
3125	2101.3	67.2	18.0
625	632.0	101.1	
125	130.0	103.9	
25	25.6	109.4	
5	5.7	111.1	
1	1.1	108.4	

CHO HCP was first spiked into 4-fold diluted M1 or M15, and then a 5-fold serial dilution was made using the diluent. The results showed a good dilution linearity with CV% <20% across the 3.5 log dynamic range.

Summary of ProteinSEQ HCP assay

- Sensitivity: ProteinSEQ HCP assay is about 5-fold more sensitive than the traditional ELISA for HCP quantitation.
- **Dynamic range:** ProteinSEQ HCP assay has a large dynamic range of 4 logs, which minimizes multiple dilutions of the samples to fit into the standard curve.
- **Quantitation efficiency:** Achieved good quantitation efficiency and precision in matrices containing 10–100 mg/ml proteins.
- **Dilution linearity:** Small variation of quantitation with the dilutions across 3.5 log dynamic range.
- **Automation:** Provides high throughput process with reproducibility and precision.