A customizable, qPCR-based immunoassay platform for accurate quantitation of protein impurities and contaminants

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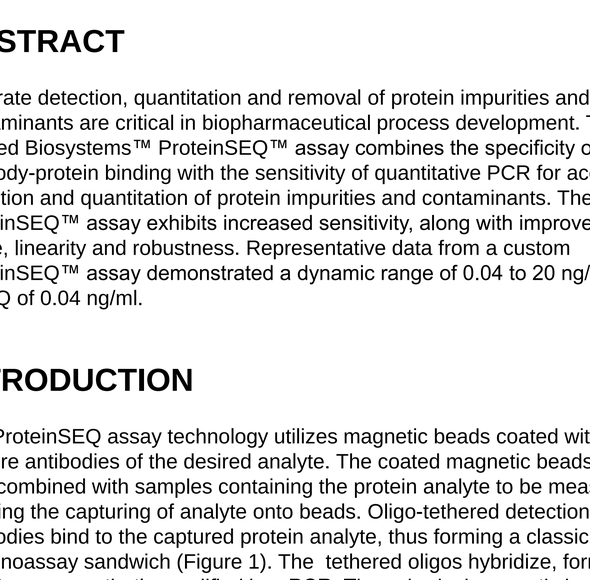
ABSTRACT

Accurate detection, quantitation and removal of protein impurities and contaminants are critical in biopharmaceutical process development. The Applied Biosystems™ ProteinSEQ™ assay combines the specificity of antibody-protein binding with the sensitivity of quantitative PCR for accurate detection and quantitation of protein impurities and contaminants. The ProteinSEQ™ assay exhibits increased sensitivity, along with improved range, linearity and robustness. Representative data from a custom ProteinSEQ™ assay demonstrated a dynamic range of 0.04 to 20 ng/ml and a LOQ of 0.04 ng/ml.

INTRODUCTION

The ProteinSEQ assay technology utilizes magnetic beads coated with capture antibodies of the desired analyte. The coated magnetic beads are then combined with samples containing the protein analyte to be measured, allowing the capturing of analyte onto beads. Oligo-tethered detection antibodies bind to the captured protein analyte, thus forming a classic immunoassay sandwich (Figure 1). The tethered oligos hybridize, forming a target sequence that's amplified by qPCR. The spherical magnetic beads enable higher density of capture antibodies to be coated compared to traditional immunoassays. Consequently, there is increased binding of protein analytes and suppression of background due to non-specific adsorption. Detection and amplification by qPCR enables signal readout many orders of magnitude higher than traditional colorimetric immunoassays, with substantially better resolution. These advantages are directly translated into a much more robust assay with unparalleled assay linearity and spike recovery/quantitation efficiency.

Figure 1. Immunoassay sandwich



Here we describe the use of the ProteinSEQ assay to quantitate CHO HCP and residual Protein A from various simulated product matrices. Additionally, we present representative data a custom ProteinSEQ assay developed for a leached protein.

MATERIALS AND METHODS

CHO HCP was quantified using the industry-standard HCP antibody from spike studies using matrices created to simulate ion-exchange elution buffer and bulk drug substance.

Protein A was quantified from spike-recovery studies using a high salt matrix. Dilution linearity was demonstrated in a high salt and simulated BDS matrix.

A custom ProteinSEQ assay was developed and validated for a leached protein using a commercially available polyclonal antibody.

All samples were processed using the ProteinSEQ assay workflow (Figure 2) with utilizes the Thermo Scientific™ Kingfisher™ Flex instrument. Real-time PCR was performed on a Applied Biosystems™ 7500 FAST real time instrument.

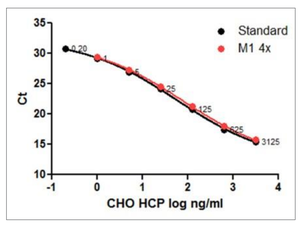
Figure 2. The ProteinSEQ Assay workflow

RESULTS

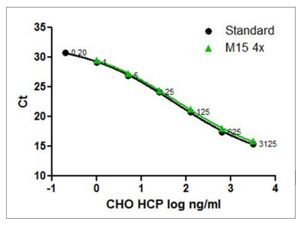
CHO HCP

CHO HCP was spiked into matrices M1 and M15, simulated ion-exchange elution buffers. The results (Figure 3) indicate good quantitation with low variation.

Figure 3. Quantiation efficiency of CHO HCP in simulated ion-exchange elution buffer



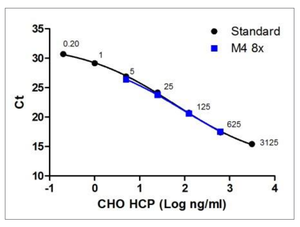
Input ng/mL	Measured ng/mL	Efficiency %	% CV
3125	2241.9	71.7	1.3
625	504.0	80.7	1.1
125	92.7	74.2	5.4
25	19.7	78.6	4.0
5	4.3	86.1	5.5
1	0.9	87.5	16.8



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 simulated bulk drug substance containing 100 mg/ml human IgG. The results (figure 4) indicate good quantitation with low variation.

Figure 4. Quantiation efficiency of CHO HCP in simulated bulk drug substance

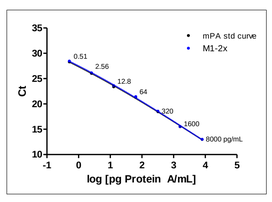


Input ng/mL	Measured ng/mL	Efficiency %	% CV
625	659.0	105.4	6.5
125	128.8	103.0	4.0
25	28.4	113.4	1.6
5	7.2	143.4	2.1

Protein A

Protein A was spiked high salt matrix. The results (Figure 5) indicate excellent spike recovery.

Figure 5. Protein A spike-recovery in high salt matrix



Protein input (pg/mL)	Protein measured	% Spike recovery	% CV
8000	7687	96%	7%
1600	1723	108%	6%
320	291	91%	6%
64	51	80%	2%
12.8	13.0	102%	8%
2.6	2.4	93%	5%
0.5	0.4	84%	6%

Dilution linearity was demonstrated in high salt matrix (table 1) and in simulated BDS (table 2).

Table 1. Dilution linearity in high salt matrix

Dilution factor	Protein A input (pg/mL)	Protein A measured	% Spike recovery	% CV
NA	8000	7325	92%	7%
1/5	1600	1768	111%	4%
1/25	320	250	78%	8%
1/125	64	52	81%	5%
1/625	12.8	13.5	105%	5%
1/3125	2.6	2.5	99%	4%
1/15625	0.5	0.5	90%	17%

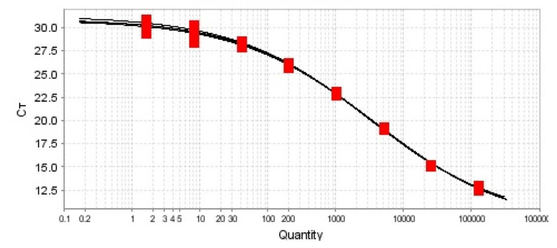
Table 2. Dilution linearity in simulated BDS

Dilution factor	Protein input (pg/mL)	Protein measured	% Spike recovery	% CV
NA	8000	7061	88%	7%
1/5	1600	1787	112%	7%
1/25	320	254	79%	10%
1/125	64	56	87%	8%
1/625	12.8	12.2	95%	6%
1/3125	2.6	2.3	90%	10%
1/15625	0.5	0.6	124%	28%

Custom leached protein

Current ELISA lacked robustness, dynamic range and sensitivity. A commercially available polyclonal antibody was identified and used for generation of ProteinSEQ reagents. The initial assay performance was evaluated by 4 independent standard curves (Figure 6).

Figure 6. Overlay of 4 standard curves of custom ProteinSEQ assay

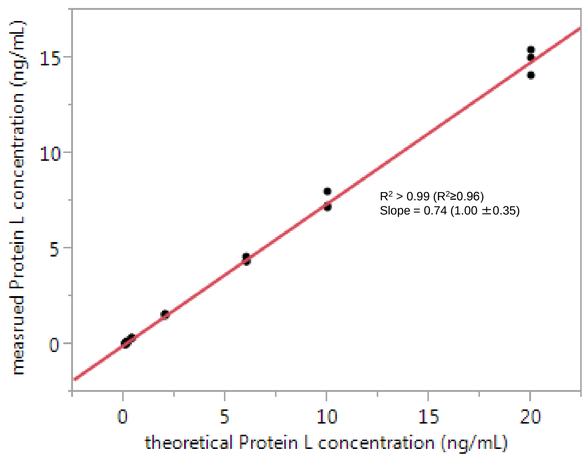


The custom ProteinSEQ assay was validated in bulk drug substance diluted 1:10. Accuracy was tested by spiking BDS with 8 spike levels from 0.04 to 20 ng/ml protein. Recoveries ranged from 70 to 80% with % cv of 3 to 12% for all spike levels.

Intermediate precision was tested by spiking BDS with 8 spike levels from 0.04 to 20 ng/ml protein. Testing was performed by three separate analysts. Average recovery for all assays was ~80% with CV below 10%.

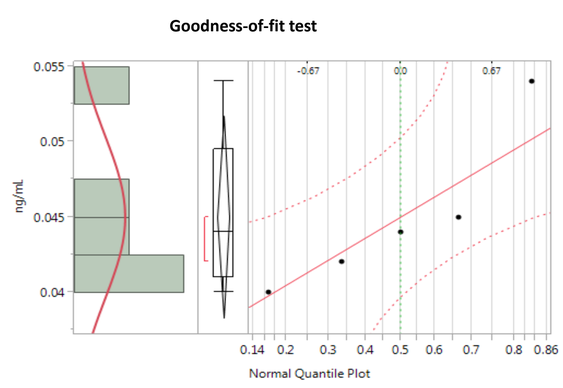
Data from accuracy and intermediate precision were used to calculate linearity (Figure 7).

Figure 7. Linearity of custom ProteinSEQ assay



Limit of Quantitation was confirmed by diluting BDS 1:10 and spiking with 0.04 ng/ml protein. Five independent replicates were tested. Mean recovery was 112% with 12%CV. Results fell within a normal distribution (Figure 8).

Figure 8. Goodness of fit of LOQ analysis



CONCLUSIONS

The ProteinSEQ assay combines the specificity of antibody-protein binding with the sensitivity of quantitative PCR to provide accurate detection and quantitation of protein impurities and contaminants. The assay provides increased sensitivity and dynamic range and is customizable for any protein analyte. Representative data from a custom ProteinSEQ assay demonstrated a dynamic range of 0.04 to 20 ng/ml and an LOQ of 0.04 ng/ml.