Rapid, reliable quantitation of Fc-Fusion protein in cell culture supernatants

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Background

The cell line development group at Biogen IDEC needed a robust assay for the measurement of Fc-fusion protein (Protein 1) in crude cell culture supernatants. The group had historically used HPLC for protein quantitation during screening and selection of promising mammalian clones at every scale-up step, from 96-well microplates to 3L-bioreactors. They wanted to replace the HPLC method due to its low throughput, cumbersome sample processing, and long run times with a higher-throughput alternative that provided accurate, reliable results.

Octet platform solution

The Octet® QK384 instrument was evaluated as an alternative to HPLC due to its many advantageous features and suitability for screening proteins in cell culture supernatant fluids. Octet systems have the ability to analyze crude samples, allowing users to bypass sample pre-processing. In addition, 96 samples could be analyzed in less than 30 minutes, expediting screening that took more than 19 hours to complete by HPLC. A higher throughput, automated screening workflow with significantly reduced analyst involvement was achieved via integration of the Octet system and a PerkinElmer (formerly Caliper Life Sciences) Sciclone robot. This document summarizes the performance of Biogen IDEC's Fc-fusion protein assay.

Assay development and results

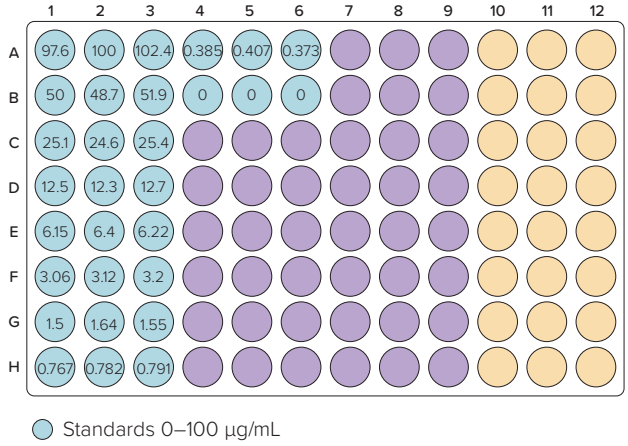
METHOD

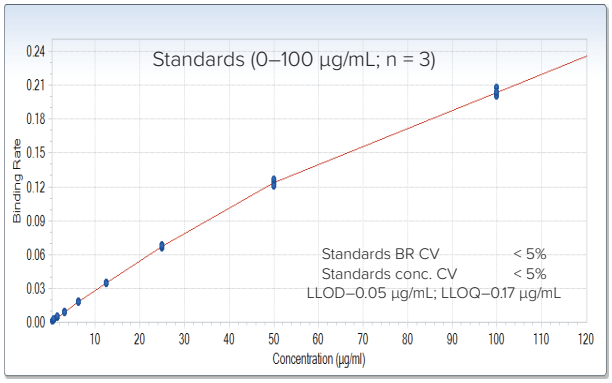
An Octet QK384 instrument integrated with a PerkinElmer Sciclone robot was used for all experiments. Samples were analyzed in 96-well, black, polypropylene, flat-bottom microplates from Greiner (Part No. 655209). Protein A and Protein G biosensors were selected because of their high specificity and selectivity for Fc-fusion protein detection in supernatants. Default settings of a 2-minute read time and 400 RPM shake speed were selected.

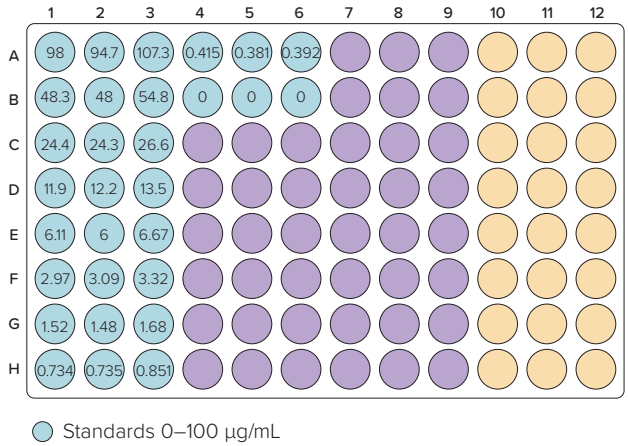
OCTET ASSAY PROVEN ROBUST AND RELIABLE

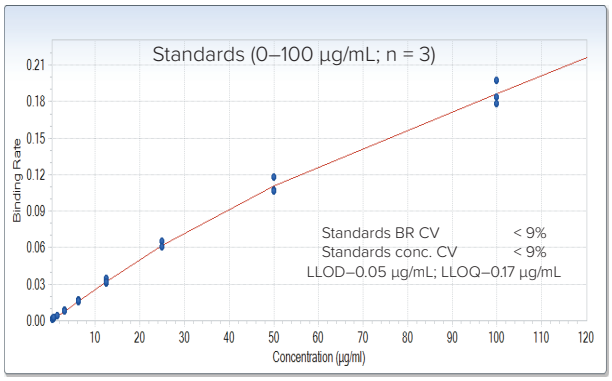
During assay development, standard curves were constructed by diluting the purified bulk drug substance at concentrations from 0 to 100 µg/mL in cell culture media (CCM). Assays were performed in triplicate, using identical well locations in three independent experiments. The average coefficient of variation (CV) for the entire standard curve range was less than 9% for the three combined runs (Figures 1A and 1B). A dynamic range of 1-100 µg/mL was found to be optimal, with acceptable recovery values and CVs. Standard curves were also assayed in different well locations in three independent experiments to account for any well-to-well variability (Figures 2A and 2B). Assay performance was similar across all well locations, demonstrating good consistency across the plate.

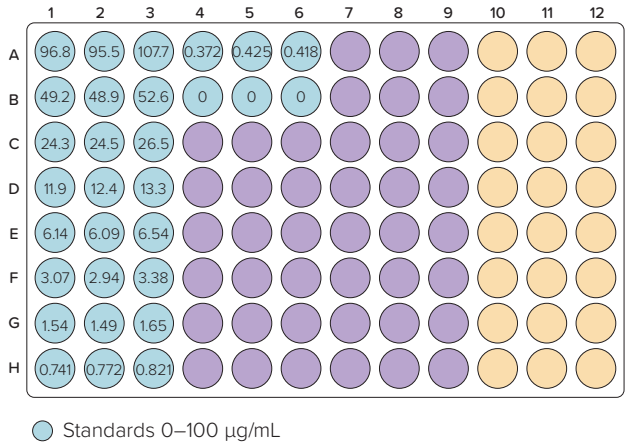
Standard curve generation in three independent experiments at same well locations











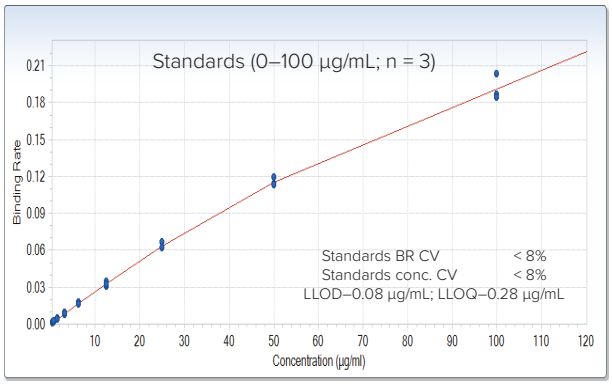


Figure 1A: Standard curves of the Fc-fusion protein assay using Protein G biosensors, obtained in three independent experiments using the same 96-well microplate well locations (BR = Binding Rate). The calculated recovery values are shown in the plate map.

Overlay of standard curves from Figure 1A

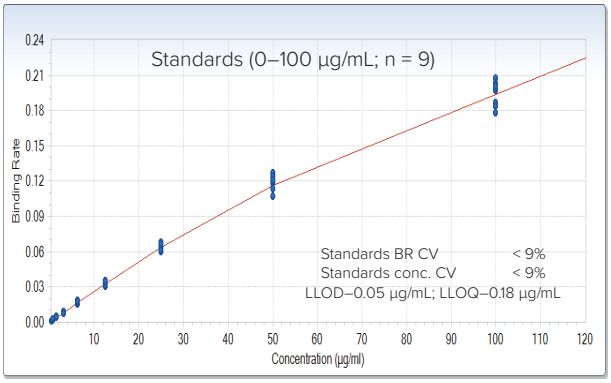


Figure 1B: Pooled standard curve data generated at same well locations in three independent experiments. The results were highly consistent across the entire plate, with CVs for all three combined runs below 10%.

CELL CULTURE MEDIA HAD NO EFFECT ON RESULTS

Interference by matrix components was checked to ensure the assay could be performed on all upstream process samples with minimal pre-processing. Standard samples were assayed in various dilutions of cell culture media: 8-fold (12.5% CCM), 4-fold (25% CCM), 2-fold dilutions (50% CCM), and neat (100% CCM) (Figure 3). The four standard curves were then overlaid and overall variability was determined at each concentration. The assay was found to tolerate up to 100% CCM, with comparable recoveries of spiked standards in all dilutions and an overall CV of <8%.

RESULTS WERE ACCURATE

The accuracy of the Octet assay was evaluated by spiking Fc-fusion protein into CCM and host cell protein (HCP) matrices at known concentrations in the low (10 µg/mL), mid (40 µg/mL) and high (80 µg/mL) range of the standard curve (Figure 4). Spike-in recoveries for the high, mid and low controls in neat CCM were 95%, 104%, and 109% respectively. Recoveries of all control samples in 2 (50%), 4 (25%) and 8 (12.5%) fold dilutions of the HCP matrix were close to 100%, demonstrating excellent accuracy. Recoveries of the control samples in neat HCP matrix were around 122%, showing values higher than acceptable levels. In all subsequent assays, standard and unknown samples were run with at least a 2-fold dilution. Minimization of matrix interference and consistency between matrices used in standard and unknown samples are important for getting accurate results.

GOOD DILUTION LINEARITY

Dilution linearity is an important feature of any assay used to screen samples containing a wide range of protein concentrations. Fc-fusion protein samples at low (S1), high (S2) and medium (S3) range concentrations were diluted 5, 10, 20, 80 and 160-fold and analyzed in triplicate (Figure 5). The CV for back-calculated values of the low concentration sample S1 was <7%. Corresponding CV values for high concentration sample S2 and medium concentration sample S3 were <5%. These results indicate excellent dilution linearity and recoveries for the Fc-fusion protein in cell culture supernatant.

GOOD CORRELATION BETWEEN OCTET DATA AND HPLC

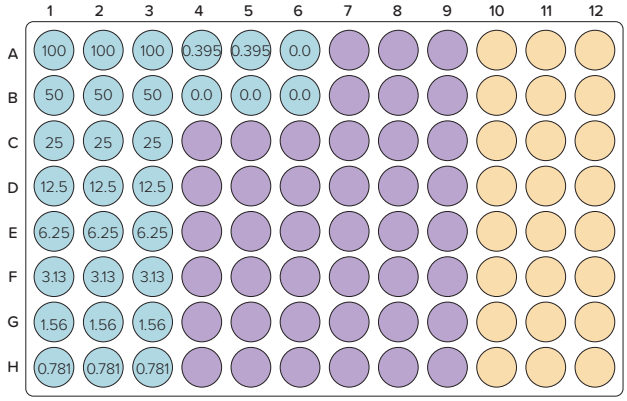
When evaluating the performance of Octet assays to complement or replace HPLC protocols, users generally perform comparative studies. If there are significant differences in sample preparation and handling between the two methods, data correlation may be affected. For this experiment, all samples to be analyzed on both platforms were centrifuged and filtered prior to analysis. In the Octet assay, early cell culture samples were run with at least a 2-fold dilution in CCM, and bioreactor samples were diluted 20- to 40-fold in CCM to ensure final analyte concentrations were within the assay's dynamic range (below 100 µg/mL). HPLC samples were run undiluted with a 50-µL injection volume.

A total of 15 samples were analyzed in the comparison study (Figure 6). Titer values measured by both techniques showed excellent comparability, falling within 12% of each other, with most samples within 10%. Analysis time for the 15 samples on the Octet system was less than 10 minutes whereas the HPLC analysis required about 180 minutes.

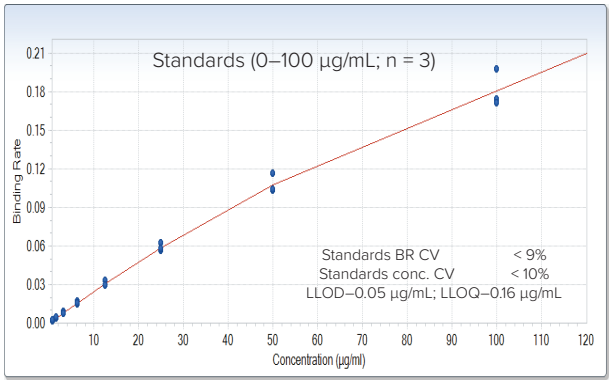
OCTET ASSAY DEMONSTRATED EXCELLENT PRECISION

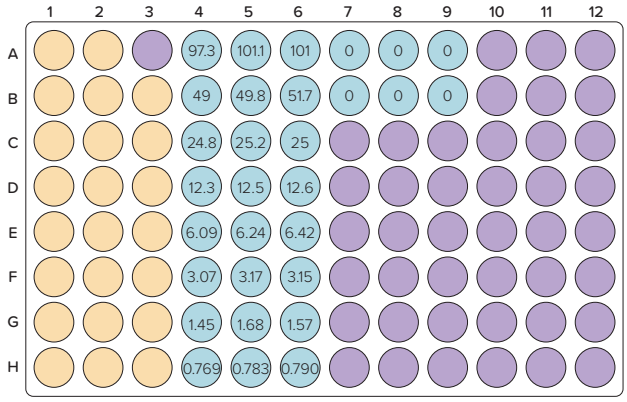
Intermediate assay precision was evaluated by comparing titer values of Fc-fusion protein control sample run on various days by several analysts, as shown in Figure 7. The overall % CV across all runs (n=54) was <5%, verifying excellent precision of the Octet assay.

Standard curve in three independent experiments at different well locations

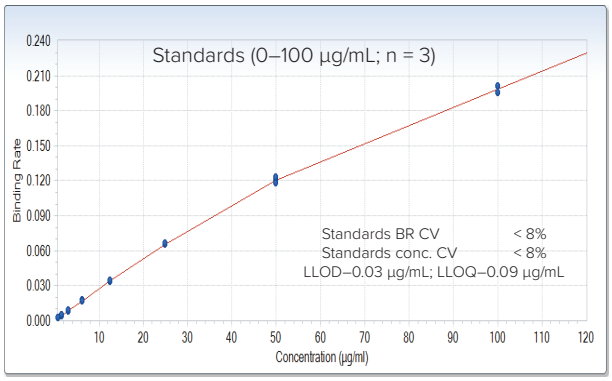


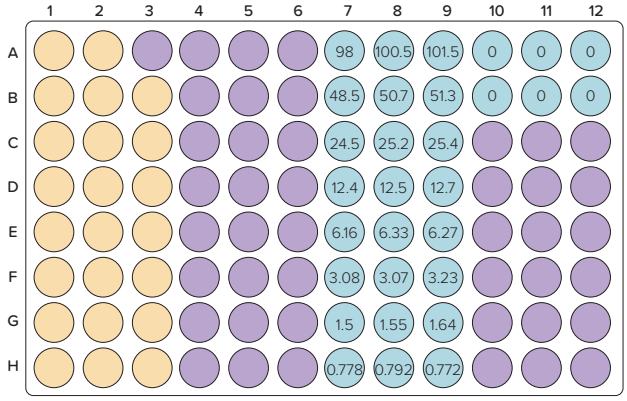
Standards 0–100 µg/mL





Standards 0–100 µg/mL





Standards 0–100 µg/mL

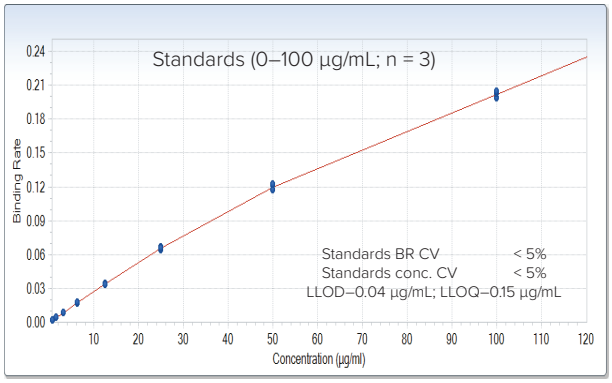


Figure 2A: Standard curves were generated in different well locations in three independent experiments to check consistency of results.

Overlay of standard curves generated in three independent experiments at different well locations

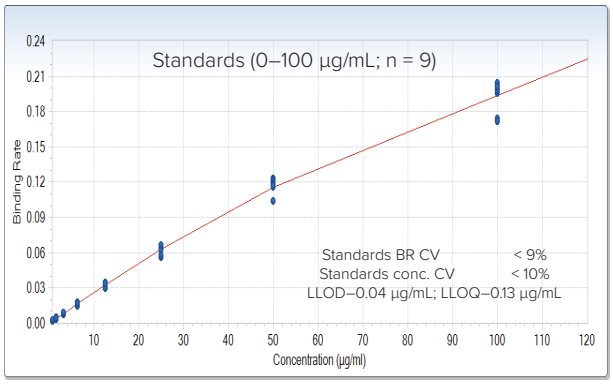


Figure 2B: Pooled standard curve data generated at different well locations in three independent experiments. The results were highly consistent across the entire plate, with a CV for all three combined runs below 10%.

Standard curve generated using various dilution of cell culture media to study media interference

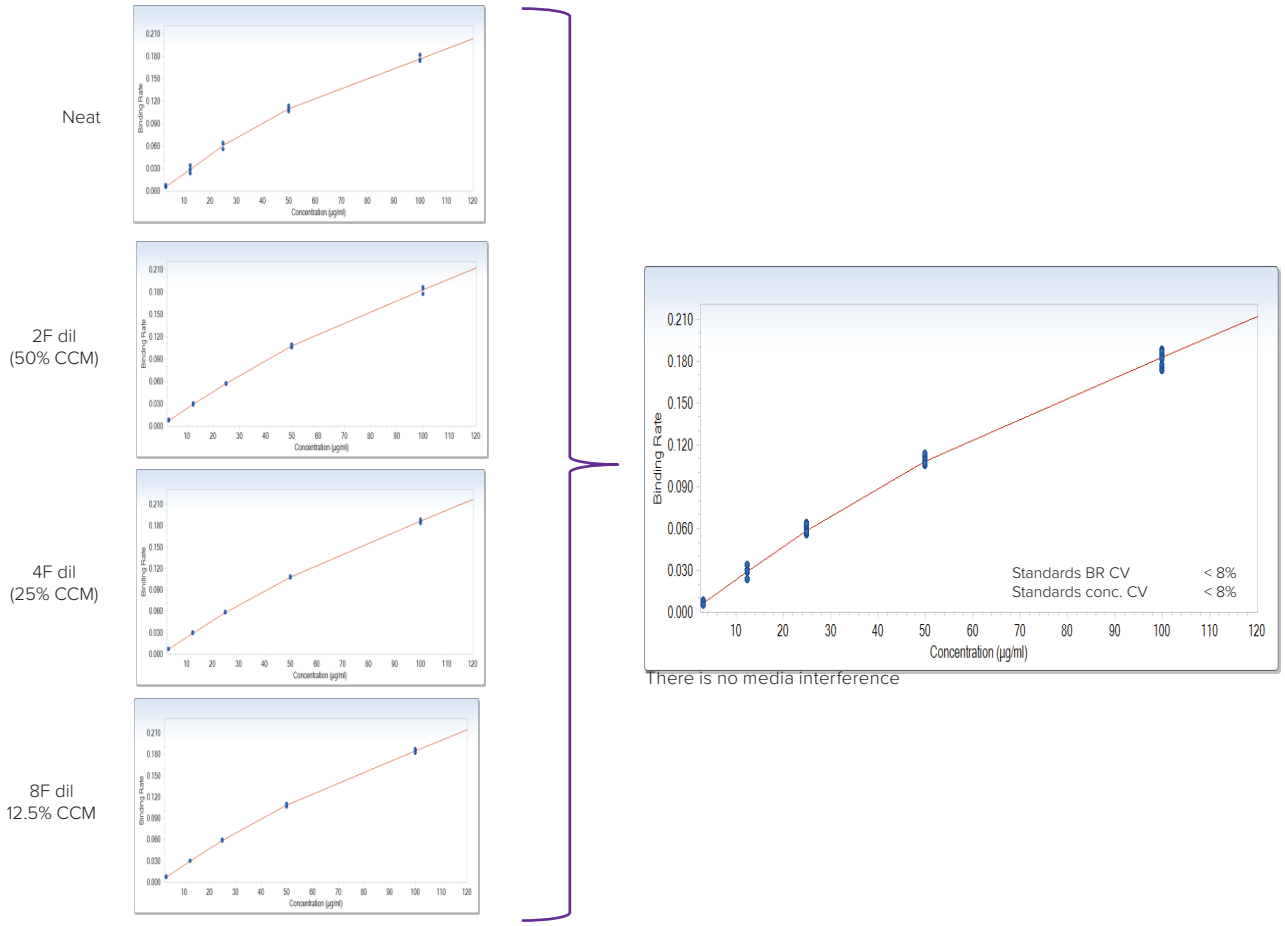


Figure 3: The assay tolerated neat (100%) CCM. 100%, 50%, 25% and 12.5% CCM had comparable recoveries of the standard curve with overall combined CV <8%.

Protein 1 sample spiked in the CCM and HCP matrix to study accuracy of the assay

Spiking study in CCM and HCP (host cell protein media)

- Purified Protein 1 was spiked to 10, 40 & 80 µg/mL in CCM
- HCP was diluted 2F, 4F & 8F; Purified Protein 1 was spiked to 10, 40 & 80 µg/mL
- Samples were analyzed in triplicate

CCM: Cell culture media

HCP: Host cell protein

100% Recovery in CCM

22% higher recovery in HCP neat

Figure 4: Fc-fusion protein was spiked at three different concentrations in CCM and HCP matrix to determine the accuracy of the assay. 100% recovery was achieved in neat CCM and diluted HCP matrices, however, 122% recovery was seen in undiluted HCP.

Protein 1 sample diluted to various folds to study linearity of the assay

- Three Protein 1 samples S1, S2, S3 were diluted 5F, 10F, 20F, 40F, 80F & 160F
- Samples were analyzed on Octet system in triplicate

Protein1	Sample 1 conc. CV	< 7%
	Sample 2 conc. CV	< 5%
	Sample 3 conc. CV	< 5%

Linearity of the assay was confirmed with CV < 7%

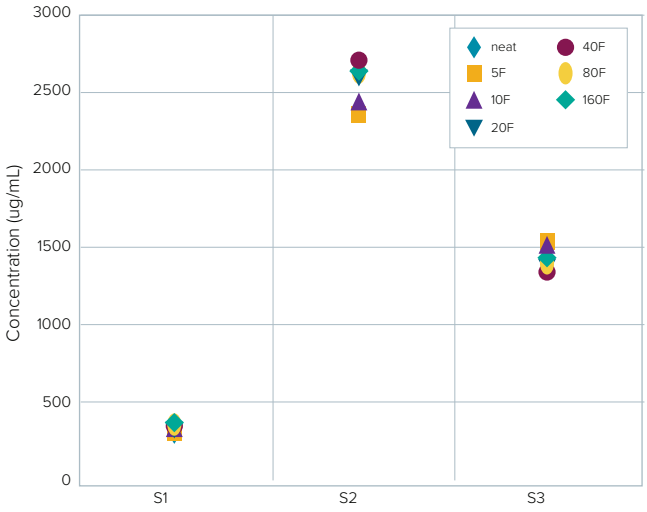
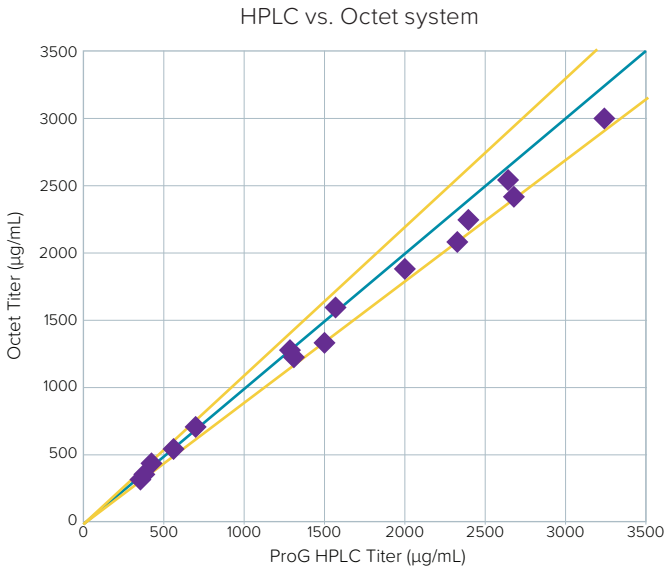


Figure 5: Dilution linearity was confirmed by diluting three concentrations of the Fc-fusion protein to various levels. Assays were performed in triplicate.

Comparison study performed between Octet system and HPLC for Protein 1



Gold lines represent variation between HPLC and Octet system <10%.

Sample ID	ProG HPLC (µg/mL)	Octet system (µg/mL)	% Difference
S1	357	318	11%
S2	2672	2395	10%
S3	388	373	4%
S4	421	448	-6%
S5	2397	2254	6%
S6	1275	1266	1%
S7	1299	1235	5%
S8	1493	1313	12%
S9	2645	2521	5%
S10	1555	1578	-1%
S11	2327	2104	10%
S12	2009	1883	6%
S13	710	708	0%
S14	551	545	1%
S15	3233	2971	8%

Figure 6: A total of 15 samples were analyzed for the comparison study. Reported concentration values showed good agreement between the two methods, falling within 12% of each other for all samples and within 10% for most samples.

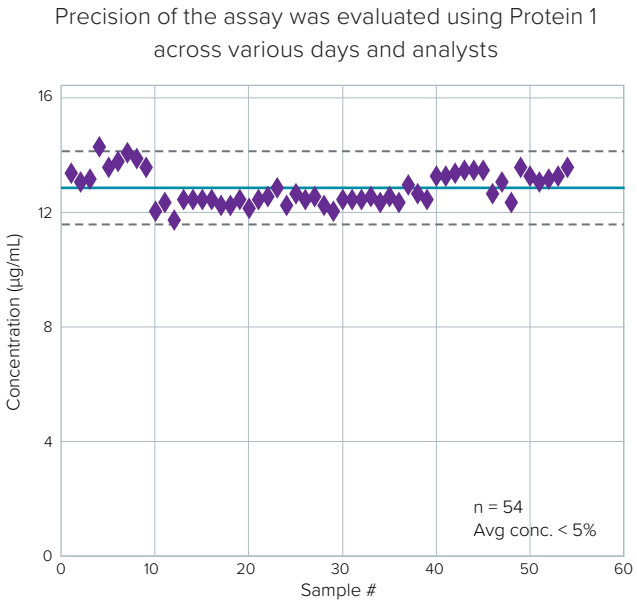


Figure 7: Intermediate precision of the Octet assay had an average CV of <5% (n=54).

Conclusion

The titer assay was developed at Biogen IDEC for Fc-fusion proteins in cell culture supernatants. The assay is currently used to quickly screen large numbers of clones and identify promising, high-producing clones during cell line development. More than 40,000 samples were run on the Octet QK384 system in one year, making it an indispensable tool in their cell line development workflow. Expanded use of the Octet system in other bioprocess applications is currently under exploration in the cell line development lab at Biogen IDEC.

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