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APPLICATION NOTE

FAST AND REPRODUCIBLE HOST CELL PROTEIN DETECTION WITH SIMPLE PLEX ASSAYS ON ELLA

USING CYGNUS TECHNOLOGIES CHO HCP 3G ANTIBODIES FOR AUTOMATED HOST CELL PROTEIN MEASUREMENTS

INTRODUCTION

Host cell proteins (HCPs) are generated by the host organism during the production of recombinant therapeutic proteins or biologics and are a major component of process-related impurities. During purification, HCPs need to be monitored and reduced at every step. Residual HCPs that go undetected can decrease product efficacy, alter its quality and/or risk triggering an immunogenic reaction when the drug is administered to the patient.

To meet increasing business demands and safety standards, fast and reproducible detection of HCPs is essential during the manufacturing process of a biotherapeutic. However, accurate in-process reporting is often complicated by varying amounts of HCPs present throughout bioproduction—initial process pools may consist of high levels of HCPs, whereas final pools typically amount to lower levels. This means you'll need to carefully and extensively dilute samples at each stage of purification to ensure detection within the dynamic range of your assay. Moreover, the sensitivity of your assay should especially enable the detection of low levels of HCPs that may be present in final pools. Lastly, look out for the presence of large amounts of recombinant protein product, which creates an intricate sample matrix that can interfere with your ability to accurately measure low abundance HCPs.

In this application note, we pair Simple Plex™ Assays on Ella™ with Cygnus Technologies third generation (3G) Chinese Hamster Ovary (CHO) antibodies to demonstrate utility for accurately and efficiently detecting and quantifying the presence of HCP contaminants from bioprocess samples. We also demonstrate that Simple Plex CHO HCP, 3G-1 assay data has a near perfect correlation with data generated using the gold standard Cygnus CHO HCP, 3G ELISA (F550-1).

WHY PAIR ELLA WITH CYGNUS CHO HCP, 3G ANTIBODIES?

All steps of the Simple Plex immunoassay on Ella are highly automated thanks to the microfluidic cartridge—everything is preloaded, even the calibration curve. Just pipette your diluted samples onto the cartridge, add wash buffer, press Start in the Ella Runner Software and walk away to the sound of automatic processing.

Inside the microfluidic cartridge are glass nano reactors (GNRs), the core of the Simple Plex immunoassay. In the Simple Plex CHO HCP 3G-1 assay (PN SPCKB-OT-003714), the GNRs are coated with a polyclonal Cygnus 3G CHO antibody that works to capture HCPs in your bioprocess samples. That same antibody is also lyophilized in the cartridge to act as the detection reagent. The Cygnus CHO antibody and ELISA kit (Cygnus Technologies PN F550-1) have been evaluated for reactivity to more than 1,000 individual HCPs present in CHO strains by state-of-the-art Antibody Affinity Extraction™ (AAE™) and mass spectrometry methods. When paired with Ella and the three GNRs within each channel of the microfluidic cartridge, you'll get both broad detection coverage to the most relevant HCPs and automatically obtain triplicate results for every sample. Moreover, the assay provides sub-ng/mL detection giving you superior assay sensitivity and data reproducibility throughout the manufacturing process. Setup takes just 10 to 15 minutes and you'll get fully processed and analyzed results in 75 minutes!

COMPARISON OF ELLA WITH CYGNUS CHO HCP 3G ELISA KIT, F550-1: ASSAY RANGE OF DETECTION AND SENSITIVITY

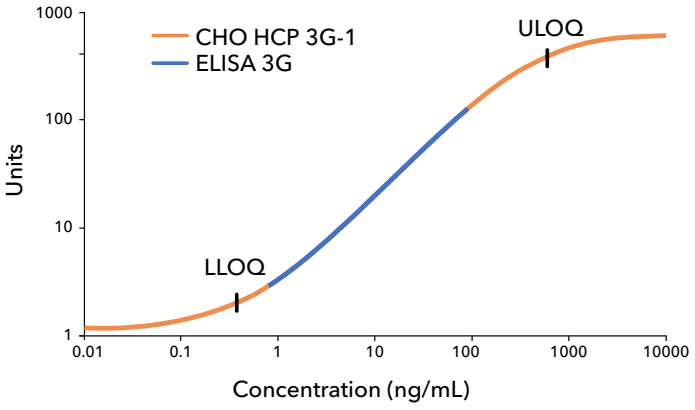

FIGURE 1. Comparison of standard curves. Standard curve of the Simple Plex CHO HCP, 3G-1 Assay (orange) and conventional Cygnus CHO HCP, 3G ELISA (blue). The Simple Plex CHO HCP, 3G-1 Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) are indicated on the standard curve.

The majority of biotherapeutic proteins are manufactured within mammalian cell-line systems, of which CHO strains are the predominant cell lines used for production, and a common originating source of HCP impurities. Although no compulsory limit has been set for the HCP level permitted, <100 ng of HCPs per 1 mg of the drug substance (PPM) is generally considered acceptable[1].

The gold standard Cygnus CHO HCP ELISA Kit, 3G (F550-1) uses the same HCP polyclonal antibody and lot number on either side of the "sandwich" and was used herein as a benchmark for the refined Simple Plex CHO HCP, 3G-1 Assay. To begin to demonstrate the utility of the Simple Plex CHO HCP, 3G-1 Assay in this setting, we compared the dynamic range of Simple Plex Assays with that of the Cygnus CHO HCP ELISA (**FIGURE 1**). When plugging the Cygnus CHO HCP antibody into the Simple Plex Assay on Ella, which uses a built-in fluorescent detection system, the dynamic range of detection for the traditional Cygnus CHO HCP 3G Assay was expanded from 1–100 ng/mL to 0.29-400 ng/mL (**FIGURE 1**). Standard curves for the colorimetric Cygnus ELISA were produced by running all standards in triplicate followed by absorbance detection using a microtiter plate reader spectrophotometer with dual wavelength capability at 450 nm and 650 nm.

Initial culture harvest samples contain high levels of HCPs which must be reduced by many orders of magnitude to reach acceptable levels in the final drug product. Routine monitoring of HCP clearance during downstream monoclonal antibody (mAb) purification using a highly sensitive method is necessary to comply with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines[1]. In **FIGURES 2** and **3**, we demonstrate that HCP levels measured by the Simple Plex 3G-1 assay strongly correlate (R^2=0.982) with those obtained by the Cygnus CHO HCP ELISA, 3G throughout the purification process.

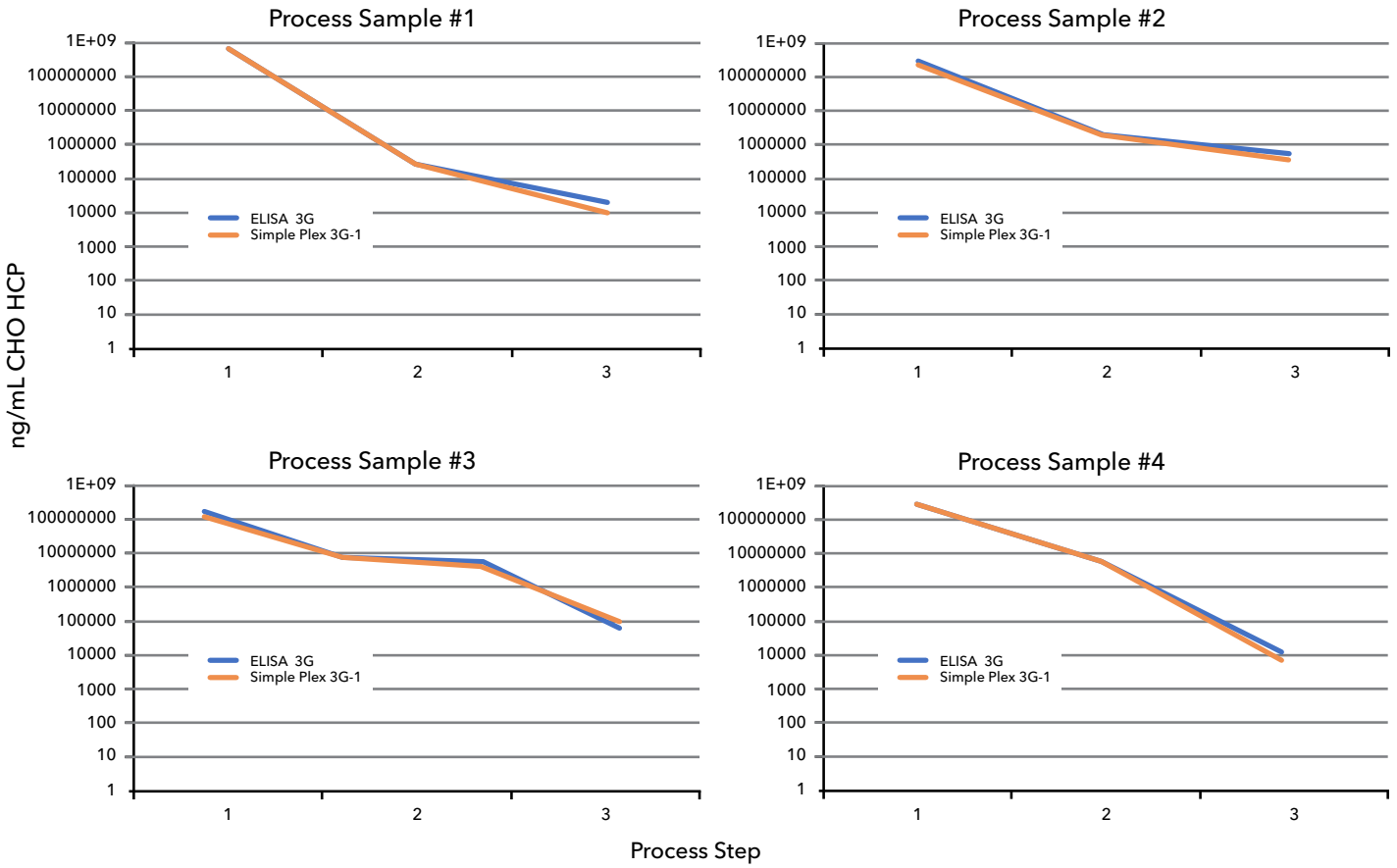

FIGURE 2. Detection of HCPs during bioproduction with Simple Plex 3G-1 and 3G ELISA. Four industrial purification processes were profiled using both the 3G plate ELISA (blue) and the 3G-1 Simple Plex assay (orange). These processes were measured at multiple steps (X-Axis) to show the elimination of CHO HCP from the drug product. Across each process the Simple Plex assay showed the same fold reduction in CHO HCP as the plate ELISA.

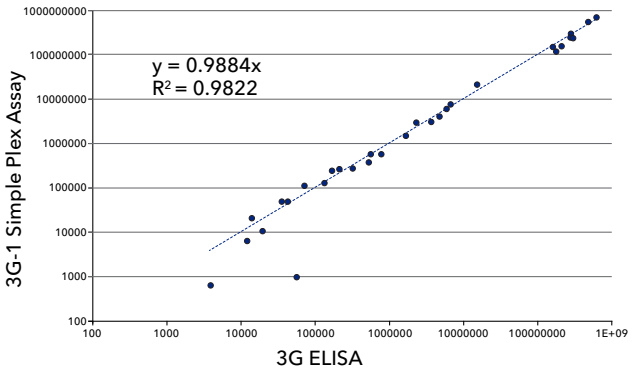

FIGURE 3. Linear correlation of the 3G-1 Simple Plex and plate ELISA assays. The aggregated Simple Plex and ELISA data from the four purification processes were plotted and a linear fit was applied to the data set. The Simple Plex 3G-1 Assay and the 3G ELISA showed a nearly perfect correlation (R^2=0.9822) across multiple steps of the purification processes.

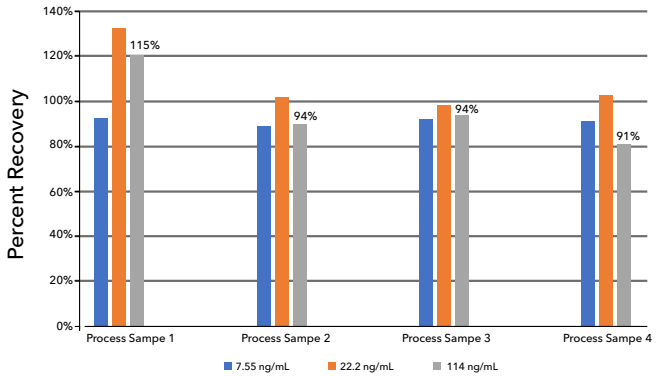

FIGURE 4. Spike recovery of in-process pools with Simple Plex Assays. In-process pooled samples were spiked with known concentrations of HCP antigen concentrate, and the percent HCP recovered was determined for each spike via the Simple Plex CHO HCP, 3G-1 Assay. Average percent recovery for the low, medium and high spike concentrations is indicated above each bar for that respective in-process pool.

SPIKE RECOVERY OF IN-PROCESS POOLS WITH SIMPLE PLEX ASSAYS

To demonstrate assay performance, mAb in-process samples of varying matrices were spiked with a low, medium or high concentration of HCP antigen concentrate, 3G (Cygnus Technologies, F553X). To interrogate the breadth of the assay range, we chose 7.55 ng/mL, 22.2 ng/mL and 114 ng/mL of HCP (FIGURE 4).

SAMPLE LINEARITY OF MULTIPLE IN-PROCESS POOLS WITH SIMPLE PLEX ASSAYS

To examine the ability of Simple Plex Assays to accurately quantify HCP levels across multiple mAb cultures and dilution ranges, six pooled in-process samples were assessed for linearity across four dilutions. Initial culture dilutions were based on values that were predicted by the Cygnus CHO HCP ELISA, 3G Kit, F550-1 to fall within the linear range of the Simple Plex CHO HCP, 3G-1 Assay. All samples were then serially diluted 1:2, 1:4, 1:8 and 1:16. The percent difference between each sample value and the previous dilution was then calculated.

Each in-process mAb sample showed good linearity at all four dilutions tested, with all percent differences falling between 80–120% (FIGURE 5). The initial dilution of these samples, as recommended by the Cygnus CHO HCP ELISA, 3G Kit, F550-1 ranged from 1:2 to 1:50,000.

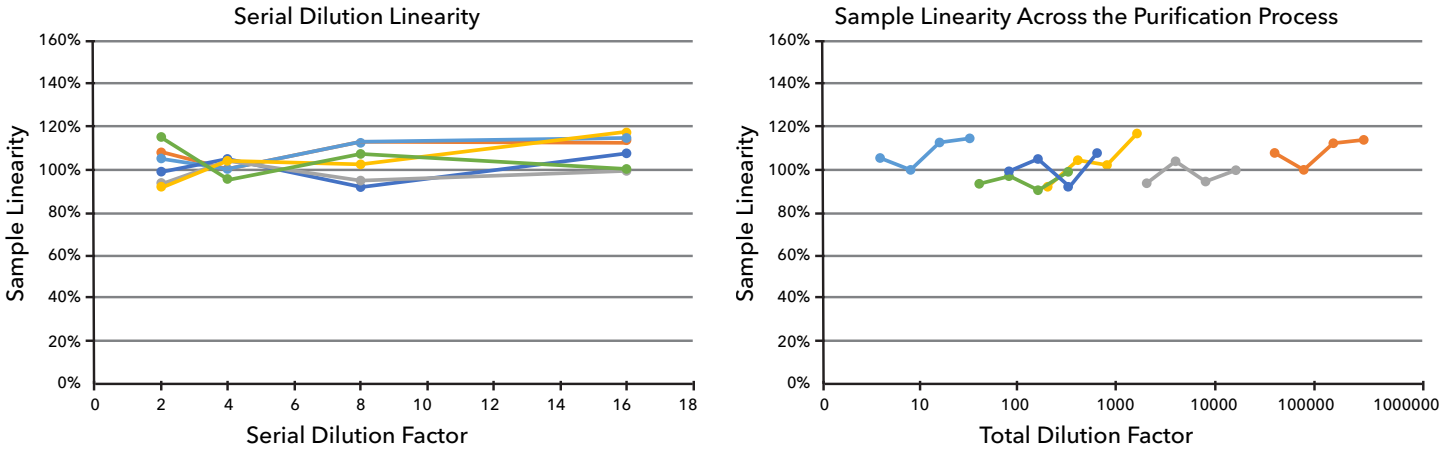

FIGURE 5. Sample linearity from harvest material to drug product. All six process samples showed good linearity across four different dilutions. All samples were within the acceptable range of 80%-120%.

MAB PROCESS	IN-PROCESS POOL 2 (HCP, ng/mL)	IN-PROCESS POOL 3 (HCP, ng/mL)	IN-PROCESS POOL 4 (HCP, ng/mL)	FINAL POOL (HCP, ng/mL)
Replicate 1	4,630	600	246	7.95
Replicate 2	4,810	568	244	7.75
Replicate 3	5,130	621	246	8.3
Average	4,856.7	598.7	245.3	8
Standard Deviation	0.23	0.22	0.29	0.075
CV (%)	5%	4%	2%	5%

TABLE 1. Precision comparison of Simple Plex CHO HCP, 3G-1 Assays. Triplicate assay values of HCP levels in Process Pool 2, Process Pool 3, Process Pool 4 and the Final Pool were analyzed via the Simple Plex CHO HCP, 3G-1.

When taken in aggregate, the sample performance showed good linearity across 5 logs of dilution and a large range of incorporated matrix (**FIGURE 5**). This demonstrates that the sensitive nature of Cygnus CHO HCP, 3G reagents translates well to the robust performance of the Simple Plex Assay format on Ella.

SIMPLE PLEX ASSAY PRECISION

Ella's hands-off CHO HCP, 3G-1 immunoassay workflow consistently delivers high-quality, reproducible data. To examine assay precision, HCP levels present within in-process pooled samples of a mAb at four progressive stages of purification were measured. Inter-assay CV values for triplicate analyses ranged from 2% to 5%, attesting to Ella's ability to report the same concentration from separately run cartridges (**TABLE 1**).

CONCLUSION

Analysis of CHO HCP with the Simple Plex 3G-1 Assay is a robust alternative to the conventional HCP ELISA approach. Ella is an automated immunoassay platform that eliminates the hands-on steps that come with running a traditional ELISA. You'll significantly decrease time to result and reduce human error, both factors that adversely impact assay reproducibility and team productivity. These attributes make Ella an ideal tool for industry environments where a good manufacturing practice (GMP) system is in place. In other words, Ella satisfies the demands of timely data generation for maintaining efficient production and overcomes the lengthy investigations and sample retests that often result from human error[2].

You also get unmatched assay sensitivity and highly reproducible results, which ensures that you get the high-quality data required to meet regulatory standards for HCP monitoring throughout bioproduction workflows. The improved range of detection achievable with Simple Plex assays is particularly useful for HCP detection, as samples need fewer dilutions to meet the linear range of the assay. With Ella, you'll get an extended linear range and improved precision with the same excellent sample performance you get with the Cygnus CHO HCP ELISA Kit, 3G.

REFERENCES

1. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidance Q9 Quality Risk Management. Available online at https://www.fda.gov/science-research/guidancedocuments-including-information-sheets-andnotices/ich-guidance-documents. Updated June 2006, Accessed October 28, 2019.

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