

SIMPLE PLEX AUTOMATES HEK 293 HCP DETECTION USING ANTIBODY REAGENTS FROM CYGNUS TECHNOLOGIES

GET REPRODUCIBLE DATA IN 90 MINUTES!



INTRODUCTION

Human Embryonic Kidney (HEK) 293 cells are often used as host cell lines for protein production or viral vector scale-up, as part of bio-therapeutic or cell and gene therapy manufacturing workflows. HEK 293 host cell proteins (HCPs) have a similar nature, risk profile, and manufacturing process optimization requirement as HCPs from other expression systems¹⁻³. As such, HEK HCPs need to be monitored and reduced at every step of the manufacturing workflow. Residual HCPs that go undetected can decrease product efficacy, alter its quality, and trigger immunotoxic reactions after administration of bio-therapeutics or cell and gene therapies³.

To meet increasing business demands and safety standards, fast and reproducible detection of HCPs is essential during viral vector and protein production. Accurate in-process reporting is complicated by the wide range of in-process matrices present throughout the purification process. The initial crude viral vector harvest may be obtained via the lysis or disruption of host cells, generating high levels of HCPs. In contrast, final purification stages contain significantly lower levels. You'll need to carefully and extensively dilute samples at the early stages of purification to ensure detection within the dynamic range of your assay. The sensitivity of your assay should enable the detection of low levels of HCPs that may be present in the final purification stages. This workflow requires a quantitative solution that delivers both sensitivity and a broad dynamic range.



WHY PAIR ELLA WITH CYGNUS TECHNOLOGIES HEK 293 HCP RESUPPLY 1 ANTIBODIES?

In this application note, we show how the Simple Plex™ HEK 293 HCP 3G Assay on the Ella™ platform consistently delivers sensitivity and a broad dynamic range. By pairing Simple Plex microfluidic immunoassay technology with the Cygnus Technologies HEK 293 HCP Resupply 1 polyclonal antibodies, we demonstrate utility for fast, accurate detection and quantitation of HEK HCP contaminants from viral vector and protein production samples.

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 nanoreactors (GNRs), the core of the Simple Plex immunoassay (FIGURE 1). In the Simple Plex HEK 293 HCP 3G assay (Cat # SPCKB-OT-007066), the GNRs are coated with the Cygnus Technologies HEK 293 Resupply 1 polyclonal capture antibody that works to capture HCPs in your viral vector production samples. The Cygnus HEK 293 HCP Resupply 1 polyclonal antibody has a broad coverage of ~90% to HEK 293 HCPs as determined by Antibody Affinity Extraction with 2D PAGE/Silver Stain and LC-MS⁴.

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 detects single-digit nanogram HCP concentrations giving you excellent assay sensitivity and data reproducibility throughout the manufacturing process. Assay setup takes just 10 to 15 minutes, and you'll get fully processed and analyzed results in 90 minutes!

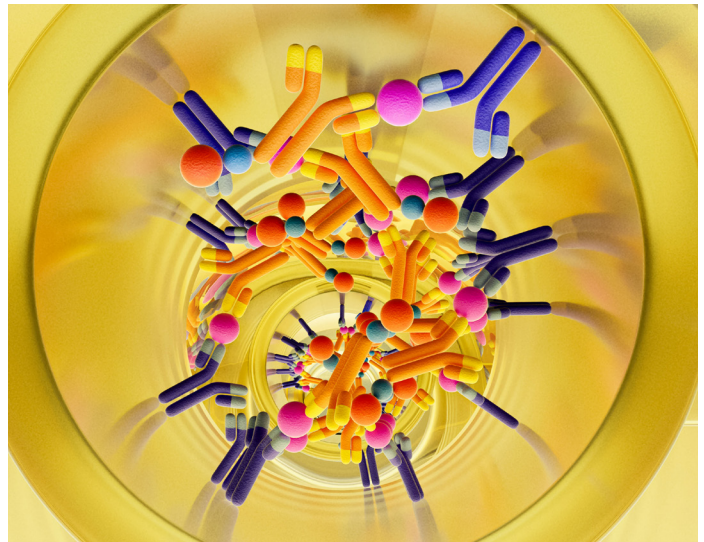


FIGURE 1. Artist's rendering of cross-section of glass nanoreactor (GNR) containing the immunoassay sandwich.

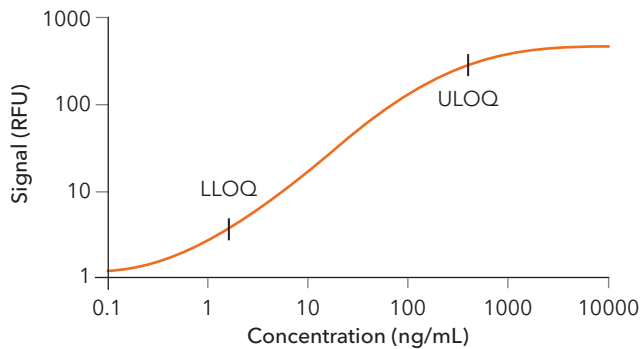


FIGURE 2. The Simple Plex HEK 293 HCP 3G assay has a lower limit of quantitation (LLOQ) of 1.64 ng/mL and an upper limit of quantitation (ULOQ) of 400 ng/mL.

THE SIMPLE PLEX HEK 293 HCP 3G ASSAY IS HIGHLY CORRELATED WITH CYGNUS HEK 293 HCP 3G ELISA

Samples of initial process stages contain high levels of HCPs which must be reduced by many orders of magnitude to reach acceptable levels in the final product. Routine monitoring of HCP clearance during viral vector and protein production using a highly sensitive method is a necessary element of risk management and compliance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines⁵. In FIGURE 3, we demonstrate that HCP levels measured by the Simple Plex HEK 293 HCP 3G assay strongly correlates ($R^2=0.98$) with those obtained by the Cygnus HEK 293 HCP ELISA Kit, 3G throughout the purification process.

THE SIMPLE PLEX HEK 293 HCP 3G ASSAY HAS A BROAD DYNAMIC RANGE

We compared the Simple Plex dynamic range to that of the Cygnus HEK 293 HCP ELISA Kit, 3G (F650S) (FIGURE 2). The dynamic range was 1.64-400 ng/mL, which is comparable to the Cygnus ELISA. Built-in factory standards undergo a rigorous quality control process in order to ensure long-term lot-to-lot consistency.

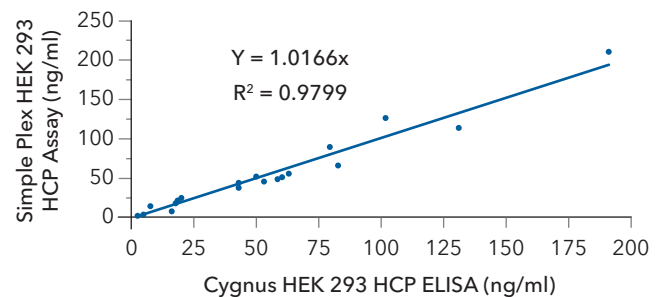


FIGURE 3. The Simple Plex HEK 293 HCP 3G assay is highly correlated ($R^2=0.9799$) with the Cygnus HEK 293 HCP ELISA Kit, 3G Resupply 1.

SPIKE RECOVERY OF IN-PROCESS POOLS WITH SIMPLE PLEX ASSAYS

To demonstrate assay performance, in-process samples of varying matrices were spiked with varying concentrations of HEK HCP antigen concentrate. The Simple Plex HEK 293 HCP 3G assay had an excellent recovery for all of the samples tested (FIGURE 4).

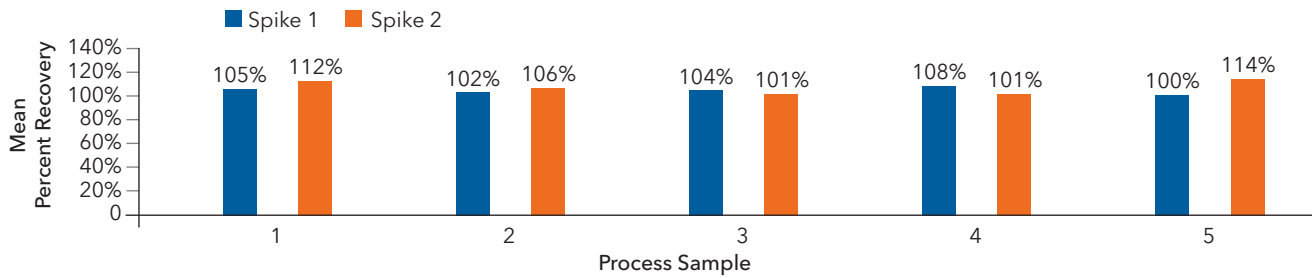


FIGURE 4. In-process pooled samples were spiked with known concentrations of HCP antigen concentrate, and the percent HCP recovered was determined for each spike via the Simple Plex HEK 293 HCP 3G assay. Average percent recovery is indicated above each bar for that respective in-process pool.

SIMPLE PLEX ASSAYS ACCURATELY QUANTIFY IN-PROCESS HEK HCP

To examine the ability of Simple Plex Assays to accurately quantify HEK HCP levels across viral vector samples and dilution ranges, we compared the Simple Plex HEK 293 HCP 3G assay to the Cygnus HEK 293 HCP ELISA Kit, 3G, using in-process testing samples (FIGURE 5). Bioprocess step samples were run at their appropriate minimum required dilution (MRD) to determine the concentration of HEK HCP at each step. Concentrations were subsequently back-calculated and plotted to visualize the decreasing amount of HCP across each process step. Our data indicate that the sensitive nature of Cygnus HEK 293 HCP 3G reagents translates well to the robust performance of the Simple Plex Assay format on Ella.

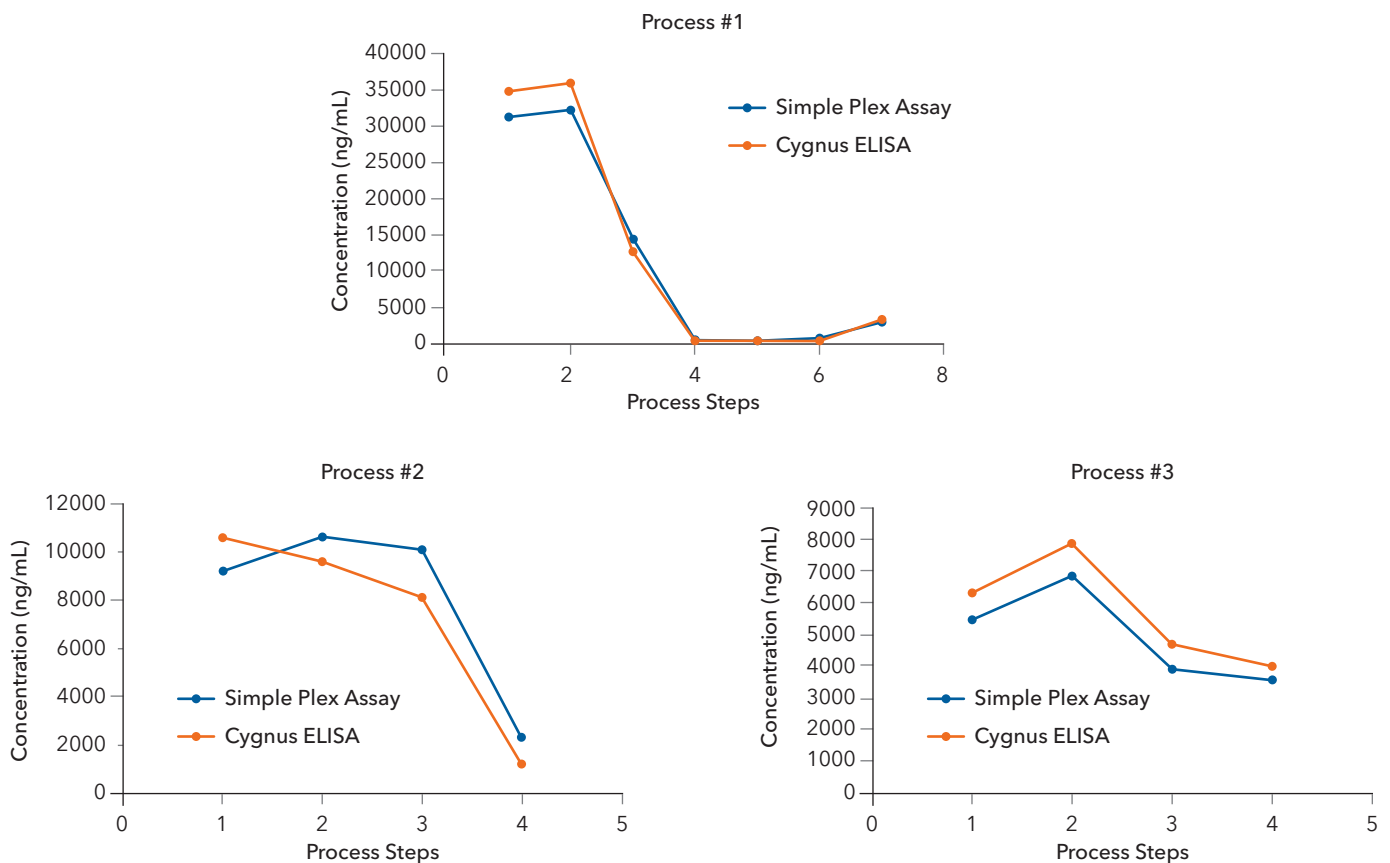


FIGURE 5. Detection of HEK HCPs during bioproduction with Simple Plex HEK 293 HCP 3G assay and Cygnus HEK 293 HCP ELISA Kit, 3G ELISA. Three industrial purification processes were profiled using the Cygnus ELISA (orange), and the Simple Plex assay (blue). These processes were measured at multiple steps (X-axis) to show the elimination of HEK HCP from the product. Across each process, the Simple Plex assay demonstrates the same fold reduction in HEK HCP as the Cygnus ELISA although some differences in absolute sample values were observed.

SIMPLE PLEX ASSAY PRECISION

Ella's hands-free HEK 293 HCP immunoassay workflow consistently delivers high-quality, reproducible data. Intra-assay and inter-assay precision are measures of variability within and between assays, respectively. To examine assay precision, HCP from low and high control samples was measured. Percent CV ranged from 10.6% to 14.3%, attesting to Ella's ability to report the same concentration within and between cartridges (TABLE 1).

	INTRA-ASSAY PRECISION	INTER-ASSAY PRECISION
Low Control	12% CV	14.3% CV
High Control	5% CV	10.6% CV

TABLE 1. Intra-assay and inter-assay precision comparison for the Simple Plex HEK 293 Assay.

CONCLUSIONS

Analysis of HEK 293 HCP 3G with the Simple Plex HEK 293 HCP 3G 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. 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.

These attributes make Ella an ideal tool for industrial 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 error⁶.

REFERENCES

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RELATED RESOURCES

- [Ella Automated Immunoassay System](#)
- [Simple Plex Assays for Viral Titer Quantification](#)
- [Simple Plex Assays for Bioprocess Development](#)
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