

APPLICATION NOTE

Sensitive quantitation of host cell protein contamination of biologics manufactured in CHO cells

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Introduction

Host cell proteins (HCPs) are produced by the cells in which biologics are manufactured. Although they may be vital to the health or growth of these cells, they are not related to the biologic itself and must be removed from the final product. Detection and removal of HCP is a time-consuming process, and levels of HCP must be monitored throughout the purification process to ensure safety of the final product.

Amersham HCPQuant CHO (supernatant) Kit is a 96-well microtiter strip format sandwich ELISA that can be used to measure residual HCP in biologics during the downstream purification process. It uses pre-immobilized anti-CHO HCP antibodies, a horseradish peroxidase (HRP)-conjugated detection antibody, and 3,3',5,5'-tetramethylbenzidine (TMB) substrate.

In this application note we show automation of wash steps using the AquaMax® 4000 Microplate Washer to reduce hands-on time with no compromise in data quality. Absorbance was detected using a SpectraMax® iD3 Multi-Mode Microplate Reader, and data were analyzed using SoftMax® Pro Software, which enables automated standard curve graphing and sample data analysis.

Benefits

- Wide dynamic range enables excellent dilution linearity, increasing confidence across the entire purification process
- Compatibility with automated plate washing increases throughput and reduces hands-on time
- High sensitivity enables detection of as little as 1 ng/mL HCP, and quantitation down to 2 ng/mL HCP

Excellent compatibility with a wide range of samples from different processes

To demonstrate compatibility with biologic therapeutics, we tested commercial drug substances and biosimilars for residual HCP using the Amersham HCPQuant CHO Kit and a competitor kit. We tested each substance in duplicate across several dilutions and performed each assay in triplicate.

We interpolated the measured absorbance values for each sample to concentration (in ng/mL) using a four-parameter logistic (4-PL) fit of the standard curve included with each kit. We converted the average concentrations to parts per million (ppm) of drug substance.

We used a t-test to calculate any statistically significant differences between the measured concentrations of samples from each kit. For each assay, we excluded from the analysis any sample absorbance values that fell outside the range covered by their corresponding standard curves. The results indicate that Amersham HCPQuant CHO (supernatant) detects more HCP in five out of nine substances tested versus the competitor kit (Fig 1).

Importance of linearity

Dilution linearity across many in-process steps helps scientists demonstrate good sample compatibility and maintain confidence in data. You should be able to calculate the same stock concentration of HCP across several dilutions of the same sample — this parameter is also known as parallelism. Parallelism in downstream steps demonstrates a robust assay and enables accurate HCP level tracing — helping you develop a good purification strategy while saving time and resources.

Here, we demonstrate sample compatibility and parallelism across several downstream purification steps for two cell lines: CHO-K1 and CHO-S.

We used a standard downstream process to purify supernatant from CHO-K1 and CHO-S cell lines expressing monoclonal antibody¹. To demonstrate dilution linearity, we tested samples from each purification step for HCP in triplicate assays across several dilutions. We performed the tests using the Amersham HCPQuant CHO (supernatant) Kit and a competitor kit.

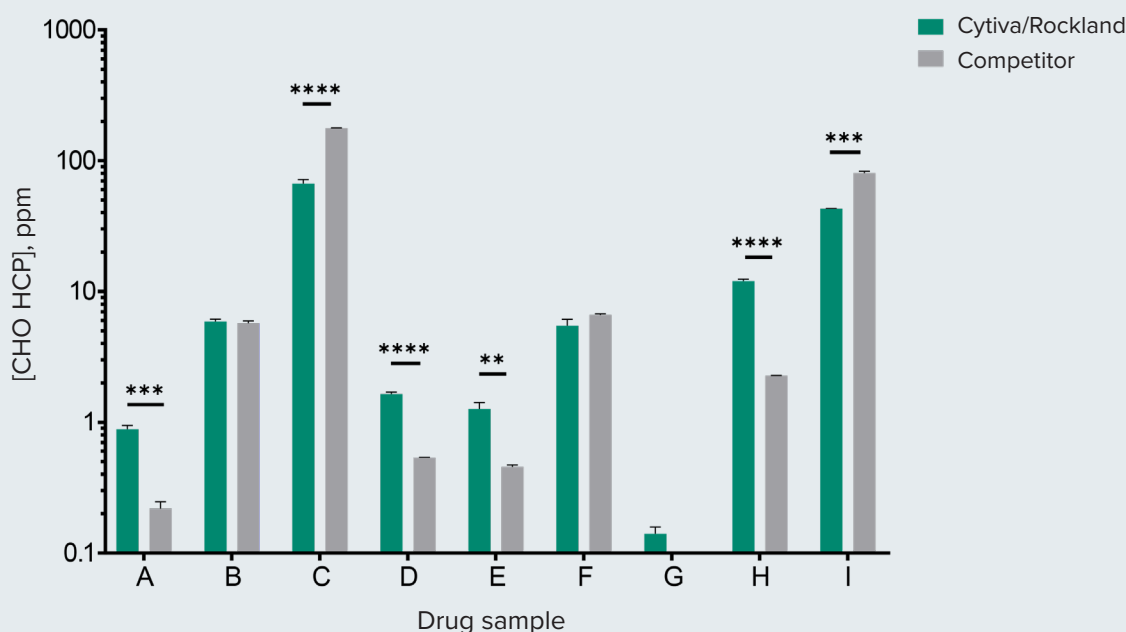


Figure 1. Residual HCP in commercial drug substances expressed as ppm (ng/mg). Asterisks denote statistical significance (t-test where ** = $P < 0.01$, *** = $P < 0.001$, and **** = $P < 0.0001$). Error bars represent standard deviation.

We interpolated the measured absorbance values for each sample to concentration (in ng/mL) using a 4-PL fit of the standard curve included with each kit and converted the average concentrations to ppm.

We used a t-test to calculate any statistically significant differences between the measured concentrations from each kit for all samples. For each assay, we excluded absorbance values from samples which were outside

their corresponding standard curves from analysis. The results indicate that Amersham HCPQuant CHO (supernatant) detected more HCP than the competitor kit at each purification step for CHO-K1 (Fig 2, Fig 3) and CHO-S (Fig 4, Fig 5). Amersham HCPQuant CHO also demonstrated excellent parallelism for both CHO-K1 and CHO-S across the entire purification process, from harvest to final purified product (Table 1).

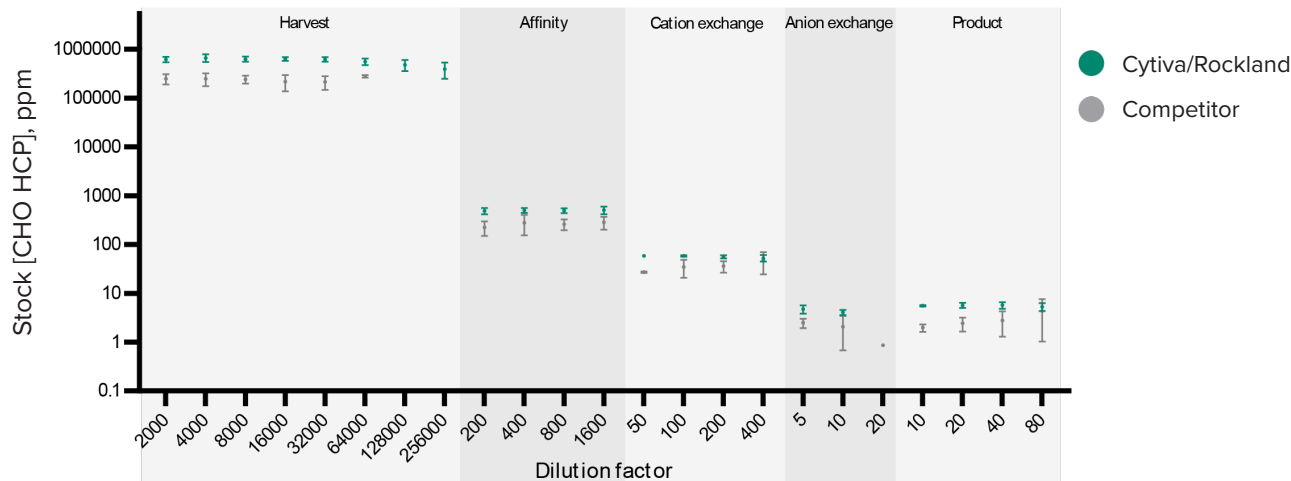


Figure 2. Parallelism across in-process purification steps for a monoclonal antibody produced in a CHO-K1 cell line. Error bars represent standard deviation.

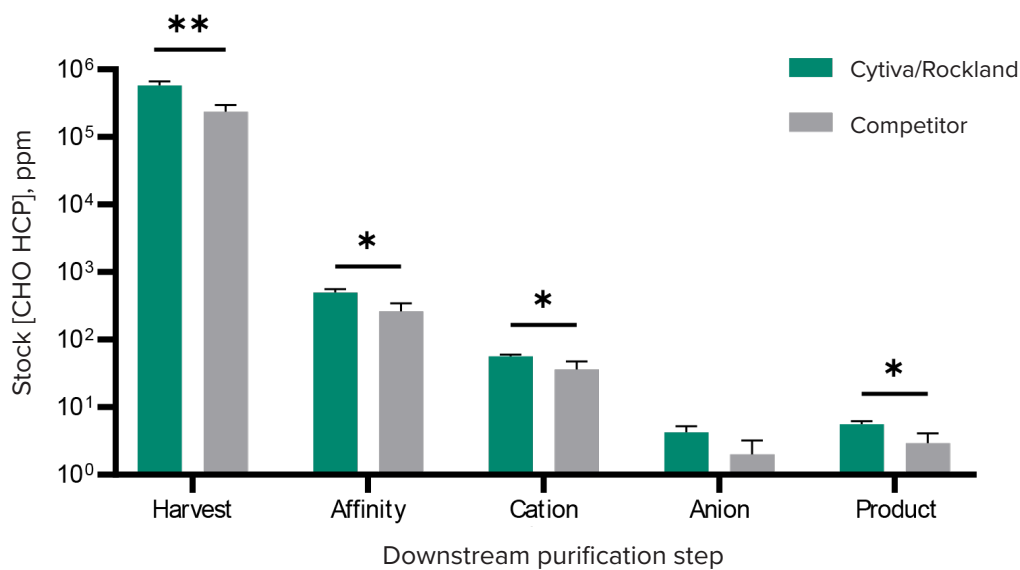


Figure 3. HCP concentration across in-process purification steps for a monoclonal antibody produced in a CHO-K1 cell line, expressed as ppm. Asterisks denote statistical significance (t-test where * = $P < 0.05$, and ** = $P < 0.01$). Error bars represent standard deviation.

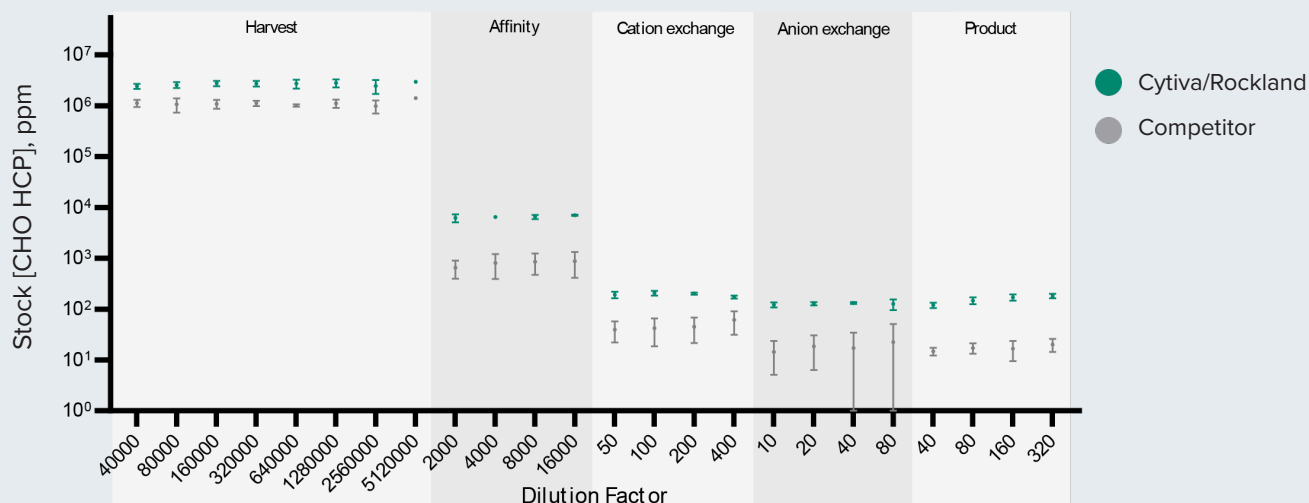


Figure 4. Parallelism across in-process purification steps for a monoclonal antibody produced in a CHO-S cell line. Error bars represent standard deviation.

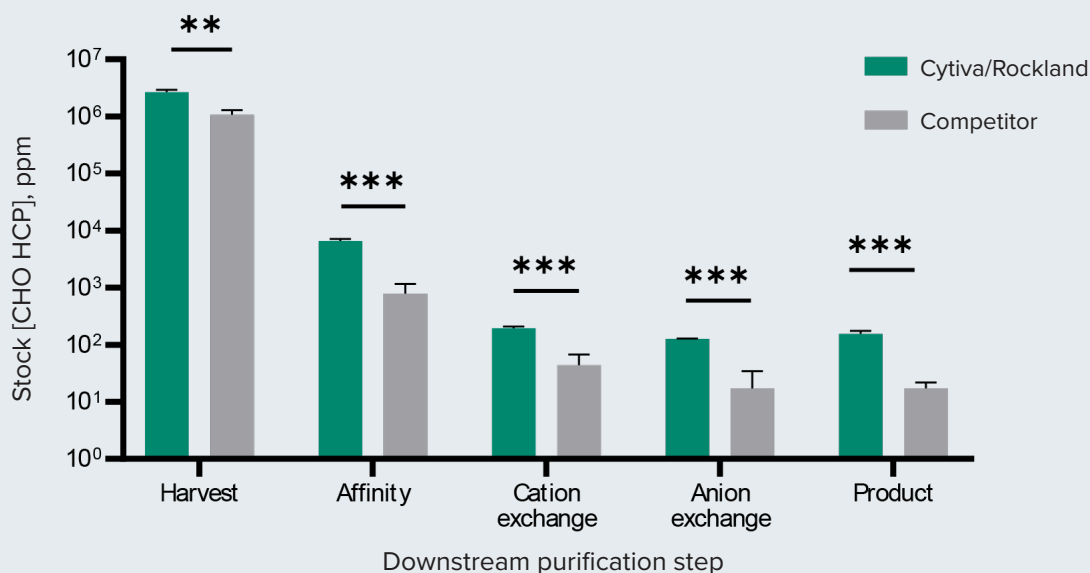


Figure 5. HCP concentration across in-process purification steps for a monoclonal antibody produced in a CHO-S cell line expressed as ppm. Asterisks denote statistical significance (t-test where ** = $P < 0.01$, and *** = $P < 0.001$). Error bars represent standard deviation.

Purification step	CHO-K1		CHO-S	
	HCPQuant	Competitor	HCPQuant	Competitor
Harvest	18.10	10.78	10.49	13.48
Affinity	8.16	14.87	7.06	14.48
Caption exchange	6.85	21.27	9.60	15.51
Anion exchange	13.74	10.00	12.59	34.95
Product	7.15	20.64	17.90	17.01

Table 1. Parallelism (expressed as the coefficient of variation, or CV%) of in-process purification steps for a monoclonal antibody produced in CHO-K1 and CHO-S cell lines. CV < 20% indicates good sample compatibility.

High sensitivity

The lower limit of detection (LLD) is the lowest concentration that can be distinguished from the background. The lower limit of quantitation (LLQ) is the lowest concentration that can be measured reliably and reproducibly.

To calculate the LLD and LLQ, we spiked known concentrations of Amersham HCPQuant protein standard into sample buffer and measured absorbance in the ELISA Kit. We calculated LLD as the concentration for which the signal was greater than three standard deviations (σ) from the mean of the zero standard [$0.106 + (3 \times 0.003) = 0.115$] (Table 2). We calculated LLQ as the lowest concentration for which the coefficient of variation (CV) was less than 20% and recovery was between 80% and 120% (Table 3). We determined LLD and LLQ from eight replicates across two plates.

We used spike assays to confirm the LLD and LLQ, testing a total of 72 replicates at 1 and 2 ng/mL across three plates.

For LLD, the average absorbance from 72 replicates of the blank plus 3σ was 0.0683, and the average absorbance from 72 replicates of the 1 ng/mL standard was 0.0723. This indicates an LLD around 1 ng/mL.

For LLQ, the CV for 72 replicates of the 2 ng/mL protein standard was 18.3% with a recovery of 91.9%, indicating an LLQ around 2 ng/mL.

Broad dynamic range

Our Amersham HCPQuant CHO (supernatant) Kit has a broad dynamic range that reduces the time and number of plates needed for linear experiments.

We used the protein standard from the HCPQuant CHO (supernatant) ELISA Kit to prepare a standard calibration curve from 200 to 1.389 ng/mL (Fig 6, Table 4). To perform the assay in triplicate, we measured in duplicate across three assays (six replicates total). The standard curve shows a broad range (1.39 to 200 ng/mL) and a strong goodness of fit ($R^2 > 0.999$).

To avoid assay repetition due to out-of-range detection, scientists need to determine the optimal dilution factors for each assay sample. For the best performance, we recommend performing two dilution series in parallel: a two-fold eight-step serial dilution, and a five-fold eight-step serial dilution. This combination enables you to identify a dilution where the sample generates a reading within the detection range.

HCP concentration (ng/mL)	6.0	5.0	4.0	3.0	2.0	1.0	0.5	0
Mean A ₄₅₀	0.184	0.168	0.156	0.149	0.132	0.118	0.112	0.106
Standard deviation (σ)	0.005	0.007	0.005	0.006	0.004	0.003	0.005	0.003
Blank + 3σ								0.115
Mean abs > blank + 3σ	Yes	Yes	Yes	Yes	Yes	Yes	No	—

Table 2. LLD

HCP concentration (ng/mL)	6.0	5.0	4.0	3.0	2.0	1.0	0.5
Mean interpolated concentration (ng/mL)	5.74	4.55	3.68	3.13	1.95	0.88	0.48
Recovery (%)	95.60	90.97	92.02	104.18	97.64	87.91	95.67
CV (%)	4.90	4.09	4.23	10.03	19.91	21.03	69.90

Table 3. LLQ

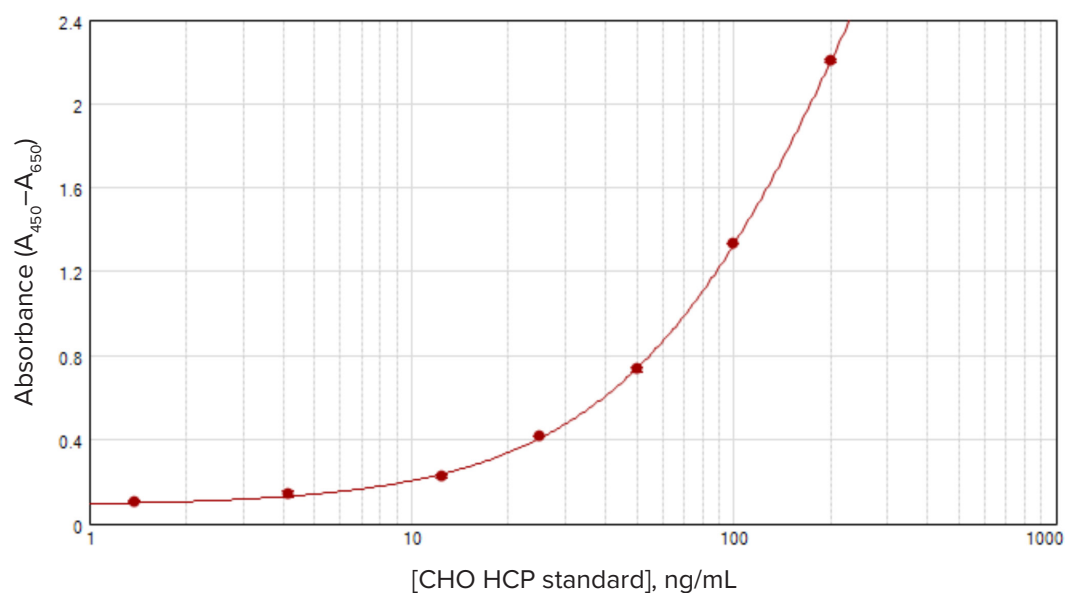


Figure 6. Standard curve (4-parameter logistic fit) of the Amersham HCPQuant CHO (supernatant) kit. Results measured on the SpectraMax iD3 reader were plotted using SoftMax Pro Software.

Concentration (ng/mL)	Mean absorbance ($A_{450} - A_{650}$)	Inter-plate variation (CV%)
200	2.369	2.315
100	1.422	2.787
50	0.758	3.073
25	0.414	3.504
12.5	0.236	2.809
4.167	0.116	5.702
1.389	0.077	7.656

Table 4. Mean absorbance and inter-plate CV of the standard curve.

A robust assay with reproducible results

Assay precision is the coefficient of variation (CV) within a single experiment (intra-assay) and across multiple experiments (inter-assay). To perform precision analyses, we spiked the assay with three sample concentrations

(160, 60, and 8 ng/mL). We calculated intra-assay precision using 10 replicates for each concentration (Table 5) and calculated inter-assay precision from the averaged means of three replicate assays (three plates) (Table 6).

Spiked concentration of HCP (ng/mL)	Calculated concentration (ng/mL)	Recovery (%)	Recovery CV%
160	165.12	103.20	3.40
60	61.77	102.95	4.10
8	9.28	115.95	4.70

Table 5. Intra-assay precision

Spiked concentration of HCP (ng/mL)	Average calculated concentration (ng/mL)	Average recovery (%)	Recovery CV%
160	154.69	96.68	4.70
60	56.86	94.77	6.72
8	7.87	98.34	5.82

Table 6. Inter-assay precision

Automated plate washing accelerates your workflow

Manual washing of ELISA plates presents a bottleneck that hinders efficient workflows and requires more tedious hands-on effort compared to automated methods. To increase throughput and reduce hands on time, we tested automated wash methods using a Molecular Devices AquaMax 4000 Microplate Washer. Assay plates were washed using a series of four repeated aspirate and dispense steps with the following settings:

Aspirate settings:

- Mode: Edge or Crosswise (work equally well here; results with Crosswise are shown)
- Rate: 5
- Descent speed: Fast
- Probe height: 0 mm

Dispense settings:

- Rate: 1 (150 mm Hg)
- Volume: 350 μ L

We used representative in-process samples from a standard downstream purification of a therapeutic monoclonal antibody and measured residual HCP at each process step, across triplicate assays. We tested the same samples using the manual washing method to act as a control. To compare the mean HCP concentrations between the washing methods, we performed a t-test on the calculated ppm from each in-process step.

The results indicate that there was no significant difference ($p < 0.05$) in the detected level of residual CHO HCP with either wash method (Figure 7). This supports the use of automated plate washing using the AquaMax 4000 washer with the Amersham HCPQuant CHO (Supernatant) ELISA.

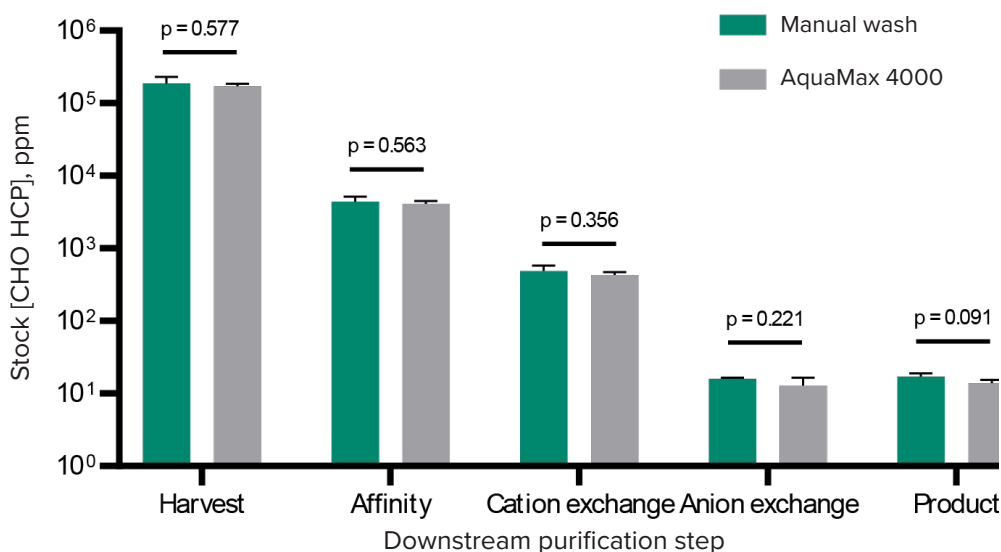


Figure 7. HCP concentration across in-process purification steps for a monoclonal antibody produced in a CHO-K1 cell line expressed as ppm. The results from each statistical comparison (t-test) are given above each group. Error bars represent standard deviation.

Conclusion

The Amersham HCPQuant CHO (Supernatant) ELISA Kit is compatible with a wide range of sample types that may be taken at different steps of the purification process, with a demonstrated dilution linearity across these steps that ensures accurate HCP level tracing for a reliable purification strategy. Highly sensitive and with a broad dynamic range, this assay reduces the time and number of plates required for linear results. Automated washing with the AquaMax 4000 plate washer, along with detection on the SpectraMax iD3 reader and analysis using SoftMax Pro software, streamline the workflow and reduce hands-on time for faster results.

References

1. Application Note: Three-step monoclonal antibody purification processes using modern chromatography media. Cytiva; 29132569 AA, May 2015.

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