

Quantitation of Host Cell Protein Impurities

Application Note

D0019867

Host Cell Protein (HCP) impurity concentrations are regulated in the manufacture of biologic drugs because of their immunogenic potential. Methods to detect and measure HCPs are complex because of the heterogeneous mixture of HCPs present in samples. Failure to accurately measure HCPs can potentially reduce therapeutic efficacy or cause adverse patient reactions and could have significant cost implications in both bioprocess development and production.

Current methodologies, such as ELISA, inadequately meet the current demands to improve productivity and efficiency as a result of increased workloads to support multiple programs with limited resources and personnel.

Gyros™ technology has been used to develop an automated immunoassay to measure HCP from a variety of host cell systems. The assay uses either generic reagents, e.g. 3rd Generation HCP (Cygnus), for broad measurement of HCPs, or specific reagents that are unique to the biologic. The assay provides substantial benefits over ELISA:

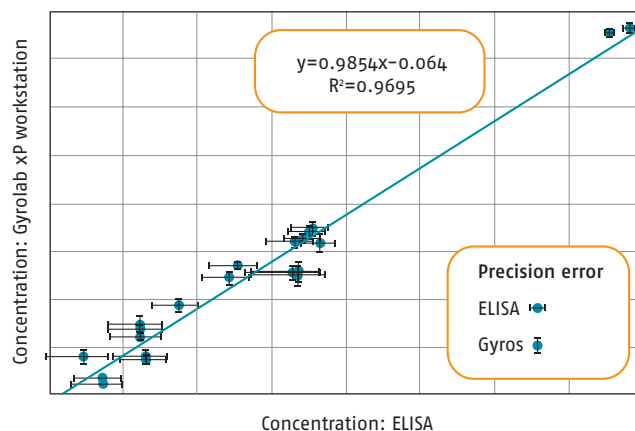
Broad dynamic range: Minimizes dilutions and the need for repeat analysis with 3–4 logs of dynamic range.

Rapid results: Data-driven decisions with results in under an hour.

Automation and throughput: Reduces manual hands-on time with up to 480 data points generated unattended.

Precision and reproducibility: Provides robustness for better consistency and easy assay transfer.

Correlation of Gyrolab with ELISA



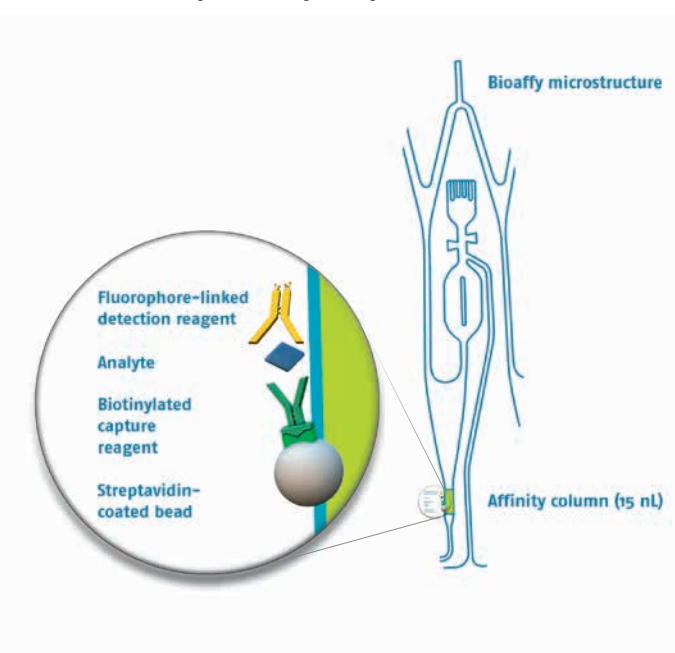
Correlation of results for samples throughout the purification of a therapeutic protein are shown. The error bars indicate that the results from Gyrolab xP workstation are less variable.

Nanoliter-scale immunoassays on Gyrolab xP workstation

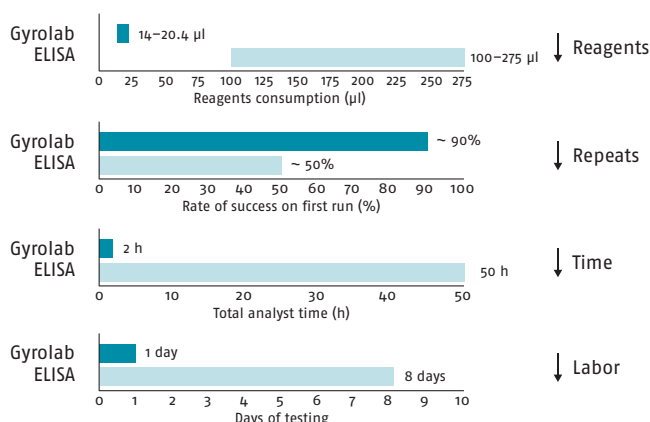
Gyrolab™ xP workstation automates the immunoassay workflow by integrating sample addition, incubation, washing and detection using nanoliter volumes in a compact (CD) format. Centrifugal force, capillary action and hydrophobic barriers combine for rapid parallel processing of up to 112 or 96 reactions in a Bioaffy 200 CD or Bioaffy 1000 CD, respectively.

The HCP assay can be run in a Gyrolab Bioaffy 1000 CD. The biotinylated anti-HCP antibody binds to streptavidin-coated beads in the affinity column. Gyrolab xP workstation transfers the sample to the CD, followed by an anti-HCP antibody labelled with Alexa 647 fluorophore. A detector records the laser-induced fluorescence from each column.

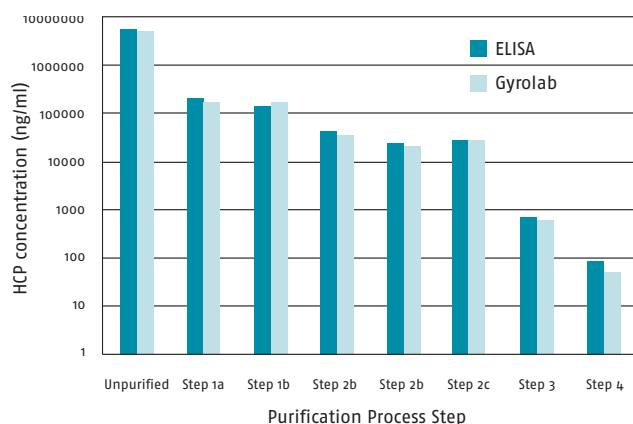
The sample is quantitated using a standard curve. Results are evaluated using Gyrolab Evaluator, or exported to a LIMS. All software programs are designed for 21 CFR part 11-compliance, ensuring that assays can be developed and transferred in regulated environments.



Increased efficiency and productivity



HCP concentrations during purification



Gyrolab and ELISA gave essentially identical results throughout the purification process.

Selectivity

Dilution Factor (DF)	MAb-A1			MAb-A2		
	CV (%)	Calc. Conc. (ng/ml)	CV DF (%)	CV (%)	Calc. Conc. (ng/ml)	CV DF (%)
100	7	10.50	9	4	13.50	10
50	9	11.05		6	13.30	
10	5	9.82		1	12.24	
2	2	9.12		5	10.80	

Two in-process samples with an estimated HCP concentration of 10,000 ng/ml were diluted (DF: Dilution Factor) and run in duplicate. The Coefficient of Variation (CV) for both samples was < 10%, indicating excellent dilutional linearity and a Minimum Required Dilution of only 2.

Comparison of methods to automate HCP analysis

HCP Assay	Manual ELISA	Tecan ELISA	Gyrolab
Assay Time/run	4.5 h (192 dp)	4.5 h (192 dp)	1 h (96 dp)
Throughput/day	192 dp	384 dp	960 dp
Hands-on Time	3 h with frequent intermission	30 min with one time operation	10 min with one time operation
Dynamic Range (ng/ml)	7–100	7–100	4–10,000
Minimum Sample (µl)	50	50	8
Assay Cost	2×	3×	1×
Overnight Run	No	Yes (up to 192 dp)	Yes (up to 480 dp)
Assay Development & Evaluation Time	More than 1 week	More than 1 week	Within 3 days

dp: Data Point

Data supplied by MedImmune Inc. and Merck & Co., Inc.

Conclusions

Gyrolab immunoassay platform delivers high precision results over a broad dynamic range, which minimizes repeat analysis of HCPs for data-driven decisions in bioprocess development and production. With rapid turnaround time to results and automation, data-driven decisions improve program efficiencies while increasing productivity with existing personnel. Finally, the nanoliter scale of the assays ensures low reagent and sample consumption at a higher throughput than either manual or automated ELISAs.

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