Gyrolab® CHO-HCP E3G Kit

For the detection of CHO Host Cell Proteins using the industry standard Cygnus CHO HCP 3G ELISA antibody

Product Information Sheet D0031773/C

Gyrolab® system delivers results comparable to ELISA with:

- Automated workflows – reduced manual operations
- Broad dynamic range – over four logs
- Fast turnaround – 96 datapoints in 75 minutes
- High throughput – up to 960 datapoints in a working day

Introduction

Gyros Protein Technologies has partnered with Cygnus Technologies to develop a sandwich immunoassay kit specific for the sensitive detection of residual Chinese Hamster Ovary Host Cell Protein (CHO-HCP) impurities based on the industry-standard 3G antibody. This antibody is reactive to more than 750 individual HCPs from conditioned media and cell lysates in both a CHO-S strain and a K1 strain. These HCPs represent more than 98% of the total mass of protein as indicated by methods orthogonal to ELISA. Assays based on the 3G antibody therefore have a high chance of detecting significant individual HCPs.

Gyrolab systems automatically analyze the dilution linearity and spike recovery data required for assay qualification, and the software simplifies the application of acceptance criteria according to current guidelines. Additionally, IgG titer can be measured in parallel to determine relative HCP levels. Combined with this and other ready-to-use kits, Gyrolab systems promise to deliver timely analytical support in the development and manufacture of recombinant antibodies and streamline the implementation of QbD principles in bioprocess development and long-term monitoring.

Gyrolab CHO-HCP assays increase productivity in bioprocess development

- Analytical results comparable to ELISA
- Automation generates 96 data points within 75 minutes without manual intervention
- Broad dynamic range minimizes dilutions needed, thus simplifying spike recovery and dilution linearity experiments
- Short turnaround time and reduced manual intervention accelerates data-driven decision making and frees up operator time for more important tasks
- Matrix insensitivity throughout the bioprocess minimizes interference and ensures robust analysis that lowers risk of repeat experiments

CYGNUS TECHNOLOGIES
part of Maravai LifeSciences

GYROS PROTEIN Technologies

The assay

Gyrolab CHO-HCP E3G Kit has been developed to quantify CHO-HCP impurities in bioprocess samples. The sandwich immunoassay is run on Gyrolab Bioaffy 1000 HC CD (Figure 1) using reagents from Cygnus Technologies and detects a broad spectrum of CHO-HCPs.

The biotinylated anti-HCP antibody is automatically introduced into a microstructure in the Gyrolab Bioaffy CD and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing CHO-HCPs are introduced into the microstructures and captured by the immobilized anti-CHO-HCP antibody. Bound HCP is then detected using an anti-HCP antibody labeled with Alexa Fluor®647. Results are evaluated using Gyrolab Evaluator, or exported to a LIMS. All Gyrolab software programs are designed for 21 CFR part 11-compliance, ensuring that assays can be developed and transferred in regulated environments.

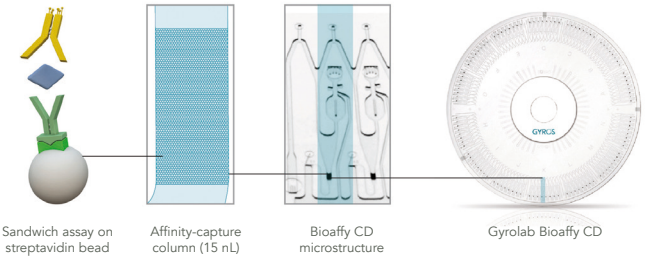

Figure 1. Sandwich Immunoassay format on a Gyrolab Bioaffy 1000 HC.

Assay performance

Broad dynamic range

Gyrolab CHO-HCP E3G Kit demonstrates a broad, four-log working range (Table 1) that minimizes the number of dilutions needed to analyze bioprocess samples with concentrations ranging from sub-g/mL down to ng/mL. LLOQ and ULOQ were established in ten runs as concentrations where Total Error (%CV + absolute %RE) < 30%, (see Table 2).

Limit of Detection (LOD) was determined as the concentration where the response equalled two standard deviations above the average blank response.

Table 1. Assay working range

LOD ng/mL	LLOQ ng/mL	ULOQ ng/mL
<1	~3	8 000

Table 2. Accuracy and precision data for five QC samples

Sample	Exp conc ng/mL	Av. measured conc (ng/mL)	Av. %CV	Av. Abs %RE	Av. %TE
QC1	8 000	8 134.5	9.4	3.8	13.2
QC2	80	83.5	4.5	5.0	9.6
QC3	5	5.0	8.3	7.0	15.3
QC5	3	3.0	11.4	5.8	17.2
QC6	2	2.0	23.0	9.8	32.9

High precision, accuracy and reproducibility

Data for standard curves run in triplicate in four runs on two instruments and by two operators are shown in Figure 2. Table 3 shows intra- and inter-run precision data for ten runs, on four instruments and by two operators.

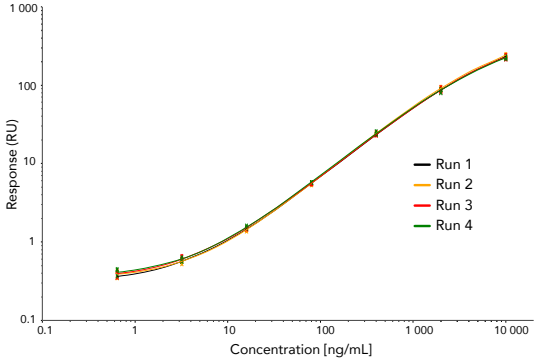

Figure 2. Overlay of four standard curves

Table 3. Inter- and intra-run precision data for the standard curve

	Expected conc ng/mL	Av. measured conc ng/mL	Average accuracy %	Intra-run %CV	Inter-run %CV
Blank	0	0	N/A	N/A	N/A
Standard 1	0.64	0.7	102	37.7	45.1
Standard 2	3.2	3.6	99	13.8	14.0
Standard 3	16	17	101	4.2	4.5
Standard 4	80	77	101	3.0	3.7
Standard 5	400	404	101	3.6	3.6
Standard 6	2 000	1 990	99	7.0	6.1
Standard 7	10 000	10 168	116	8.1	7.7

Specificity

Specificity was established using samples containing 10 mg/mL of Remicade® spiked with CHO-HCP levels in the range of 4–2500 ng/mL. The results in Table 4 demonstrate excellent recovery of HCP levels in the presence of high IgG concentrations.

Table 4. CHO-HCP spiked into samples containing 10 mg/mL IgG (Remicade)

Sample Name	Expected conc ng/mL	Measured conc ng/mL	%CV n=3	%RE
Remicade QC 1	2 500	2 819	12.1	12.8
Remicade QC 2	100	112	6.2	12.3
Remicade QC 3	4	4.5	14.0	13.6

Dilutional linearity

Dilutional linearity is a critical assay validation parameter for HCP assays and demonstrates antibody excess for the range of HCPs in the samples. Gyrolab system software automatically analyzes dilutional linearity and spike recovery data according to preset acceptance criteria and presents graphs for visual data assessment. Dilutional linearity data for two bioprocess samples are shown in Table 5 and Figure 3. In this example, dilutional linearity was assessed using a ratio of the maximum deviation from the highest measured concentration between each sample of 20%.

Table 5. Dilutional linearity

Sample Series	Dilution Factor	Back-calculated conc ng/mL	%CV	Recovery (%)
Harvest	20	151 056	5.6	87.2
	80	151 742	10.2	87.6
	320	153 505	3.7	88.6
	1 280	173 184	1.2	100.0
	5 120	160 886	0.9	92.9
	20 480	168 808	10.4	97.5
	81 920	157 700	10.6	91.1
Final Product	2	358	14.3	100.0
	4	339	10.2	94.6
	8	315	5.3	88.0
	16	332	1.4	92.6
	32	334	2.1	93.2
	64	337	14.5	94.1

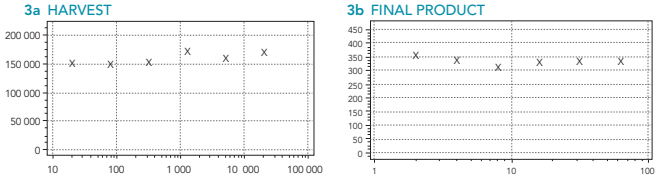

Figure 3a and 3b. Dilutional linearity graphs automatically generated by Gyrolab software

Spike recovery

Two bioprocess samples (harvest and final product) were spiked with known amounts of HCP over a dilution range to test the validity of the analysis (see Table 6). The results demonstrate excellent recovery of the spiked concentration in the dilution range.

Table 6. Results from spike recovery experiment

Sample	Dilution Factor	Unspiked conc ng/mL	Expected conc ng/mL	Measured conc ng/mL	CV Spiked conc [%]	Spike Recovery [%]
Harvest Spike conc: 140 000 ng/mL	40	144 894	284 894	263 447	1.0	85
	160	153 848	293 848	303 466	1.4	107
	640	165 437	305 437	310 995	0.6	104
	2 560	163 008	303 008	314 013	0.5	108
	10 240	162 174	302 174	301 170	3.5	99
	40 960	137 054	277 054	286 453	5.7	107
Final Product Spike conc: 300 ng/mL	4	364	664	675	1.5	104
	8	383	683	684	1.2	100
	16	361	661	663	7.3	101
	32	359	659	629	3.7	90
	64	323	623	648	3.9	108

Comparison with ELISA

Gyrolab CHO-HCP E3G Kit and Cygnus CHO HCP ELISA show comparable results for samples collected from different stages of a two-step purification process (see Figure 4). Figure 5 shows the correlation between the results from the two methods, with a correlation coefficient of 0.98.

Note: HCP immunoassays are complex assays that attempt to simultaneously measure hundreds of potential HCP contaminants and may at best be considered semi-quantitative. Variations may be observed when comparing results from two different methods and results may vary depending on the nature of individual samples.

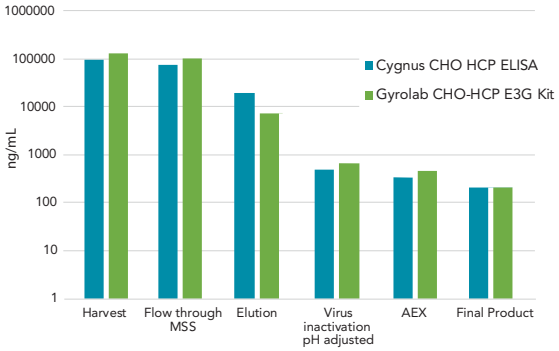

Figure 4. Gyrolab CHO-HCP E3G Kit delivers data that is comparable with ELISA with benefits of a broader dynamic range, shorter turnaround time, and higher throughput

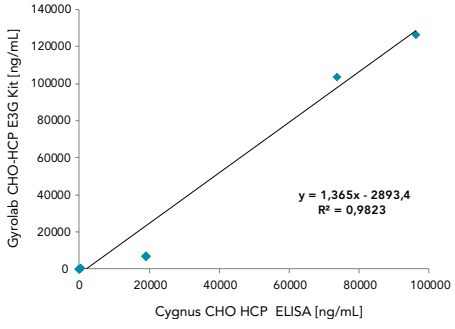

Figure 5. Correlation between Gyrolab CHO-HCP E3G Kit and Cygnus CHO HCP ELISA in six bioprocess samples

Abbreviations: MRD, Minimun Required Dilution; LOD, Limit Of Detection; LLOQ, Lower Limit Of Quantitation; ULOQ, Upper Limit Of Quantitation; RE, Relative Error, TE, Total Error; SD, Standard Deviation; CV, Coefficient of Variation

Ordering Information

Gyrolab CHO-HCP E3G Kit

Product Number: P0020605

Gyrolab CHO-HCP E3G Kit Contents

	Quantity
Gyrolab CHO-HCP E3G Kit Reagents	1 of each
Gyrolab Bioaffy 1000 HC CD	1
PCR plate 96	3
Microplate foil	3
Gyrolab Wash Buffer pH 11	1

Gyrolab CHO-HCP E3G Kit Reagents

Reagent A	Capture Reagent, Biotinylated anti CHO-HCP, ready to use solution, 60 µL
Reagent B	Detection Reagent, Fluorophore-labeled anti CHO-HCP, ready to use solution, 60 µL
Reagent C	CHO-HCP Standard, 50 µL at 20 µg/mL
Reagent D	Wash Buffer 1, 1.5 mL
Reagent E	Wash Buffer 2, 1.5 mL
Reagent F	Sample Dilution Buffer, 25 mL

Storage conditions

Gyrolab Bioaffy 1000 HC CD

Refrigerate at +4 °C to +8°C, unopened package.

Shelf life (unopened package): Minimum 12 months after delivery.

Gyrolab CHO-HCP E3G Kit Reagents

Refrigerate at +4°C to +8°C. Do not freeze.

Shelf life (unopened package): see product label.

Gyrolab CHO-HCP E3G CD50 Kit

Product Number: P0020606

Gyrolab CHO-HCP E3G CD50 Kit contains sufficient reagents and consumables to generate 4800 data points and is manufactured upon order.

Please contact your local sales specialist for more details

RELATED PRODUCTS

Gyrolab CHO-HCP Kits

P0020246 Gyrolab CHO-HCP Kit 1
(contains a different 3G antibody from Cygnus Technologies)
See Product *Information Sheet D0024206 for more details*

Gyrolab Protein A and MabSelect SuRe Kits

Quantification of leached native Protein A or MabSelect SuRe during purification processes. The kits contain sufficient reagents to generate 96 datapoints.

P0020456 Gyrolab Protein A Kit for MabSelect SuRe™ Ligand

P0020457 Gyrolab Protein A Kit for Native Protein A

Gyrolab huIgG Titer Kits

Quantification of human IgG in cell supernatants during cell line development.

P0020382 Gyrolab huIgG Kit - High Titer (96 data points)

P0020381 Gyrolab huIgG Kit – Low Titer (112 data points)

P0020379 Gyrolab huIgG Standard (IgG1)

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