Instruction For Use

Gyrolab® CHO-HCP E3G Kit

Product number	Product name
P0020605	Gyrolab CHO-HCP E3G Kit
P0020606	Gyrolab CHO-HCP E3G CD50 Kit

1. Product description

Gyrolab CHO-HCP E3G Kit is a ready-to-use kit for analysis of host cell proteins (HCPs) in bioprocesses for biotherapeutic drug substances produced in chinese hamster ovary (CHO) cell lines.

P0020605 Gyrolab CHO-HCP E3G Kit is a single CD kit containing all reagents, CDs, buffers and consumables required for 96 datapoints. P0020606 Gyrolab CHO-HCP E3G CD50 Kit is manufactured upon order and contains all reagents, CDs, buffers and consumables required for 50 CD runs. The reagents come packed in 10 plastic bags with each bag containing reagent volumes in vials that are sufficient for 5 CD runs.

2. Intended use

This document describes a protocol to quantify CHO-HCP impurities in therapeutic antibodies on Gyrolab® immunoassay platforms, using Bioaffy 1000 HC and ready-made reagents provided in a kit format.

This instruction for use can be used to perform CHO-HCP analysis using both product configurations.

Gyrolab CHO-HCP E3G Kit are for research use only and not intended for diagnostic use.

3. Introduction

Host Cell Proteins (HCPs) are produced by the host cell during the manufacture of recombinant proteins. HCP impurities present in the final product may potentially increase the risk for adverse effects in patients treated with therapeutic proteins. During cell culture, the concentration of HCP proteins increases to a maximum in harvest preparations. The level of residual HCP is then reduced to acceptable levels (<100 ppm) during product purification.

Gyrolab CHO-HCP E3G Kit is based on the CHO HCP antibody and antigen used in the industry standard ELISA from Cygnus Technologies. The affinity purified goat antibody is broadly reactive to more than 750 individual HCPs from conditioned media and cell lysates in both a CHO-S strain as well as a K1 strain. These 750+ HCPs represent more than 98% of the total mass of protein as indicated by protein detection methods orthogonal to ELISA.

4. Assay description

The CHO-HCP immunoassay is based on the well-established sandwich principle, where biotinylated capture antibody is introduced into a microstructure in the CD to saturate a capture column packed with porous beads that are coupled with streptavidin. Subsequently, samples containing CHO-HCP are volume defined and introduced into the microstructures where CHO-HCP antigens are captured in the capture column. Finally, a detecting reagent labeled with a suitable fluorophore is added. The integrated fluorescent signal represents the collective response from the CHO-HCP reaction.

5. Limitations

Partial Use of Kits

For single CD kits we recommend no more than two times partial use of kit reagents and CD that have been removed from its pouch. Use within one week. We do not recommend partial use of single CD's from the CD50 configuration of the kit.

Assay qualification

We recommend that you qualify and validate the assay for its intended use to ensure that the assay protocol provides acceptable performance, such as accuracy, precision and dilution linearity before using it to report CHO-HCP impurities in protein preparations.

6. Storage and Stability

Reagents

Gyrolab CHO-HCP Kit E3G reagents must be stored at +4 to +8°C to maintain functionality. Gyrolab Wash Buffer pH 11 can be stored at +4 to+28°C.

Unopened CD package

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

Opened CD package

CDs must be used within one week of opening. Return partially used CDs to original CD box and pouch. Re-seal. Store dark, dry and at room temperature.

7. Reagents, Methods & Materials

P0020605 Gyrolab CHO-HCP E3G Kit

Content	Product number
Gyrolab CHO-HCP E3G Kit Reagents: Contents see below	
Gyrolab CD: One (1) Gyrolab Bioaffy 1000 HC	
Wash Station Solution 2: Gyrolab Wash Buffer pH 11	P0020087
96-well plate: Three 0.2 mL skirted PCR plates	P0004861
Microplate Foil: Three Microplate Foils	P0003313

Gyrolab CHO-HCP E3G Kit Reagents Contents

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, 1500 µL	
Reagent E: Wash Buffer 2, 1500 µL	
Reagent F: Sample Dilution Buffer, 25 mL	P0020489

P0020606 Gyrolab CHO-HCP E3G CD50 Kit

This product contains all reagents, CDs, buffers and consumables required for 50 CD runs from the same manufacturing lot. The reagents come packed in 10 plastic bags.

Content	Product number
Gyrolab CHO-HCP E3G CD5 Kit Reagents: Contents see below	
Gyrolab CD: Fifty (50) Gyrolab Bioaffy 1000 HC	
Wash Station Solution 2: Fifty (50) vials of Gyrolab Wash Buffer pH 11	**P0020087**
96-well plate: One hundred and fifty (150) of 0.2 mL skirted PCR plates	**P0004861**
Microplate Foil: One hundred and fifty (150) Microplate Foils	**P0003313**

Gyrolab CHO-HCP E3G CD5 Kit Reagents.

A total of 10 plastic bags with reagents are included.
Each plastic bag contains:

Reagent A: Capture Reagent, Biotinylated anti CHO-HCP, ready to use solution, 250 µL
Reagent B: Detection Reagent, Fluorophore-labeled anti CHO-HCP, ready to use solution, 250 µL
Reagent C: CHO-HCP Standard, 375 µL at 20 µg/mL
Reagent D: Wash Buffer 1, 2000 µL
Reagent E: Wash Buffer 2, 2000 µL
Reagent F: Sample Dilution Buffer, 25 mL P0020489

Other Materials and Components required but not provided

- Gyrolab System
- Gyrolab Evaluator (version 3.3 or later)
- PBS-T; Bioaffy Pump Liquid and Bioaffy Wash Station Solution – see Gyrolab User Guide
- Pipettes or pipetting equipment with disposable polypropylene tips
- Disposable polypropylene test tubes
- Lab centrifuge
- Vortex mixer

Gyrolab Method

The Gyrolab method **'Gyrolab CHO-HCP kit method v2'** must be installed on the instrument being used for analysis. The method can be downloaded from www.gyrosproteintechnologies.com/user-zone

Please observe that for Gyrolab systems with software versions lower than 8.0, the method requires the use of three microtiter plates. These should be loaded with:

- Reagents
- Standard, controls and samples (one or several plates depending on number of samples)
- Wash buffers

respectively.

8. Preparation of reagents

Note! Briefly spin all vials in a micro-centrifuge before opening to collect all liquid at the bottom.
Note! Ensure that pipetting routines are designed to minimize contamination, e.g. use clean pipettes and new tips. Contamination may reduce the utility/shelf life of the reagent.

Capture Reagent

The Capture Reagent (**Reagent A**) is ready-made and is transferred directly to the microtiter plate according to the Gyrolab Control Loading List.

Detection Reagent

The Detection Reagent (**Reagent B**) is ready-made and is transferred directly to the microtiter plate according to the Gyrolab Control Loading List.

9. Standard curve

Table 1 shows an example of how to prepare a standard curve assuming the stock solution of CHO-HCP (**Reagent C**) is 20 µg/mL. We recommend preparing the CHO-HCP standard curve in the range 0.64–10 000 ng/mL. Dilute the standard in Sample Dilution Buffer (**Reagent F**).

Table 1. Example of a standard curve for CHO-HCP

	CHO-HCP Concentration (ng/mL)	Volume CHO-HCP stock (µL)	Volume higher standard conc. (µL)	Volume Reagent F (µL)
Std 1	10 000	25		25
Std 2	2 000		10	40
Std 3	400		10	40
Std 4	80		10	40
Std 5	16		10	40
Std 6	3.2		10	40
Std 7	0.64		10	40
Blank	0		0	40

QC samples

Table 2 shows an example of how to prepare QC samples from a stock solution of CHO-HCP at 20 µg/mL. The QC samples should be prepared by diluting the CHO-HCP stock to the appropriate concentrations using **Reagent F**.

Table 2. Dilution of QC samples to different CHO-HCP concentrations

	CHO-HCP Concentration (ng/mL)	Volume CHO-HCP stock (µL)	Volume higher QC conc. (µL)	Volume Reagent F (µL)
HQC	8 000	10		15
Intermediate dilution	*800*		*5*	*45*
MQC	80		5	45
LQC	8		5	45

10. Sample preparation

All samples to be analyzed for CHO-HCP impurities are recommended to be centrifuged at 12,000 g for 4 min to remove any particulates that may be present. Transfer the sample into a new tube. Aspirate the sample with caution to avoid any sediment. Dilute the sample in Reagent F. If the centrifugation step is omitted, extra caution might be required when evaluating the data, as spikes in the column profiles or possibly also clogged microstructures could occur.

The working range of the Gyrolab CHO-HCP E3G Kit is between 3 and 8 000 ng/mL which means that samples may need less dilution to hit the working range of the assay, compared to in an ELISA assay. However, samples should be analyzed at different dilutions to investigate the dilution linearity of the assay. For instance, a sample may initially be diluted 1:5 followed by additional dilutions of 1:2, 1:4 and 1:8, to generate a set of sample dilutions covering the range 1:5 to 1:40. Always dilute at least as much as required to get the sample into the quantifiable range of the assay.

For HCP assays in general, dilution linearity is an important parameter and must be considered when evaluating assay performance. Different assays can be expected to vary in dilution linearity for individual samples or processes.

11. Preparation of plate for wash buffers

For Gyrolab Control Software v 8.0 and above: Place wash buffers in MP according to loading list.
For Gyrolab Control Software versions below 8.0: Wash buffers (reagents D and E) shall be placed in a separate MP (100 µL in each well) in wells selected by the user. As an example, a full CD run will require 3 wells of Reagent D and 2 wells of Reagent E.

12. Preparation of Wash Station Solution 2

1. Dissolve one package (10 g) of Gyrolab Wash Buffer pH 11 powder in 1 L of deionized or distilled water for a one-CD run or dissolve two packages (20 g) of Gyrolab Wash Buffer pH 11 powder in 2 L of deionized or distilled water for a five-CD run
2. Filter the solution through a 0.22 or 0.45 µm filter.

Note!

- Prepare fresh wash solution weekly
- For research use only

13. Data analysis

The data is evaluated in Gyrolab Evaluator version 3.3 or later. Open the run and clock “Quantification”. In “Analysis Setup” default settings are recommended:

- do not include blanks in curve fitting
- five parameter logistic curve
- weight on response
- limit of detection factor: 2

Select “LOQ matrix”: diluted and enter 3 ng/mL as LLOQ and 8 000 ng/mL as ULOQ (or values established in-house). Acceptance criteria for %CV and bias can be added for highlighting values outside these criteria.

14. Performance characteristics

Precision and Accuracy

The standard curve in Figure 1 was generated by preparing CHO-HCP standard curves ranging from 10 000 to 0.64 ng/mL. Four standard curves were run in triplicate in Bioaffy 1000 HC on two Gyrolab systems by two operators. The back-calculated intra- and inter-assay precision data are illustrated in Table 3 as well as the back-calculated accuracy of standard points.

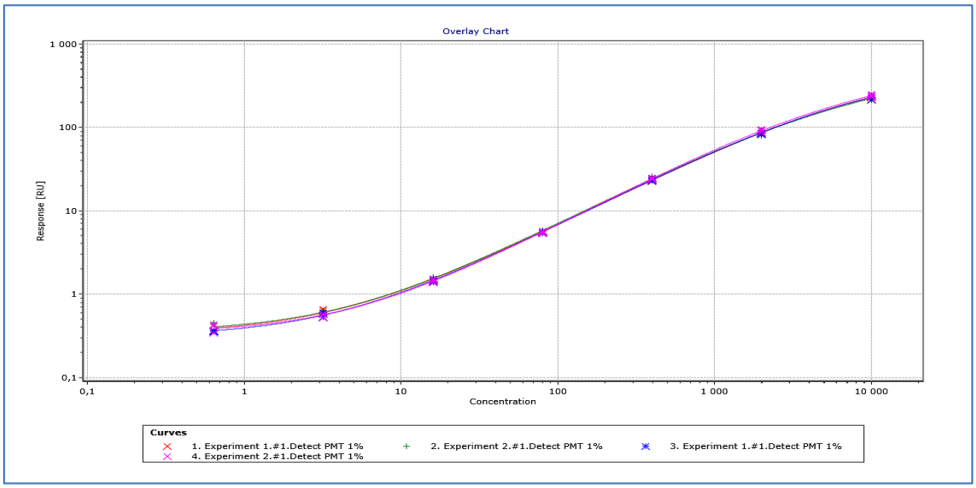


Figure 1. *Four Standard curves of freshly prepared CHO-HCP antigen in Reagent F. Each antigen concentration was determined in triplicate*

Table 3. Data from ten separately prepared standard curves in triplicate analyzed on four different Gyrolab instruments by two operators

Standard Point	Concentration (ng/mL)	Average Response	Average Accuracy (%)	Average Intra-Assay CV (%)	Inter-Assay CV (%)
Blank	0	0.383			
1	0.64	0.413	116	37.7	45.1
2	3.2	0.623	99	13.8	14.0
3	16	1.60	101	4.2	4.5
4	80	6.17	101	3.0	3.7
5	400	26.1	101	3.6	3.6
6	2000	96.7	99	7.0	6.1
7	10000	258	102	8.1	7.7

15. LOD, LLOQ, ULOQ of Gyrolab CHO-HCP E3G Kit

LOD was determined as 2 SD above the blank from ten standard curves in triplicate (30 data points) using the Gyrolab CHO-HCP E3G Kit. LOD was consistently lower than 1 ng/mL.

LLOQ and ULOQ were determined by analyzing several QC samples in the lower and upper range of the assay, respectively. The lowest (LLOQ) and highest (ULOQ) HCP concentration that gave a Total Error (absolute %RE + %CV) < 30% were assigned as LLOQ and ULOQ, respectively. The working range of the Gyrolab CHO-HCP assay is summarized in Table 4.

Table 4. Assay characteristics of Gyrolab CHO-HCP E3G Kit

Assay Characteristics (ng/mL)	
LOD	<1
LLOQ	~3
ULOQ	8 000

16. Dilution Linearity

Dilutional linearity is a critical assay validation parameter for HCP immunoassays. A dilutional linearity study demonstrates the important condition of antibody excess for the array of contaminants in your samples. Dilutional linearity involves performing a series of dilutions within the analytical range of the assay. These dilutions are then assayed and a dilution corrected concentration is determined at each dilution.

If the antibody is in a limiting concentration the apparent HCP concentration for a sample increases with increasing dilution. In most cases a dilution will be reached where the dilution corrected value remains essentially constant.

The results in Table 5 and Figures 2a–b, demonstrate typical data that can be observed from dilutional linearity experiments using Gyrolab CHO-HCP kits. In the example, two CHO-HCP samples originating from a small-scale bioprocess was tested for CHO-HCP impurities and analyzed for dilution linearity.

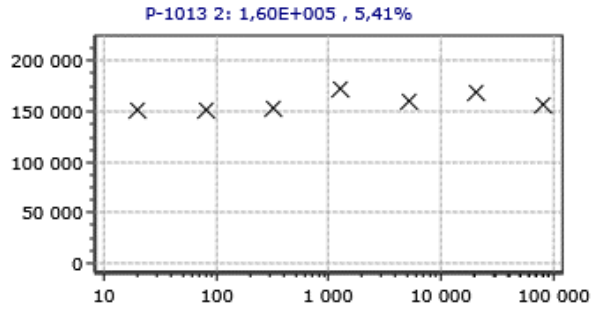
- Harvest from cell culture of an IgG1 producing recombinant CHO cell line
- Final product after an additional anion-exchange chromatography step

Samples were diluted in Reagent F to fit the working range of the Gyrolab CHO-HCP E3G Kit assay. In this example, dilutional linearity was established when the ratio of the maximum deviation from the highest measured concentration was less than 20%. Dilutional linearity data can be automatically analyzed using preset acceptance criteria in Gyrolab Control software version above 8.0.

Table 5. Dilution linearity of two samples originating from a small-scale bioprocess. Samples were diluted to fit the working range of the assay. Concentrations are in ng/mL

Sample Series	Dilution Factor	Unspiked Conc, Neat	Unspiked Conc, Diluted	CV Unspiked Conc [%]	Conc vs Max [%]	CV (%) on back calculated dilutions
Harvest	20	151056	7553	5.6	87.2	5.4
	80	151742	1897	10.2	87.6	
	320	153505	480	3.7	88.6	
	1280	173184	135	1.2	100.0	
	5120	160886	31	0.9	92.9	
	20480	168808	8	10.4	97.5	
	81920	157700	2	10.6	91.1	
Final product	2	358	179	14.3	100.0	4.1
	4	339	85	10.2	94.6	
	8	315	39	5.3	88.0	
	16	332	21	1.4	92.6	
	32	334	10	2.1	93.2	
	64	337	5	14.5	94.1	

2a. Harvest



2b. Final Product

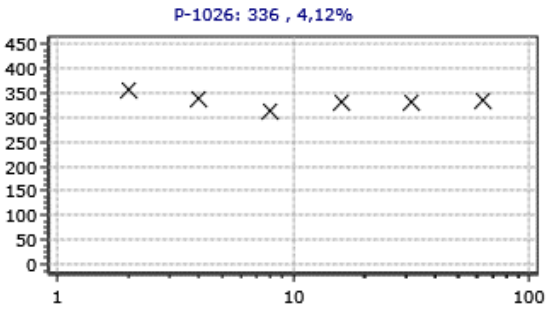


Figure 2a–b. *Dilution linearity of back-calculated CHO-HCP concentrations in samples from harvest and final product after anion exchange chromatography as assessed using a Gyrolab CHO-HCP E3G Kit. The graphs are automatically generated in Gyrolab Evaluator version 3.5.1 or higher. The unit on the x-axis is dilution and on the y-axis ng/mL*

17. IgG interference

Gyrolab CHO-HCP E3G Kit has been tested for compatibility with recombinant intact IgG. A pharmaceutical grade of recombinant antibody produced in a SP2/0 cell line was tested at 10 mg/mL in Reagent F in the Gyrolab CHO-HCP E3G Kit without any indication of interference.

Disposal procedures

Gyrolab CDs and microplates shall be disposed of in accordance with federal, state and local environmental control regulations. The user is responsible for waste disposal and for providing suitable waste containers. Packaging material can be disposed through combustion for energy recovery after that.

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