Instruction For Use

Gyrolab™ CHO-HCP Kit 1 CD50

Product number	Product name
P0020424	Gyrolab CHO-HCP Kit 1 CD50

1. Product description

Gyrolab CHO-HCP Kit 1 CD50 is a custom-made product that contains Gyrolab CHO-HCP Kit 1 (P0020246) reagents sufficient for 50 CDs, from the same manufacturing lot. Gyrolab CHO-HCP Kit 1 CD50 is manufactured upon order.

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.

Gyrolab CHO-HCP Kit 1 CD50 is 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. HCPs may contaminate the final product and potentially increase the risk for adverse effects in patients treated with therapeutic proteins. Today, most manufacturing processes for therapeutic antibodies utilize Chinese Hamster Ovary cells (CHO). 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.

Immunoassays for HCP face many challenges. Typically, 100s of different HCPs are produced during cell culture. The read-out from an HCP assay should represent the collective contributions of all reactive HCPs in the sample. These proteins may vary in abundance, physicochemical and immunogenic properties, and contribute differently to the collective response of the assay. The response of different HCP assays therefore depends on their component antibody preparations and the process under study.

4. Assay description

Gyros has developed several assays for CHO-HCP impurities. These assays may differ in utility between specific processes. It is important to evaluate if an assay is suitable for a given process. You must determine this by using relevant samples from the bioprocess.

The CHO-HCP immunoassays are 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

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

Gyros CHO-HCP Kit 1 CD50 reagents must be stored at +4 to +8°C to maintain functionality.

Unopened CD package

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

7. Reagents, Methods & Materials

Gyrolab CHO-HCP Kit 1 CD50 is a custom-made product that contains Gyrolab CHO-HCP Kit 1 (P0020246) reagents sufficient for 50 CD runs, from the same manufacturing lot. This custom-made product also contains an increased amount of HCP standard which enables additional spike recovery experiments. The reagents come packed in 10 plastic bags. Each bag contains reagents sufficient for 5 CDs.

Gyrolab CHO-HCP Kit 1 CD50 content	Product number
Gyrolab CHO-HCP Kit 1 CD50 Reagents. Contents see below	
Gyrolab CD Fifty (50) Gyrolab Bioaffy 1000 HC CDs	
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
Foil One hundred and fifty (150) Microplate Foils	P0003313

Gyrolab CHO-HCP Kit 1 CD50 Reagents.

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

Reagent A
Capture Reagent, Biotinylated anti CHO-HCP, 5x stock solution, 60 µL

Reagent B
Detection Reagent, Fluorophore-labeled anti CHO-HCP, 5x stock solution, 60 µL

Reagent C
CHO-HCP Standard, 375 µL at 20 µg/mL

Reagent D
Wash Buffer 1, 1800 µL

Reagent E
Wash Buffer 2, 1800 µL

Reagent F
Sample Dilution Buffer, 25 mL

Reagent G
Detection Reagent Dilution Buffer, 400 µL

Other Materials and Components required but not provided

- Gyrolab xPlore or Gyrolab xP workstation (Control Software version 5.4 or later)
- 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 (PP) test tubes, such as microcentrifuge tubes or matrix tubes
- Lab centrifuge
- Vortex mixer

Gyrolab Method

The Gyrolab method 'Gyrolab CHO-HCP kit method' must be installed on the instrument being used for analysis. The method can be downloaded from www.gyros.com/user-zone.
Note, that if a Gyrolab Control Software version lower than version 7.1 is used, a CD design file for Bioaffy 1000 HC must be imported. The CD design file can be downloaded from www.gyros.com/user-zone.
For information on how to import a method and a CD design with the Admin Tool see User Guide section F2.2.4 and F2.2.7 for CD design.

Please observe that 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 (this plate will be added just before the run is started)

8. Preparation of Wash Station Solution 2

Use freshly prepared solution. For research use only

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 µm filter.

9. Preparation of reagents

Note! Briefly spin all vials in a micro-centrifuge before opening to collect all liquid in the bottom of the tubes.
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 reagents.

Capture Reagent

The Capture Reagent (**Reagent A**) is diluted 1:5 in **Reagent D**. Ensure that the volume required is in accordance with the Gyrolab Control Loading List. Prepare the dilutions in propylene tubes. Vortex before transferring to the PCR plate.

Table 1 *Example of dilution of Capture Reagent*

Intended Run	Total Volume Required (µL)	Volume Reagent A stock (µL)	Volume Reagent D (µL)
1 CD	50	10	40
5 CDs	250	50	200

Detection Reagent

The Detection Reagent (**Reagent B**) is diluted 1:5 in Detection Reagent Dilution Buffer (**Reagent G**). Ensure that the volume required is in accordance with the Gyrolab Control Loading List. Prepare the dilutions in propylene tubes. Vortex before transferring to the PCR plate.

Table 2 *Example of dilution of Detection Reagent*

Intended Run	Total Volume Required (µL)	Volume Reagent B stock (µL)	Volume Reagent G (µL)
1 CD	50	10	40
5 CDs	250	50	200

Standard curve

Table 3 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. Make the standard in PP tubes and dilute the standard in Sample Dilution Buffer (**Reagent F**). Vortex between all steps.

Table 3 *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 4 shows an example of how to prepare QC samples from a stock solution of CHO-HCP at 20 µg/mL. Prepare the QC's in PP tubes by diluting the CHO-HCP stock to the appropriate concentrations using **Reagent F**. Vortex between all steps.

Table 4 *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)
QC1	8 000	10		15
QC2	80		5	495
QC3	5		5	75

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 following into the tip. Dilute the sample in Reagent F in PP tubes and vortex before transferring to the PCR plate. 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 CHO-HCP kit is between 2 and 8 000 ng/mL which means that samples may need less dilution to hit the working range of the 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 1:80. Always dilute at least as much as required to get the sample into the quantifiable range of the assay. When spiking samples, spike with a concentration of the same order of magnitude as the HCP concentration expected in the samples, as shown in Table 5.

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.

Table 5 *Example of serial dilution of a spiked sample (approximately 10 µg/mL HCP expected in sample)*

Serial Dilution	**Volume higher sample conc. [µl]**	**Volume Reagent F [µL]**	**Volume CHO-HCP stock (µL)**	**Spiked HCP conc [ng/mL]**	**Back-calculated spike [ng/mL]**
1:10	10	85	5	1000	10000
1:20	20	20		500	10000
1:40	20	20		250	10000
1:80	20	20		125	10000

Table 6. *Example of serial dilution of an unspiked sample*

Serial Dilution	**Volume higher sample conc. [µl]**	**Volume Reagent F [µL]**
1:10	10	90
1:20	20	20
1:40	20	20
1:80	20	20

11. Preparation of plate for wash buffers

Wash buffers (reagents D and E) shall be placed in a separate MP (100 µL in each well). As an example, a full CD run will require 3 wells of Reagent D and 2 wells of Reagent E.

12. 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
- Matrix settings: neat

Add 2 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.

13. Performance characteristics

13.1 Precision and Accuracy

The CHO-HCP assay was evaluated by preparing CHO-HCP standard curves ranging from 10 000 to 0.64 ng/mL. Six runs of fresh standard curves were tested in triplicate in Bioaffy 1000 HC on two Gyrolab xP instruments. The back-calculated intra- and inter-assay precision data are illustrated in Table 7 as well as the back-calculated accuracy of standard points.

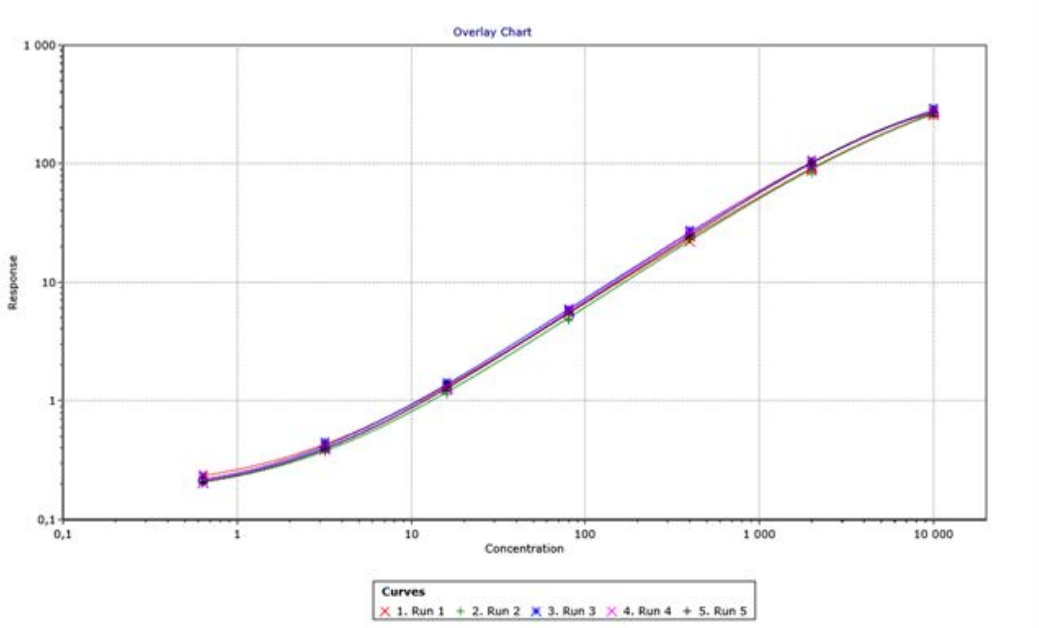


Figure 1. *Standard curves of freshly prepared CHO-HCP antigen in Reagent F from five different runs in Bioaffy 1000 HC. Each antigen concentration was determined in triplicate.*

Table 7. *Data from standard curves from freshly prepared CHO-HCP antigen analyzed on five occasions on two Gyrolab instruments*

Standard point	Concentration (ng/mL)	Average Response n=15	Back-calculated Accuracy (%)	Intra-assay CV (%) n=3	Inter-assay CV (%) n=15
Blank	0	0.17	N/A	N/A	N/A
1	0.64	0.22	101	8.4-33	24
2	3.2	0.41	102	1.9-10	7.2
3	16	1.3	99	1.7-8.9	5.5
4	80	5.5	100	0.9-6.3	4.5
5	400	25	101	1.6-6.5	4.2
6	2000	98	100	3.9-8.5	5.5
7	10000	274	101	2.7-18	8.2

13.2 LOD, LLOQ, ULOQ of Gyrolab CHO-HCP Kit 1

LOD was determined from measurements of standard points and blanks in 6 separate runs (18 data points) using Gyrolab CHO-HCP Kit 1. The lowest standard point (0.64 ng/mL) was consistently higher than the average blank + 2 SD.

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 (% R.E. + %CV) < ± 30 % were assigned as LLOQ and ULOQ, respectively. LLOQ must exceed the calculated LOD. The working range of the Gyrolab CHO-HCP assay is summarized in Table 8.

Table 8. *Assay characteristics of Gyrolab CHO-HCP Kit 1*

Assay Characteristics (ng/mL)	
LOD	0.64
LLOQ	~2
ULOQ	8 000

14. Dilution Linearity

Three CHO-HCP samples originating from a small-scale bioprocess were tested for the presence of CHO-HCP impurities and analyzed for dilution linearity.

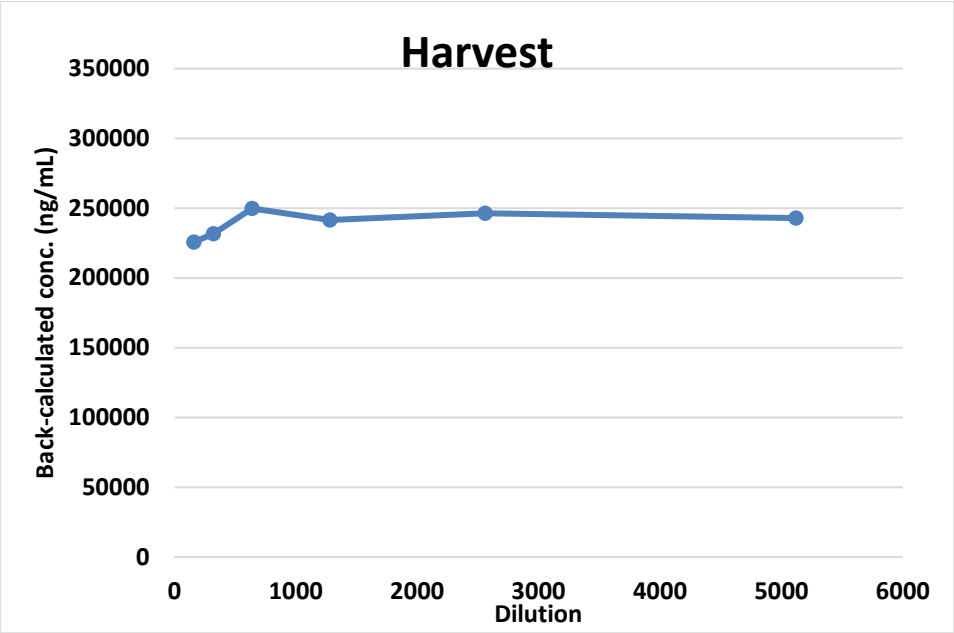
- Harvest from cell culture of an IgG1 producing recombinant CHO cell line
- Final product after an additional anion-exchange chromatography step

Samples were diluted in Reagent F to fit the working range of the CHO-HCP assay. The results are illustrated in Table 9 and Figures 2a–b.

Table 9. *Dilution linearity of two samples originating from a small-scale bioprocess. Samples were diluted to fit the working range of the assay.*

Sample	Dilution	CV (%) (n=2)	Average Back-calculated Concentration (ng/mL)	CV (%) on Back-calculated Dilutions
Harvest	160	4.0	225754	3.8
	320	6.2	231603	
	640	3.9	249782	
	1280	1.6	241404	
	2560	1.7	246275	
	5120	2.4	242820	
Final Product	4	4.2	529	7.7
	8	0.1	562	
	16	9.0	552	
	32	6.7	496	
	64	4.8	483	
	128	26.3	463	

2 a)



2 b)

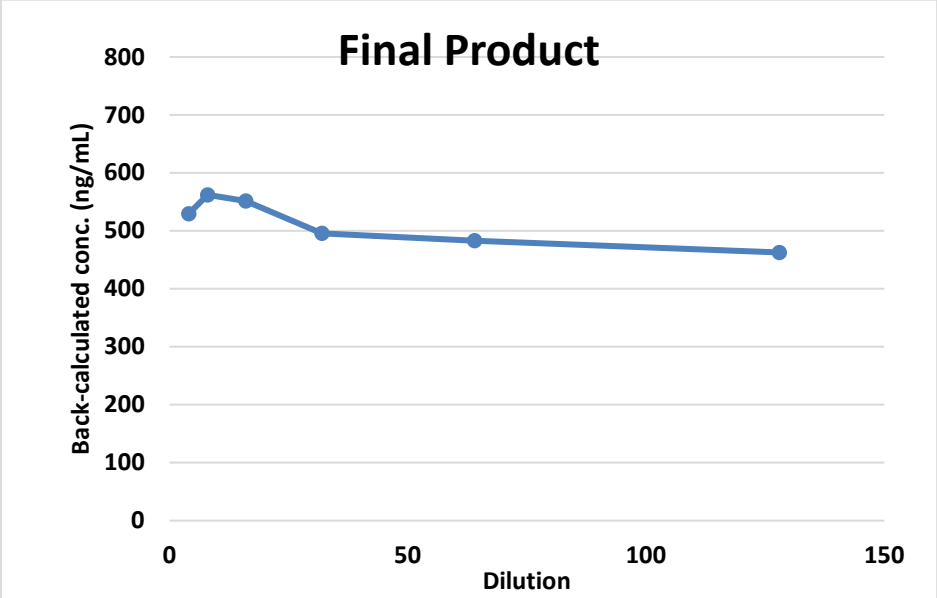


Figure 2a–b. *Dilution linearity of back-calculated CHO-HCP concentrations in samples from Harvest and Final preparation after anion exchange chromatography as assessed using Gyrolab CHO-HCP Kit 1.*

15. IgG interference

The CHO-HCP Kit 1 CD50 assay 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 CHO-HCP Kit 1 CD50 without any indication of interference.

16. Troubleshooting

Please visit user zone at www.gyros.com for current troubleshooting recommendations.

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.