
Product number	Product name
P0020247	Gyrolab CHO-HCP Kit 2
P0020248	Gyrolab CHO-HCP Kit 3
P0020249	Gyrolab CHO-HCP Kit 4
P0020250	Gyrolab CHO-HCP Kit 5

1. 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 2 to 5 are for research use only and not intended for diagnostic use.

2. 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, the majority of 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.

3. Assay description

Gyros Protein Technologies has developed several kits for determination of CHO-HCP impurities (Gyrolab CHO-HCP Kits 1 to 5). The kits are based on different reagents and may differ in utility between specific processes. It is important that you evaluate if the assay is suitable for a given bioprocess by using relevant samples.

The CHO-HCP immunoassay is based on the well-established sandwich principle, where biotinylated capture antibody is introduced into a microstructure in the Gyrolab Bioaffy 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.

4. 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.

5. Storage and Stability

Reagents

Gyros CHO-HCP Kit 2 to 5 reagents must be stored at +4 to +8°C to maintain functionality.

Unopened CD package

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

6. Reagents, Methods & Materials

Gyrolab CHO-HCP Kit content

Product number

Gyrolab CHO-HCP Kit Reagents

Kit Reagents 2, 3, 4 or 5, contents see below

Gyrolab CD

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

Foil

Three Microplate Foils

P0003313

Gyrolab CHO-HCP Kit Reagents contents

Reagent A

Capture Reagent, Biotinylated anti CHO-HCP, 60 µL

Reagent B

Detection Reagent, Fluorophore-labeled anti CHO-HCP, 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

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 test 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 must be installed if it is not already in the database. The method can be downloaded from the Gyrolab User Zone at www.gyrosproteintechnologies.com

For information on how to import a method and CD design with the Admin Tool see User Guide Sections F.2.2.4 and F2.2.7, or contact Gyros Protein Technologies.

Please note that the method requires the use of three microtiter plates loaded with:

- Reagents, standards and controls
- Samples
- Wash buffers (this plate will be added just before the run is started)

7. Preparation of reagents

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

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.

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. 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 4 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 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

8. Sample preparation

All samples to be analyzed for CHO-HCP impurities should be centrifuged at 12 000 g for 4 minutes to sediment any particulates. Transfer the sample to 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, due to the risk of particulates causing spikes in the column profiles or clogged microstructures.

The working range of the CHO-HCP kit is 1–8 000 ng/mL, which means that samples may need to be diluted less to fall in the working range of the assay. However, samples should be analyzed at different dilutions to investigate the dilutional linearity of the assay. For example, 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, dilutional linearity is an important parameter and must be considered when evaluating assay performance. Different assays can be expected to vary in dilutional linearity for individual samples or processes.

9. Preparation of plate for wash buffers

Wash buffers (reagents D and E) should be placed in a separate MP (100 µL in each well). For example, a full CD run will require 3 wells of Reagent D and 2 wells of Reagent E. The wash plate is not visible in the loading list, but will appear when executing the run.

10. 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.
2. Filter the solution through a 0.22 µm or 0.45 µm filter.

Note!

- Prepare fresh wash solution before execution of run.
- The kit is for research use only

11. Data analysis

The data is evaluated in Gyrolab Evaluator version 3.3 or later. Open the run and click 'Quantification'. In 'Analysis Setup', the 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 1 ng/mL as LLOQ (Lower Limit of Quantitation) and 8 000 ng/mL as ULOQ (Upper Limit of Quantitation; or values established in-house). Acceptance criteria for %CV (Coefficient of Variation) and bias can be added to highlight values outside these criteria.

12. Performance characteristics

12.1 Precision and Accuracy

The Gyrolab CHO-HCP Kits 2 to 5 were 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. Results from the evaluation of Gyrolab CHO-HCP Kit 3 are illustrated in Figure 1. The back-calculated intra- and inter-assay precision data are illustrated in Table 5 as well as the back-calculated accuracy of standard points.

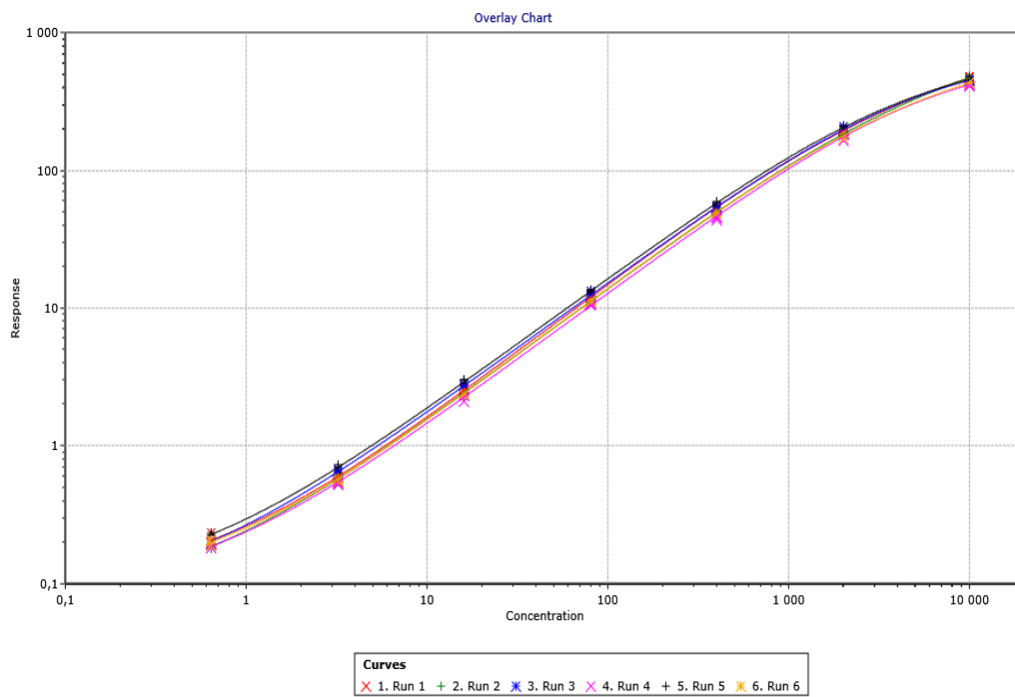


Fig 1. Standard curves of freshly prepared CHO-HCP antigen in Reagent F were run on 6 different occasions in Bioaffy 1000 HC using Gyrolab CHO-HCP Kit 3 on two Gyrolab instruments. Each antigen concentration was determined in triplicate.

Instruction For Use
Gyrolab® CHO-HCP Kit 2 to 5

Table 5a-d. Data from standard curves from freshly prepared CHO-HCP antigen analyzed on six occasions using Gyrolab CHO-HCP Kit 2 to 5 on two Gyrolab instruments. Intra assay CV% shows the range of CVs observed over six runs.

Table 5a. Gyrolab CHO-HCP Kit 2

Standard Point	Concentration (ng/ml)	Average Response n=18	Back-calculated Accuracy (%)	Intra assay CV (%) n=3	Inter assay CV(%) n=6
Blank	0	0.18	N/A	N/A	N/A
1	0.64	0.25	107	7.2-62.3	33
2	3.2	0.49	100	3.8-19.6	8.8
3	16	1.7	99	0.7-9.1	4.7
4	80	7.6	102	0.5-5.8	3.5
5	400	34	101	0.7-5.2	3.9
6	2 000	130	99	0.9-8.8	5.3
7	10 000	370	102	2.4-16.1	7.5

Table 5b. Gyrolab CHO-HCP Kit 3

Standard Point	Concentration (ng/ml)	Average Response n=18	Back-calculated Accuracy (%)	Intra assay CV (%) n=3	Inter assay CV(%) n=6
Blank	0	0.11	N/A	N/A	N/A
1	0.64	0.21	102	4.5-20.2	9.8
2	3.2	0.6	99	3.0-7.5	4.6
3	16	2.6	100	1.5-6.5	3.7
4	80	12	102	1.5-5.2	3.5
5	400	52	99	0.2-4.9	3.3
6	2 000	190	100	4.1-9.6	5.8
7	10 000	450	101	0.2-8.1	4.0

Table 5c. Gyrolab CHO-HCP Kit 4

Standard Point	Concentration (ng/ml)	Average Response n=18	Back-calculated Accuracy (%)	Intra assay CV (%) n=3	Inter assay CV(%) n=6
Blank	0	0,064	N/A	N/A	N/A
1	0.64	0.12	105	2.6-31.1	16.0
2	3.2	0.33	99	0.2-14.3	8.7
3	16	1.3	100	1.9-5.6	5.1
4	80	6.3	102	2.0-5.6	3.5
5	400	28	100	1.2-5.8	4.3
6	2 000	110	99	3.0-9.3	4.9
7	10 000	290	102	3.8-13.6	7.6

Table 5d. Gyrolab CHO-HCP Kit 5

Standard Point	Concentration (ng/ml)	Average Response n=18	Back-calculated Accuracy (%)	Intra assay CV (%) n=3	Inter assay CV(%) n=6
Blank	0	0.079	N/A	N/A	N/A
1	0.64	0.15	149	11.8-35.9	28.4
2	3.2	0.31	98	0.8-8.6	6.0
3	16	1.3	100	1.6-7.2	4.2
4	80	6	101	4.5-6.8	4.8
5	400	28	101	1.0-4.0	2.6
6	2 000	110	98	1.3-4.8	3.1
7	10 000	290	102	3.0-10.6	5.8

12.2 LOD, LLOQ, ULOQ of Gyrolab CHO-HCP Kit 2 to 5

LOD (Limit of Detection) was determined from measurements of standard points and blanks in six separate runs (18 data points) using Gyrolab CHO-HCP Kit 2 to 5. 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 (% Relative Error + %CV) < ± 30 % were assigned as LLOQ and ULOQ, respectively. LLOQ must exceed the calculated LOD. The working ranges of the Gyrolab CHO-HCP Kit 2 to 5 assays are summarized in Table 6.

Table 6. Assay characteristics of Gyrolab CHO-HCP Kit 2 to 5.

	Gyrolab CHO-HCP Kit 2 to 5 Assay Characteristics (ng/mL)			
	Kit 2	Kit 3	Kit 4	Kit 5
LOD	0.64	0.64	0.64	0.64
LLOQ	~3	~1	~1	~3
ULOQ	8 000	8 000	8 000	8 000

13. Dilutional Linearity

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

- Harvest from cell culture of an IgG1-producing recombinant CHO cell line
- A sample after purification using MabSelect SuRe™ LX
- Final product after an additional anion-exchange chromatography step

Samples were diluted in Reagent F to fit the working ranges of the CHO-HCP Kit 2 to 5 assays. The results are illustrated in Table 7 and Figures 2a–c.

Table 7. Dilutional linearity of 3 samples originating from a small-scale bioprocess using Gyrolab CHO-HCP Kit 2–5. Samples were diluted to fit the working range of the assay.

Sample	Dilution	Gyrolab CHO-HCP Kit 2			Gyrolab CHO-HCP Kit 3			Gyrolab CHO-HCP Kit 4			Gyrolab CHO-HCP Kit 5		
		CV(%) n=3	Average Back-calculated concentration (ng/ml)	CV(%) on Back-calculated dilutions	CV(%) n=3	Average Back-calculated concentration (ng/ml)	CV(%) on Back-calculated dilutions	CV(%) n=3	Average Back-calculated concentration (ng/ml)	CV(%) on Back-calculated dilutions	CV(%) n=3	Average Back-calculated concentration (ng/ml)	CV(%) on Back-calculated dilutions
Harvest	34	5.4	113 000	6.7	5.4	62 100	9.9	2.4	68 200	9.3	1.9	85 600	11
	1 700	4.9	129 000		3.9	75 800		2.1	81 600		3.4	108 000	
	3 400	4.5	128 000		0.8	76 300		6.8	83 700		2.8	105 000	
	6 800	5.6	133 000		6.4	77 100		1.8	83 000		6.6	107 000	
Post - MabSelect SuRe	3	2.7	3 970	11	3.5	2 500	13	3.1	5 930	21	2.2	9 430	21
	160	11	4 900		3.3	3 380		5.9	9 310		4.7	15 400	
	320	8.3	5 160		3.4	3 320		3.1	9 420		7.1	15 500	
	640	11	5 050		6.0	3 310		3.3	9 920		5.9	15 000	
Final Product	3	1.0	247	5.5	5.1	248	2.5	6.8	287	2.2	7.8	248	4.8
	7	2.0	264		7.2	254		10	297		5.5	254	
	13	7.8	233		8.1	242		7.3	282		9.5	264	
	27	3.6	260		11	255		10	288		10	235	

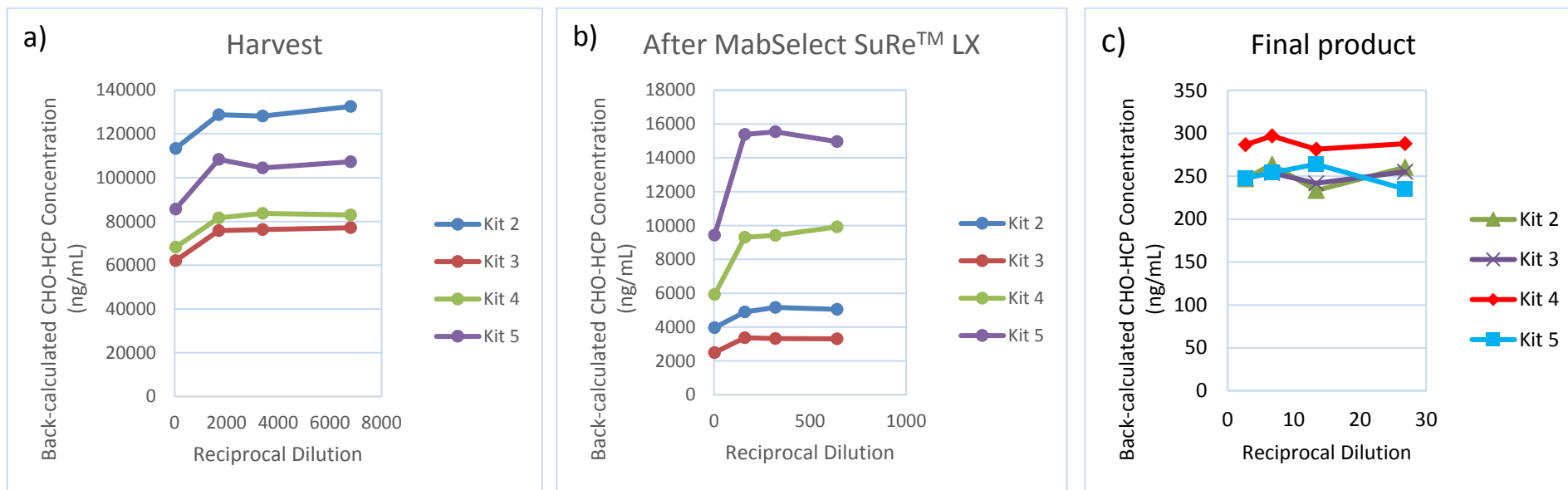


Figure 2a–c. Dilutional linearity of back-calculated CHO-HCP concentrations, as assessed using Gyrolab CHO-HCP Kit 2–5, in samples from harvest, purification with MabSelect SuRe LX, and final preparation after anion exchange chromatography.

14. IgG interference

Gyrolab CHO-HCP Kit 2–5 assays have been tested for compatibility with recombinant intact IgG. A pharmaceutical grade recombinant antibody produced in a SP2/0 cell line was tested at 10 mg/mL in Reagent F in Gyrolab CHO-HCP Kit 2–5 and showed no indication of interference.

Disposal procedures

Gyrolab CDs and microplates should 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 of through combustion for energy recovery.

15. Troubleshooting

Please visit the User Zone at www.gyrosproteintechnologies.com for current troubleshooting recommendations.

16. Disposal procedures

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