Instruction For Use

Gyrolab® CHO-HCP Kit 1

GYROS PROTEIN Technologies

Product number	Product name
P0020246	Gyrolab CHO-HCP Kit 1
P0020424	Gyrolab CHO-HCP Kit 1 CD50

1. Product description

Gyrolab CHO-HCP Kit 1 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.

P0020246 Gyrolab CHO-HCP Kit 1 is a single CD kit containing all reagents, CDs, buffers and consumables required for 96 datapoints.
P0020424 Gyrolab CHO-HCP Kit 1 CD50 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 Kit 1 are for research use only and not intended for diagnostic use.

3. Assay description

Gyrolab CHO-HCP Kit 1 is based on the well-established sandwich principle and utilizes a goat antibody from Cygnus Technologies. The 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

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 the user to qualify and validate the kit for its intended use to ensure that the kit protocol provides acceptable performance, such as accuracy, precision and dilutional linearity, before using it to report CHO-HCP impurities in protein preparations. If the qualification studies indicate deficiencies in performance for specific bioprocess samples, coverage studies may need to be performed. For such services, please contact Cygnus Technologies.

5. Storage and Stability

Reagents

Gyrolab CHO-HCP Kit 1 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

6. Reagents, Methods & Materials

P0020246 Gyrolab CHO-HCP Kit 1

This product contains all reagents, CDs, buffers and consumables required for 96 datapoints.

Content	Product number
Gyrolab CHO-HCP Kit 1 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 Kit 1 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

P0020424 Gyrolab CHO-HCP Kit 1 CD50

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 Kit 1 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 Kit 1 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, 1900 µL
Reagent E: Wash Buffer 2, 1800 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

7. 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.

8. 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

9. 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 Kit 1 is between 2 and 8 000 ng/mL which means that samples may need less dilution to hit the working range of the assay, compared to 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, see Table 4. 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 HCP concentration of the same order of magnitude as the HCP concentration expected in the sample, as shown in Table 3.

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 3. *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 4. *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

10. 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.

11. 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

12. Data analysis

The data is evaluated in Gyrolab Evaluator version 3.3 or later. Open the run and click “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 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

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.

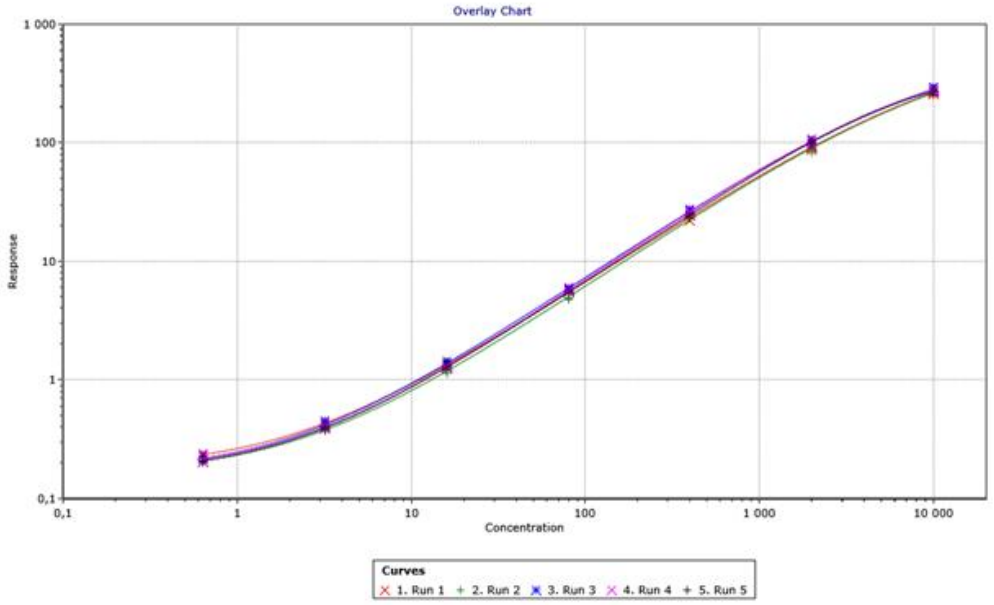


Fig 1. *Standard curves of freshly prepared CHO-HCP antigen in Reagent F from five different runs in Bioaffy 1000 HC. Each antigen concentration was determined in triplicate.*

Table 5. *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

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

LOD was determined from measurements of standard points and blanks in five separate runs (15 data points) using Gyrolab CHO-HCP Kit 1.

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 6.

Table 6. *Assay characteristics of Gyrolab CHO-HCP Kit 1*

Assay Characteristics (ng/mL)	
LOD	1.5
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.

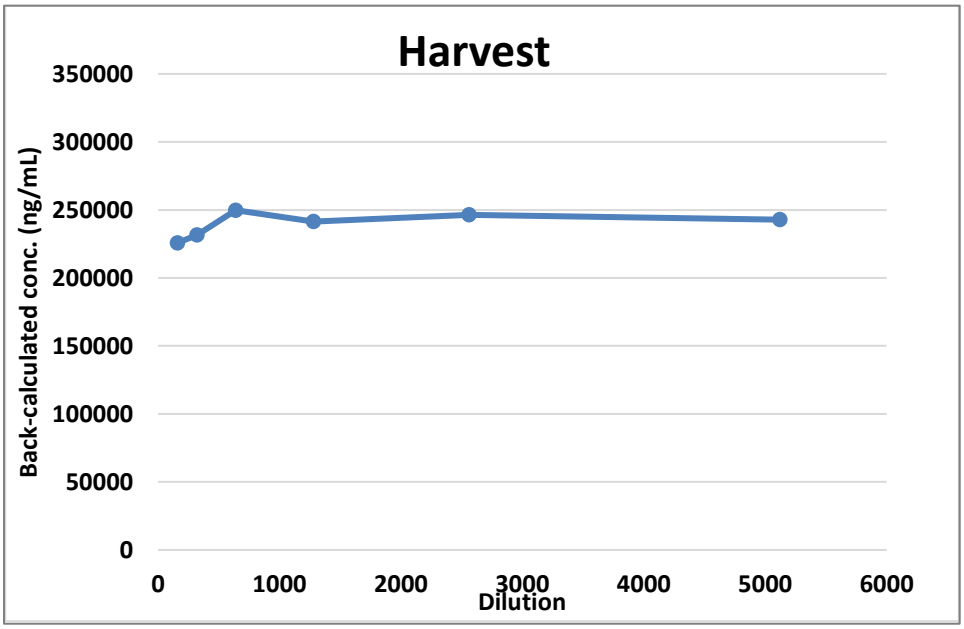
- Harvest from cell culture of an IgG1 producing recombinant CHO cell line
- Final product after an additional anion-exchange chromatography step

Samples were diluted in Reagent F to fit the working range of the CHO-HCP assay. The results are illustrated in Table 7 and Figures 2a–b.

Table 7. *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)

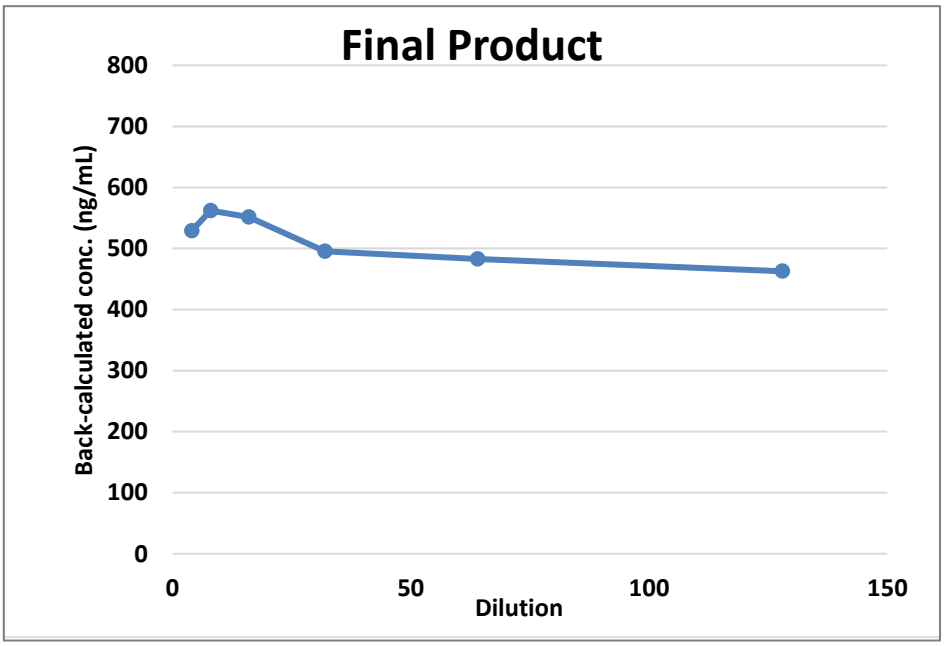


Figure 2a–b. *Dilution linearity of back-calculated CHO-HCP concentrations in samples from Harvest and Final preparation after anion exchange chromatography as assessed using Gyrolab CHO-HCP Kit 1.*

15. IgG interference

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

16. Troubleshooting

Please visit the kit guidelines on www.gyrosproteintechnologies.com/gyrolab-user-zone for more information and tips or contact your local field application specialist for support.

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.