

HEK 293 HCP Solution for Gyrolab®

For the detection of HEK 293 host cell protein impurities in bioprocess samples

Product Information Sheet

D0039652/A

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



Introduction

HEK 293 expression systems are commonly used in the production of adeno-associated virus (AAV) and lentiviral vectors for cell and gene therapy applications as well as in manufacturing of some vaccines and biopharmaceuticals. Host cell protein (HCP) impurity levels must be monitored throughout the purification process and in final product, and should be reduced to the lowest practical levels since HCPs may be immunogenic, interfere with drug efficacy or impact drug stability. Thus, there is a need for analytical tools that provide broad dynamic range, automation, fast turn-around time, and small sample volume to meet the high demands on limited manufacturing capacity and small viral vector batches.

The HEK 293 HCP Assay Reagent set for Gyrolab®, developed by Cygnus Technologies, is exclusively designed and optimized to be used together with the Gyrolab Bioaffy™ 1000 HC Assay Toolbox. The reagent set is based on the antibodies generated against a mild lysate of HEK 293 cells extracted by a procedure similar to what is used to harvest virus. These antibodies are broadly reactive to essentially all HCPs that could potentially co-purify with a drug product. The reagent set has been tested and validated by Cygnus Technologies and Gyros Protein Technologies to generate high quality results on Gyrolab systems.

HEK 293 HCP Solution for Gyrolab increases productivity in bioprocess development:

- 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

The Gyrolab solution

HEK 293 HCP Solution for Gyrolab, with assay reagent set from Cygnus Technologies, has been developed to quantify HEK 293 HCP impurities in bioprocess samples. The sandwich immunoassay is run on Gyrolab Bioaffy 1000 HC CD (Figure 1) and detects a broad spectrum of HEK 293 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 HEK 293 HCPs are introduced into the microstructures and captured by the immobilized anti-HEK 293 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.

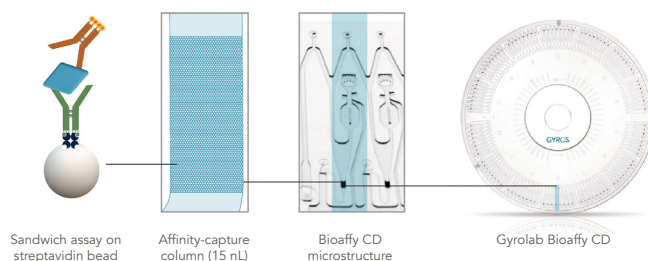


Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy 1000 HC CD

Assay performance - evaluated by Cygnus Technologies

Broad dynamic range

The HEK 293 HCP Assay Reagent Set for Gyrolab (G650S), when used with the Gyrolab Bioaffy 1000 HC Assay Toolbox (P0020667), demonstrates a broad, three-log working range (Table 1) that minimizes the number of dilutions needed to analyze bioprocess samples from all stages of the purification process.

Table 1. Reagent Set working range

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
~ 0.5	~ 4	~ 8 000

LOD is determined as the concentration where the response equals two standard deviations above the average blank response.

LLOQ and ULOQ are defined as the lowest and highest concentration respectively for which the CV is typically <20%.

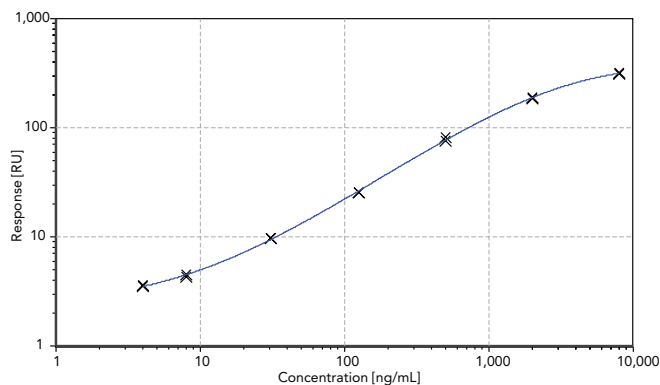


Figure 2. Typical standard curve data from an assay run

Accuracy and precision

Data for standard curves and QC samples over the working range are shown in Figure 2 and Table 2.

Both intra (n=20 replicates) and inter-assay (n=10 assays) precision were determined on 4 pools with low (~5 ng/mL), medium (~50 ng/mL and 200 ng/mL), and high HCP concentrations (~1000 ng/mL)

Table 2. Accuracy and precision data for four QC samples

Sample Name	Expected conc (ng/mL)	Average conc (ng/mL)*	Intra-run CV (%; n=20)	Inter-run CV (%; n=10)
QC1	5	4.68	15.2	11.4
QC2	50	50.32	3.6	7.0
QC3	200	199.2	4.9	5.8
QC4	1 000	1 089.2	5.4	3.4

* Three independent preparations of each sample were tested, and 20 replicates were measured for each sample.

Dilution linearity

Cygnus Technologies also evaluated the dilution linearity for 13 samples from various points in the purification process of a viral vaccine product. All samples demonstrated acceptable dilution linearity when diluted within the analytical range of the assay (Table 3).

Table 3. Dilution linearity data

Sample Series	Dilution Factor	Average Value (ng/mL)	Dilution Corrected Value (ng/mL)	% Change from Previous Dilution	Average Dilution Corrected Value (ng/mL)
1	10	121	1 210	N/A	1 247
	20	64	1 288	6	
	40	33	1 312	2	
	80	16	1 248	5	
	160	7.4	1 178	6	
320	< LLOQ	< LLOQ	< LLOQ		
2	50	255	12 750	N/A	11 318
	100	120	12 000	6	
	200	60	11 940	1	
	400	28	11 200	6	
	800	13	10 240	9	
	1 600	6.1	9 776	5	
3 200	< LLOQ	< LLOQ	< LLOQ		
3	500	205	102 500	N/A	96 530
	1 000	102	102 000	0	
	2 000	52	103 400	1	
	4 000	26	102 800	1	
	8 000	11	90 400	12	
	16 000	4.9	78 080	14	
	32 000	< LLOQ	< LLOQ	< LLOQ	
4	2	131	261	N/A	278
	4	75	301	15	
	8	34	273	9	
	16	16	259	5	
	32	9.3	298	15	
	64	< LLOQ	< LLOQ	< LLOQ	
5	2	129	258	N/A	278
	4	68	272	5	
	8	32	257	6	
	16	16	249	3	
	32	8.3	264	6	
	64	5.7	366	39	
128	< LLOQ	< LLOQ	< LLOQ		
6	1 000	55	54 800	N/A	56 750
	2 000	26	52 800	4	
	4 000	16	63 300	20	
	8 000	7.0	56 100	11	
16 000	< LLOQ	< LLOQ	< LLOQ		
7	300	23	6 890	N/A	7 393
	600	14	8 390	22	
	1 200	5.8	6 900	18	
	2 400	< LLOQ	< LLOQ	< LLOQ	
8	2	> ULOQ	> ULOQ	> ULOQ	67 100
	4	> ULOQ	> ULOQ	> ULOQ	
	8	6 825	54 600	N/A	
	16	4 313	69 000	26	
	32	2 206	70 600	2	
	64	1 159	74 200	5	
	128	573	73 400	1	
256	301	77 100	5		
9	2	209	417	N/A	339
	4	94	374	10	
	8	38	302	19	
	16	19	301	0	
	32	9.4	301	0	
	64	< LLOQ	< LLOQ	< LLOQ	
10	2	9.5	19	N/A	20
	4	5.5	22	16	
	8	< LLOQ	< LLOQ	< LLOQ	
11	100	240	24 000	N/A	21 800
	200	112	22 300	7	
	400	56	22 300	0	
	800	26	20 600	8	
	1 600	12	19 300	6	
	3 200	7.0	22 300	16	
	6 400	< LLOQ	< LLOQ	< LLOQ	
12	500	32	16 100	N/A	14 567
	1 000	14	14 300	11	
	2 000	6.7	13 300	7	
	4 000	< LLOQ	< LLOQ	< LLOQ	
13	1 000	94	94 300	N/A	86 340
	2 000	41	82 300	13	
	4 000	22	87 100	6	
	8 000	12	93 300	7	
	16 000	4.7	74 700	20	
	32 000	< LLOQ	< LLOQ	< LLOQ	

Comparison with ELISA

The 13 samples were also analyzed using Cygnus HEK 293 HCP ELISA, 3G, Cat. No. F650S. HEK 293 HCP Solution for Gyrolab and Cygnus ELISA kit produced comparable results (Figure 3).

Note: Results from ELISA and Gyrolab assays may not be comparable for all bioprocess samples due to variations in sample nature and differences in the measuring techniques. In addition, it should be noted that HCP immunoassays are semiquantitative and only measure relative amounts between samples.

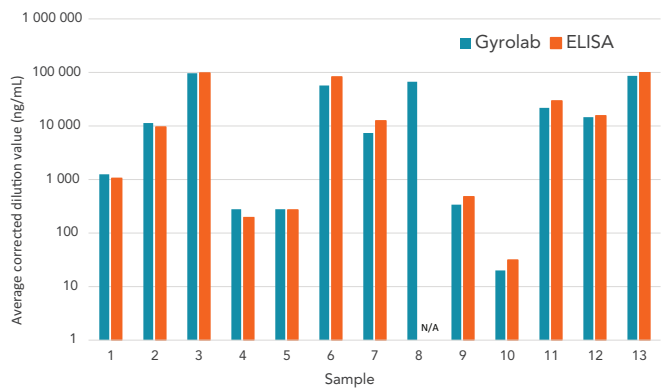


Figure 3. Comparison of results for HEK 293 HCP Solution for Gyrolab and Cygnus ELISA kit for samples from various points in the purification process of a viral vaccine product.

Spike recovery

The % recovery is calculated as the total HCP measured in the spiked sample divided by the sum of the amount of material spiked plus the contribution from any endogenous HCP at that dilution. Acceptable recovery is defined as $\pm 20\%$ from nominal concentration. Recoveries in samples used in the determination of Dilutional Linearity were all within the acceptable limits, ranging from 80 to 116%.

Abbreviations: LOD, Limit Of Detection; LLOQ, Lower Limit Of Quantitation; ULOQ, Upper Limit Of Quantitation; SD, Standard Deviation; CV, Coefficient of Variation.

Ordering Information

Product Number	Product name	Description	Supplier
G650S	HEK 293 HCP Assay Reagent Set for Gyrolab*	Contains anti-HEK 293 HCP capture and detection reagents and HEK 293 HCP antigen concentrate. Sufficient quantities to generate 96 data points (1 CD).	Order from Cygnus Technologies
P0020667	Gyrolab Bioaffy 1000 HC Assay Toolbox	Contains 1 CD and all reagents and consumables needed to generate 96 data points.	Order from Gyros Protein Technologies
P0020668	Gyrolab Bioaffy 1000 HC Assay Toolbox CD50	Contains 50 CD's and all reagents and consumables needed to generate 4800 data points.	Order from Gyros Protein Technologies
P0020670	Gyrolab HCP Sample Dilution Buffer 25 mL	Extra sample dilution buffer for Gyrolab Bioaffy 1000 HC Assay Toolbox.	Order from Gyros Protein Technologies

*Derived from the same antibodies and antigen used in the Cygnus 3rd Generation HEK 293 HCP ELISA kit, (Cygnus Cat no F650S).

Content

Gyrolab Bioaffy 1000 HC Assay Toolbox

Each toolbox contains buffers and consumables for one (1) or fifty (50) CDs, for generation of 96 or 4800 data points, respectively.

Storage conditions

Gyrolab Bioaffy 1000 HC Assay Toolbox

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

Shelf life (unopened package): see product label

RELATED PRODUCTS

Gyrolab CHO-HCP Kits

For determination of levels of host cell proteins in bioprocess samples expressed in CHO (Chinese hamster ovary) cell lines.

P0020605B	Gyrolab CHO-HCP E3G Kit
P0020606B	Gyrolab CHO-HCP E3G CD50 Kit
P0020246	Gyrolab CHO-HCP Kit 1
P0020424	Gyrolab CHO-HCP Kit 1 CD50

Gyrolab E. coli HCP Kit

For determination of levels of host cell proteins in bioprocess samples from *Escherichia coli* expression systems.

P0020625	Gyrolab E.coli HCP Kit
P0020629	Gyrolab E.coli HCP CD50 Kit
P0020637	Gyrolab E.coli HCP Standard
P0020647	Gyrolab E.coli HCP Sample Dilution Buffer

Gyrolab Protein A Kit

For quantification of leached MabSelect™ SuRe™ during purification processes.

P0020456	Gyrolab Protein A Kit for MabSelect SuRe Ligand (96 datapoints)
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Gyrolab p24 Kit

For quantification of p24 protein in manufacturing of lentiviral vectors.

P0020659	Gyrolab p24 Kit
P0020665	Gyrolab p24 CD50 Kit
P0020664	Gyrolab p24 Standard
P0020674	Gyrolab p24 Sample Dilution Buffer

Gyrolab AAVX Titer Kit

For AAV product titer determination in process development and in the manufacturing of AAV viral vectors.

P0020695	Gyrolab AAVX Titer Kit
P0020697	Gyrolab AAVX Titer CD50 Kit
P0020699	Gyrolab AAVX Titer Kit Sample Dilution Buffer

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