

Sf9 HCP 3G Solution for Gyrolab®

For the detection of Sf9 host cell protein impurities in bioprocess samples

Product Information Sheet

D0048668/A

- Automated workflows – reduced manual operations
- Broad dynamic range – over three logs
- Fast turnaround – 96 data points in 100 minutes
- High throughput – up to 960 data points in a working day



Introduction

The utilization of insect-derived Sf9 cells presents an alternative approach to HEK293 cell line methodology for AAV production. Unlike the plasmid transfection requirement of HEK293 cells, AAV production with Sf9 cells can be initiated through transduction via a baculovirus expression vector (BEV) system. This characteristic renders Sf9 cells more advantageous for large-scale and high-density serum-free suspension culture as compared to HEK293 cells. Additionally, the necessity to monitor host cell protein (HCP) impurity levels persists throughout the purification process and in the final product of AAV-based therapeutics. The imperative to reduce HCPs to the lowest feasible levels stems from their potential immunogenicity, interference with drug efficacy, or impact on drug stability. Consequently, there arises a demand for analytical tools that offer a broad dynamic range, automation, rapid turnaround time, and minimal sample volume. These requirements are essential to cope with the stringent demands on limited manufacturing capacity and small viral vector batches.

The Sf9 HCP 3G Assay Reagent set for Gyrolab®, developed by Cygnus Technologies, is exclusively designed and optimized to be used together with the Gyrolab Bioaffy™ 4000 HC Assay Toolbox. It is derived from the same antibodies and antigen used in the Cygnus Sf9 HCP 3G ELISA kit, Cat# F1040. The reagents are generic in the sense that they are intended to react with essentially all the host cell proteins (HCPs) that could contaminate the product independent of the purification process. The antibodies have been generated in goats and antigen affinity purified using Sf9 HCPs found in protein-free conditioned media. The reagent set has been qualified by Cygnus Technologies and tested by Gyros Protein Technologies to generate high quality results on Gyrolab systems.

Sf9 HCP 3G Solution for Gyrolab increases productivity in bioprocess development:

- Automation generates 96 data points within 100 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

Sf9 HCP 3G Solution for Gyrolab, with Sf9 HCP 3G assay reagent set from Cygnus Technologies, has been developed to quantify Sf9 HCP impurities in bioprocess samples. The sandwich immunoassay is run on Gyrolab Bioaffy 4000 HC CD (Figure 1) and detects a broad spectrum of Sf9 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 Sf9 HCPs are introduced into the microstructures and captured by the immobilized anti-Sf9 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.

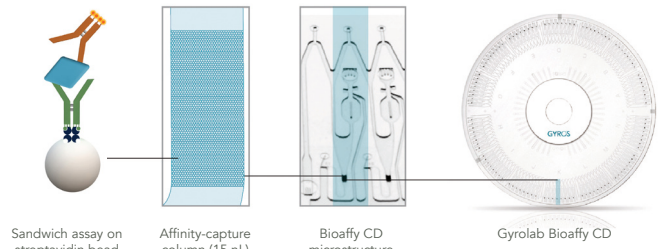


Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy 4000 HC CD.

Assay performance - evaluated by Cygnus Technologies

Broad dynamic range

The Sf9 HCP 3G Assay Reagent Set for Gyrolab (G1040), when used with the Gyrolab Bioaffy 4000 HC Assay Toolbox (P0020852), 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)
~ 0.4	~ 1

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

LLOQ is defined as the lowest concentration for which the CV and nominal value is typically +/-25%. The %CV for 18 replicates of the 1 ng/mL standard was 20% and the nominal recovery was 96%. We experimentally determined the LLOQ as ~1 ng/mL in assay diluent.

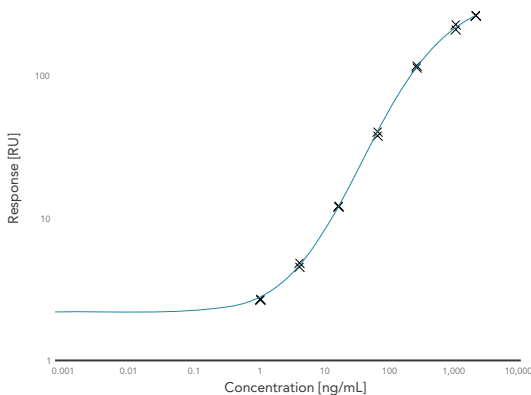


Figure 2. Typical standard curve data from an assay run.

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 QC samples with low (1.25 ng/mL), medium (~50 ng/mL and 500 ng/mL), and high HCP concentrations (~1500 ng/mL).

Table 2. Accuracy and precision data for four QC samples.

Sample Name	Expected conc (ng/mL)	Intra-run CV (%; n=20)	Inter-run CV (%; n=10)
QC1	1.25	12.5	13.6
QC2	50	7.8	4.9
QC3	500	7.1	5.5
QC4	1500	9.6	6.8

* 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 3 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	5000	41	202500	N/A	201150
	10000	19	194000	1	
	20000	9.9	197200	0	
	40000	5.2	206800	1	
	80000	2.4	195200	1	
	160000	1.3	211200	2	
	320000	<LLOQ	<LLOQ	<LLOQ	
	640000	<LLOQ	<LLOQ	<LLOQ	
2	1	4	4	N/A	5.0
	2	3.1	6	11	
	4	1.3	5	5	
	8	<LLOQ	<LLOQ	<LLOQ	
	16	<LLOQ	<LLOQ	<LLOQ	
	32	<LLOQ	<LLOQ	<LLOQ	
	64	<LLOQ	<LLOQ	<LLOQ	
	128	<LLOQ	<LLOQ	<LLOQ	
3	5000	55	272500	N/A	279450
	10000	27	273000	0	
	20000	13	258000	1	
	40000	7.1	282000	2	
	80000	3.6	285600	0	
	160000	1.9	305600	22	
	320000	<LLOQ	<LLOQ	<LLOQ	
	640000	<LLOQ	<LLOQ	<LLOQ	

Comparison with ELISA

The 3 samples were also analyzed using Cygnus Sf9 HCP 3G ELISA, Cat F1040. Sf9 HCP 3G 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.

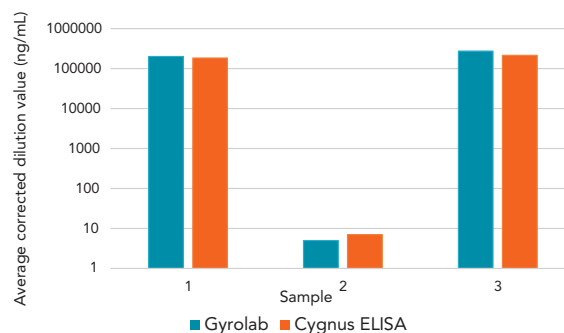


Figure 3. Comparison of results for Sf9 HCP 3G Solution for Gyrolab and Cygnus ELISA kit for samples from various points in the purification process of a viral vaccine product.

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 90-107%.

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

Ordering Information

Product Number	Product name	Description	Supplier
G1040	Sf9 HCP 3G Assay Reagent Set for Gyrolab*	Contains anti-Sf9 HCP capture and detection reagents and Sf9 HCP antigen concentrate. Sufficient quantities to generate 96 data points (1 CD).	Order from Cygnus Technologies
P0020852	Gyrolab Bioaffy 4000 HC Assay Toolbox	Contains 1 CD and all buffers and consumables needed to generate 96 data points.	Order from Gyros Protein Technologies
P0020853	Gyrolab Bioaffy 4000 HC Assay Toolbox CD50	Contains 50 CD's and all buffers 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 Assay Toolboxes.	Order from Gyros Protein Technologies

*Derived from the same antibodies and antigen used in the Cygnus Sf9 HCP ELISA Kit, 3G (F1040).

Content

Gyrolab Bioaffy 4000 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 4000 HC Assay Toolbox

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

Shelf life (unopened package): see product label

Related products

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