E. coli HCP 2G Solution for Gyrolab®

For the detection of *Escherichia coli* host cell protein impurities in recombinant proteins

Product Information Sheet D0043228/A

- Automated workflows – reduced manual operations
- Broad dynamic range – over three logs
- Fast turnaround – 96 data points in 80 minutes
- High throughput – up to 960 data points in a working day

Introduction

Recombinant expression in *Escherichia coli* (*E. coli*) is a relatively simple and cost-effective method for production of therapeutic proteins. Optimizing downstream purification involves reducing the level of host cell protein (HCP) impurities that can induce toxic or immunogenic reactions in patients. HCP levels are therefore a critical quality attribute that must be monitored using analytical tools that detect any critical contaminants with broad dynamic range, automation, and fast turn-around time.

The *E. coli* HCP 2G Assay Reagent Set for Gyrolab, developed and sold by Cygnus technologies, has been exclusively designed and optimized for use together with the Gyrolab Bioaffy™ 1000 HC toolbox on Gyrolab systems. The reagents are intended to react with essentially all the HCPs that could contaminate the product independent of the purification process. This reagent set has been fully tested and validated by Cygnus Technologies to generate high quality results on Gyrolab systems and can be used as a process development tool to monitor the optimal removal of host cell contaminants as well as in routine final product release.

E. coli HCP 2G Solution for Gyrolab increases productivity in bioprocess development:

- Automation generates 96 data points within 80 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

GYROS PROTEIN Technologies

E. coli HCP 2G Solution for Gyrolab

E. coli HCP 2G Solution for Gyrolab, with assay reagent set from Cygnus Technologies, has been developed to quantify *E. coli* HCP impurities in bioprocess samples. The sandwich immunoassay is run on Gyrolab Bioaffy 1000 HC CD (Figure 1) and detects a broad spectrum of *E. coli* 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 *E. coli* HCPs are introduced into the microstructures and captured by the immobilized anti-*E. coli* 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.

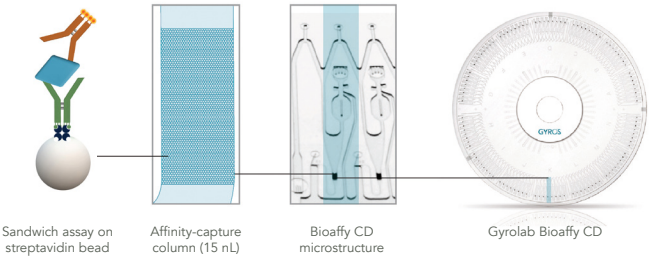

Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy 1000 HC

Assay performance

Broad dynamic range

The *E. coli* HCP 2G Assay Reagent Set for Gyrolab (G1020), when used with the Gyrolab Bioaffy 1000 HC Assay Toolbox, delivers a broad, three-log working range (Table 1, Figure 2) that minimizes the number of dilutions needed to analyze bioprocess samples.

Table 1. Assay working range

LOD (ng/mL)	LLOQ (ng/mL)
~ 3.5	~ 5

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

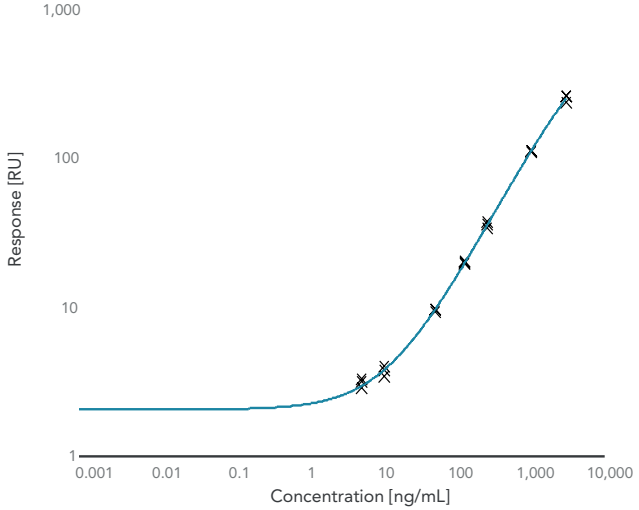

Figure 2. Typical standard curve data from an assay run

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

Precision

Intra- (n=20 replicates) and inter-assay (n=10 assays) precision was determined on four pools with low (~7.2 ng/mL), medium (~75 ng/mL and 750 ng/mL), and high HCP concentrations (~2,253 ng/mL; Table 2).

Table 2. Accuracy and precision data for four QC samples

Concentration (ng/mL)	Intra-assay CV (%) n=20	Inter-assay CV (%)
7.2	17.8	8.4
75	5.2	4.0
740	6.0	3.6
2,253	5.7	4.7

Spike recovery

Recovery was evaluated by spiking 500 ng/mL of the same HCP preparation used to make standards into both in-process samples to their established MRDs. The % recovery was calculated as the total measured HCP value 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 80–120%. Recoveries in samples were all within the acceptable limits, ranging from 90% to 114%.

Dilution linearity

Cygnus Technologies evaluated the dilution linearity for two samples from various points in purification processes for products expressed in *E. coli* cells. Once samples were diluted within the analytical range of the assay both samples demonstrated dilution linearity (Table 3).

Table 4. Dilutional linearity data

Sample	Dilution Factor	Mean value ng/mL	Dilution Corrected Value (ng/mL)	Change from Previous Dilution (%)	Average Dilution Corrected Value (ng/mL)
1	10	76	762	N/A	834
	20	40	804	6	
	40	20	816	1	
	80	12	952	17	
	160	<LLOQ	<LLOQ	N/A	
2	2	133	266	N/A	269
	4	67	269	1	
	8	32	255	5	
	16	17	278	9	
	32	8.6	276	1	
	64	<LLOQ	<LLOQ	N/A	

Comparison with ELISA

Four (4) samples were also analyzed using Cygnus *E. coli* HCP ELISA Kit, 2G (F1020). *E. coli* HCP 2G Solution for Gyrolab and Cygnus ELISA kit produced comparable results (Figure 3).

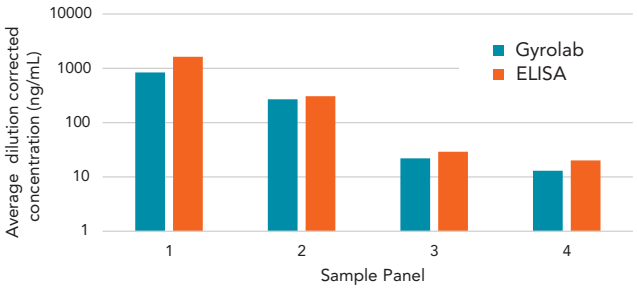

Figure 3. Comparison of Gyrolab assay and ELISA

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.

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
G1020	*E. coli* HCP 2G Assay Reagent Set for Gyrolab	Contains anti-*E. coli* HCP capture and detection reagents and *E. coli* HCP antigen concentrate. Quantities enough to generate 96 data points (1 CD).	Cygnus Technologies
P0020667	Gyrolab Bioaffy 1000 HC Assay Toolbox	Contains 1 CD and all buffers and consumables needed to generate 96 data points.	Gyros Protein Technologies
P0020668	Gyrolab Bioaffy 1000 HC Assay Toolbox CD50	Contains 50 CDs and all buffers and consumables needed to generate 4800 data points.	Gyros Protein Technologies
P0020670	Gyrolab HCP Sample Dilution Buffer 25 mL	Extra sample dilution buffer for Gyrolab Bioaffy 1000 HC Assay Toolbox.	Gyros Protein Technologies

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

Scan the QR code to learn more about our other ready-to-use kits and solutions used for bioprocess analytics:

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