Gyrolab® HEK293 HCP Kit Reagents

For the detection of host cell protein (HCP) impurities from Human Embryonic Kidney cells (HEK293) in bioprocess samples

Product Information Sheet D0051391/A

- Broad coverage of impurities – limits the risk of not detecting important HCPs
- Robust assays – low volume reagent requirements of Gyrolab mean precious antibodies last longer compared to traditional ELISA
- Precise and accurate results – Enhanced generic 360-HCP approach with Gyrolab automated pipetting reduces repeats
- Automates HCP detection – 10 minutes hands-on time and results in 90 minute run time

Introduction

HEK293 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 the 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.

Gyros Protein Technologies has partnered with BioGenes GmbH to develop the Gyrolab® HEK293 HCP Kit Reagents, specific for the sensitive detection of residual HEK293 HCPs. The antibodies were developed by using either the supernatant (SN) or the cell lysate (CL) derived from HEK293 mock fermentation as antigen fractions for antibody generation in rabbits and utilized an optimized purification strategy. These antibodies are considered generic for the HCPs that could potentially co-purify with drug product with the coverage assessed by 2D DIGE analysis.

Gyrolab HEK293 HCP assays increase productivity in bioprocess development and manufacturing:

- Automated Gyrolab assay generates 96 data points within 90 minutes without manual intervention, reducing risk of variability due to errors and speeding up workflows
- Broad dynamic range minimizes sample dilutions and assay repeats
- Matrix insensitivity minimizes interference and ensures robust analysis of all types of bioprocess samples
- Short turnaround time accelerates data-driven decision making

Process specific reagents for optimal performance

The Gyrolab HEK293 HCP Kit Reagents are available in two different types; HEK293 HCP Type SN (Supernatant) and HEK293 HCP Type CL (Cell Lysate). This allows selection of the kit most appropriate for the specific process.

- The antibodies in the Gyrolab HEK293 HCP Type SN Kit Reagents provide ≈74% coverage of HEK293 HCP antigen as determined by 2D DIGE analysis
- The antibodies in the Gyrolab HEK293 HCP Type CL Kit Reagents provide ≈85% coverage of HEK293 antigen as determined by 2D DIGE analysis

GYROS PROTEIN Technologies

The Gyrolab solution

Gyrolab HEK293 HCP Kit Reagents have been developed to quantify HEK293 impurities in bioprocess samples. The sandwich immunoassay is run on Gyrolab Bioaffy 4000 HC microfluidic disc (Figure 1) using reagents from BioGenes GmbH and detects a broad spectrum of HEK293 HCPs.

The biotinylated anti-HCP antibody is automatically introduced into a microstructure in the Gyrolab Bioaffy microfluidic disc and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing HEK293 HCPs are introduced into the microstructures and captured by the immobilized α-HEK293 HCP antibody. Bound HCP is then detected using an anti-HCP antibody labeled with Alexa Fluor™647. Results are evaluated using Gyrolab Evaluator software, 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.

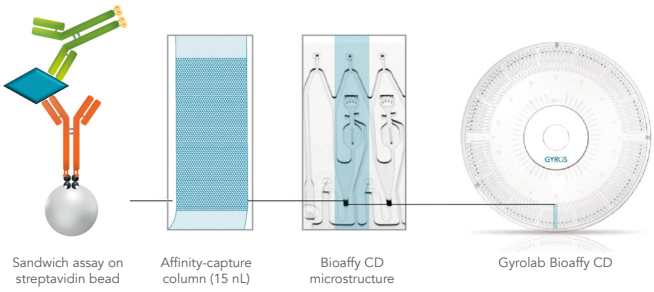

Figure 1. Sandwich Immunoassay **format on a Gyrolab Bioaffy 4000 HC disc.**

Assay performance data

Broad dynamic range

The performance of the Gyrolab HEK293 HCP Type SN Kit Reagents and Gyrolab HEK293 HCP Type CL Kit Reagents have been assessed using the Gyrolab Bioaffy 4000 HC Assay Toolbox (P0020852) and demonstrate a broad working range (Table 1). This minimizes the number of dilutions needed to analyze bioprocess samples from all stages of the purification process.

Table 1. Gyrolab HEK293 HCP Kit Reagents working range.

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
<2	~ 3	~ 1,500

The limit of detection (LOD) was determined as 2 SD above the blank.

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) concentration with CV <25%, Relative Error <25% and Total Error (absolute %RE + %CV) ≤40% were assigned as LLOQ and ULOQ, respectively.

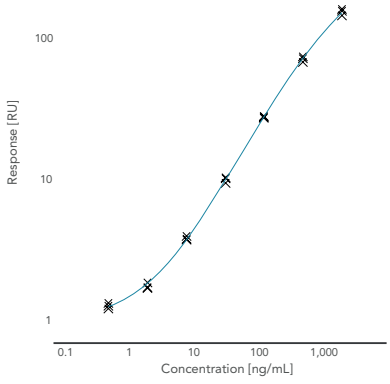

Figure 2. A typical example of a HCP standard curve from a Gyrolab run using the Gyrolab HEK293 HCP Type SN Kit Reagents. Each standard sample was analyzed in triplicate.

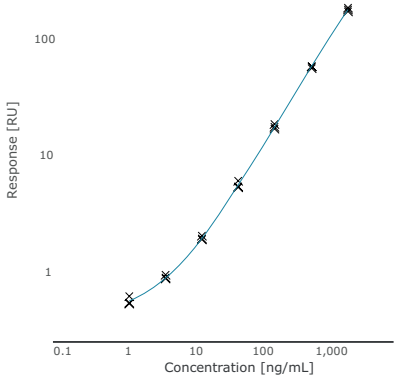

Figure 3. A typical example of a HCP standard curve from a Gyrolab run using the Gyrolab HEK293 HCP Type CL Kit Reagents. Each standard sample was analyzed in triplicate.

Accuracy and precision

Data for standard curves are shown in Figure 2 and Figure 3. Intra- and inter-run precision were determined for three QC samples with different concentrations of HEK293 HCP and are summarized in Table 2 and Table 3.

Table 2. Data from Gyrolab HEK293 HCP Type SN Kit Reagents. Intra- and inter-run precision data for QC samples covering the assay working range. The QC samples were run in triplicate in six separate runs on four different instruments by two operators.

Sample	Nominal conc. (ng/mL)	Intra-run CV	Inter-run CV (%; n=6)
ULOQ	1,500	4.4 – 11.5	8.1%
MQC	50	1.3 – 10.0	8.3%
LLOQ	3	4.4 – 20.8	16.4%

Table 3. Data from Gyrolab HEK293 HCP Type CL Kit Reagents. Intra- and inter-run precision data for QC samples covering the assay working range. The QC samples were run in triplicate in six separate runs on four different instruments by two operators.

Sample	Nominal conc. (ng/mL)	Intra-run CV	Inter-run CV (%; n=6)
ULOQ	1,500	4.1 – 10.5	7.8%
MQC	30	2.5 – 9.7	11.4%
LLOQ	3	2.9 – 22.2	24.7%

Ordering Information

Product Number	Product name	Description
P0021075	Gyrolab HEK293 HCP Type CL Kit Reagents	Includes capture reagent, detect reagent, and stock standard solution for 1 full disc run
P0021076	Gyrolab HEK293 HCP Type CL Standard	Stock standard solution (200 µL, 2000 ng/mL)
P0021078	Gyrolab HEK293 HCP Type SN Kit Reagents	Includes capture reagent, detect reagent, and stock standard solution for 1 full disc run
P0021079	Gyrolab HEK293 HCP Type SN Standard	Stock standard solution (200 µL, 2000 ng/mL)
P0020852	Gyrolab Bioaffy 4000 HC Assay Toolbox	Gyrolab Bioaffy 4000 HC, wash buffers, HCP sample dilution buffer, plates, foils
P0020670	Gyrolab HCP Sample Dilution Buffer	Sample dilution buffer (25 mL)

Type SN (Supernatant); Type CL (Cell Lysate)

Storage conditions

Gyrolab HEK293 HCP Kit Reagents and **Gyrolab HEK293 HCP Standard** are shipped refrigerated.

Storage of Kit Reagents and Standard at ≤ -70°C

Related products

Scan the QR-code to learn more about our other ready-to-use kits for bioprocess impurity and titer analysis.