Pharma&Biotech

Lonza

Development of residual host cell protein assays for recombinant microbial biopharmaceuticals

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Introduction

Lonza is a global custom manufacturing company serving the needs of the life-science industry. Today biotechnology plays a key role in Lonza's success, enabling the production of biopharmaceuticals, nutraceuticals, agrochemicals and APIs.

The consistency and efficiency of a biopharmaceutical purification process determines drug quality, including which concentrations and specific types of residual host cell or process contaminants may remain. Host cell proteins (HCPs) are a complex mixture of proteins with significantly diverse molecular and immunological properties. Analyses of crude samples from biotechnology processes are often required in order to demonstrate that residual host cell impurities are eliminated or reduced during purification. At last stages of development, as the processes are finalized, HCP content should be in a defined range at low levels.

At Lonza the HCP assays are used to detect *E.coli* impurities. The commercially available Cygnus HCP ELISA kit (#F410) is commonly applied for this purpose. Here, we compare HCP assays performed by Gyrolab® and the commercial Cygnus ELISA kit. Furthermore, we analyze In Process Control (IPC) HCP samples by using Gyrolab® with a HCP (strain) specific antibody.

How sensitive are Cygnus ELISA reagents on Gyrolab®

To determine the HCP content of a drug substance sample, Cygnus HCP antibodies (#AP117) were labeled with biotin (capture) and Alexa Fluor (detection). Subsequently, the optimal concentration of detection antibody (25 nM) was determined (data not shown). The linear range of a standard curve was assessed (figure 1).

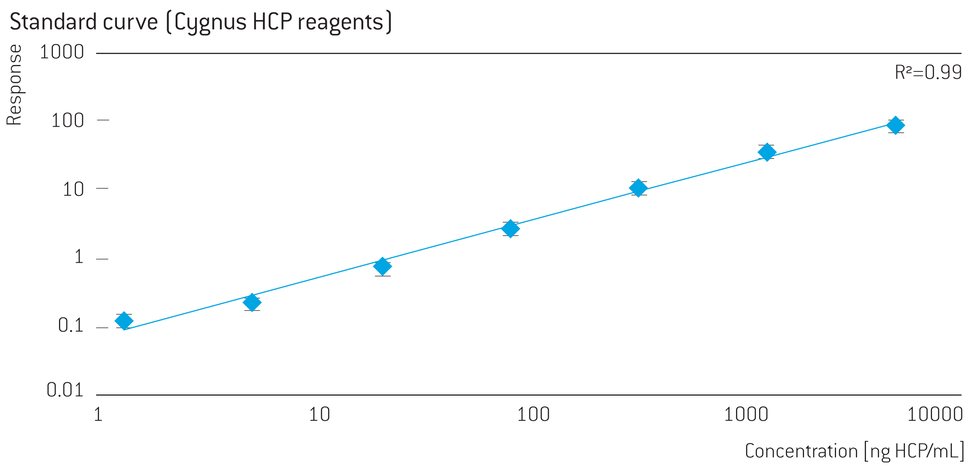

Figure 1: HCP Standard curve (Cygnus HCP reagents) based on three independent experiments
HCP concentration [ng/mL] is logarithmically plotted versus response values. Error bars show the standard deviation of each threefold determination.

The Linear range was determined from 2 – 5'000 ng HCP/mL (R2=0.99). Acceptance criteria for the individual values was the S/B ratio (Signal to Background ratio) of ≥ 2.

A traditional Cygnus ELISA vs. Gyrolab®

A bulk drug substance sample was measured in threefold triplicates (9 values) in three independent experiments on Gyrolab®. Spike recovery (acceptance criteria: 75-125%) was determined to assess matrix effects.

Table 1: HCP content of bulk drug substance (final stage of process) analysed by Gyrolab®

HCP content [ng/mL]	Spike recovery [100%]	Valid dilution factors
41.9	85 – 87	2; 4; 8
52.6	75 – 84	*4; 8
52.4	80 – 81	2; 4; 8
	Mean: 49.0 ng/mL (RSD 12.5%)	

* in one experiment 2 triplicates of the 1:2 dilution were out of the standard curve and were defined as outliers.

Comparing the results of a conventional Cygnus kit ELISA (data not shown) and Gyrolab® data show similar HCP levels.

Table2: Comparison of traditional ELISA and Gyrolab® data

	Traditional ELISA	Gyrolab® approach
Sensitivity	3 ng HCP/mL	2 ng HCP/mL
Linear range	3 – 100 ng HCP/mL	2 – 5'000 ng/mL
Dilution factors	13.2; 17.6	2, 4, 8
RSD	11.4%	12.5%
HCP conc. (mean)	59 ng/mL	49 ng/mL

For the traditional ELISA required dilutions are slightly higher than for Gyrolab® due to potential matrix effects. However, HCP levels obtained from Gyrolab® and ELISA differ about 20 %. These results are comparable with each other because this is within the ELISA inherent variation range (typically ≤ 35 %).

Sensitivity with strain specific HCP antobodies on Gyrolab®

Alternatively, HCP specific antibodies could be used to avoid insufficient binding of commercially generated HCP antibodies or product-HCP antibody interaction. Here we tested a strain specific HCP antibody system, labeled with biotin and Alex Fluor, respectively.

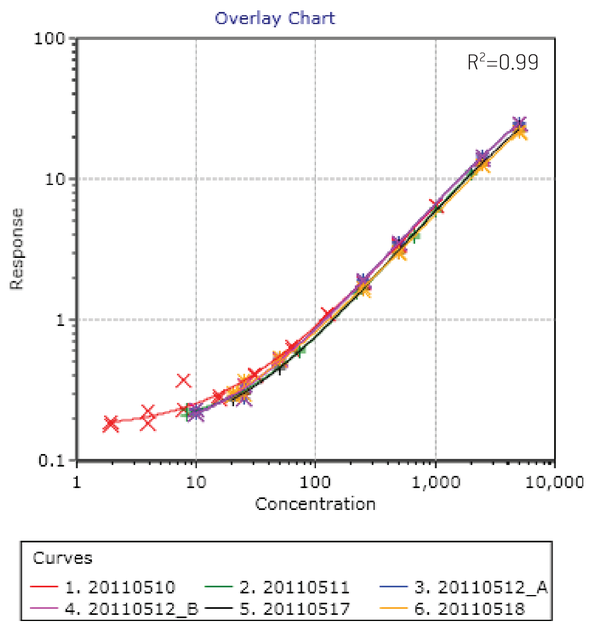

Figure 2: HCP-Standard curve with strain specific antibodies
HCP concentration in ng/mL is plotted logarithmically
versus response values, curves resulted out of six runs with different HCP concentrations. At a concentration of 25 ng/mL or higher the S/B ratio was above 2, the dynamic range was set from 25 to 5'000 ng/mL.

Different ranges of HCP concentrations were tested to define the linear range and reproducibility was determined over 6 completely independent runs (figure 2).

Results while using HCP strain specific antibodies

Currently, only IPC samples were tested (process development still ongoing). Reproducibility of the standard curve is high and the linear range was set to 25-5'000 ng/mL (see above). A traditional ELISA with the HCP strain specific antibodies shows a linear range from 0.1 to 32 ng/mL (data not shown). Subsequently, very high sample dilutions (up to 1:25'600) were necessary, which ultimately lead to higher calculation errors.

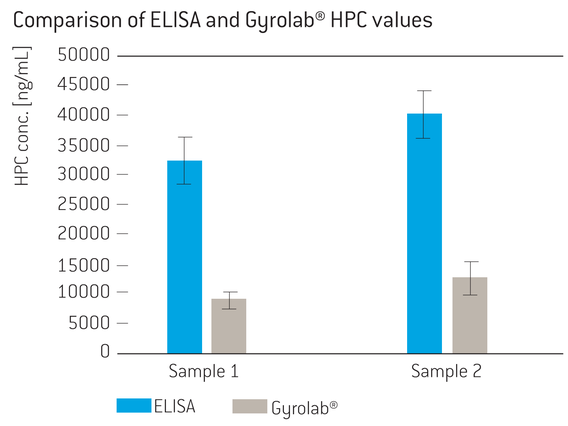

Figure 3: HCP concentration determination of In-Process samples by ELISA and Gyrolab
Samples were measured with HCP ELISA (two runs) and Gyrolab® (three runs) by using strain specific antibodies.

Consequently, the large dynamic range of the Gyrolab® system enables low samples dilutions, i.e. dilutions of 1:20 were sufficient. Additionally, the spike recovery was tested 78-85 % (data not shown)

Gyrolab® – Pros and thought-provoking topics

- Large dynamic range for HCP determination
- Lower sample dilution is required (calculation error is lower)
- Fast sample turnaround
- High throughput application
- Intuitive handling of software tools
- Professional support – fast/helpful answers

- Labeled reagents must be qualified after each new labeling – long term storage?
- Outliers of replicates are commonly observed (i.e. during second round of CD usage)
- %RSD in sample triplicates differs if more than one CD is used
- Sample preparation is time intensive without e.g. a pipetting robot