BioGenes

Development of a Generic HCP ELISA for the Measurement of Impurities Derived from the HEK293 Expression System

HEK293 | 360-HCP ELISA Kits (Types SN and CL)

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HEK293 (human embryonic kidney 293) cell lines offer a particularly beneficial expression system for the manufacturing of therapeutic recombinant proteinsor viral vectors[1, 2]. The ability to perform human-like posttranslational modifications of proteins and high transfection efficiencies make them an excellent choice for vaccine development or cell and gene therapy[3, 4].
In consistence with our multi-kit approach, we developed two kit types for the detection of HEK293-HCPs: Type SN and Type CL. The two kit types are based on either the supernatant (SN) or the cell lysate (CL) derived from HEK293 mock fermentation which served as antigen preparations for antibody development. Polyclonal antibodies were generated by immunization of rabbits followed by affinity purification using the respective HEK293-HCP mock material. The antigen samples and HCP antibodies were comprehensively characterized as part of this study.

Table 1: Sample details

Sample ID	Description
HEK293-HCP (SN)	Mock HCP from HEK293 cell line from cell culture supernatant
HEK293-HCP (CL)	Mock HCP from HEK293 cell line from cell lysate
Antiserum pool (SN)	Antiserum pool of rabbits immunized with HEK293-HCP (SN), supplemented with LMW HCP
Antiserum pool (CL)	Antiserum pool of rabbits immunized with HEK293-HCP (CL), supplemented with LMW HCP
HCP antibody (SN)	Rabbit antibody prepared by purification of antiserum pool (SN) using immobilized HEK293-HCP (SN)
HCP antibody (CL)	Rabbit antibody prepared by purification of antiserum pool (CL) using immobilized HEK293-HCP (CL)

Antibody generation, purification and modification

HEK293-HCP-specific antisera were raised by immunization of rabbits with HEK293-HCP (SN or CL). An additional group was immunized using a suitable low molecular weight (LMW) HCP fraction mainly containing small proteins (<50 kDa) to improve immune response towards small proteins which are generally less immunogenic. The individual rabbit antisera were pooled and the LMW HCP antiserum pool was added proportionally to both antiserum pools (SN and CL). These final antiserum pools were each affinity-purified using the homologous antigen fraction as affinity matrix to produce the HCP specific capture antibodies (SN and CL) in large-scale. Subsequently, a part of both capture antibody preparations was conjugated to biotin to prepare suitable detector antibodies for the HCP ELISA developed according to the common sandwich setup.

Antibody testing by 1D western blotting

The performance of both antibody preparations was qualitatively analyzed by 1D Western blotting. HEK293-HCP (SN and CL) was each subjected to electrophoresis and subsequently transferred onto nitrocellulose membranes. HCP-specific immunostaining was carried out using the purified HCP antibodies each in comparison to their corresponding antiserum pool (Figures 1 and 2). Very similar immunostaining patterns were observed in both cases when comparing each HCP antibody with the corresponding antiserum pool. A complex band pattern over the entire molecular weight range was obtained for the SN-specific antiserum and antibodies, respectively. In contrast, rather distinct signals at different molecular weights were visible for the respective CL-specific samples.

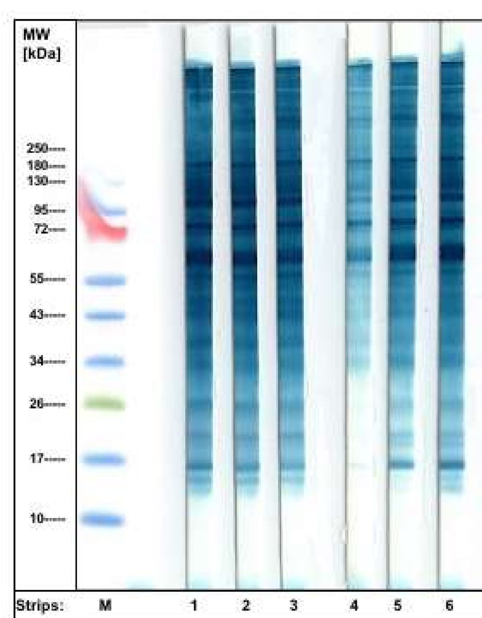

Figure 1: Western blot of HEK293-HCP (SN) immunostained with antiserum pool (SN) and HCP antibody (SN)
The molecular weight (MW) is indicated on the left (pre-stained protein standard, M). Protein amount of HCP per strip: 5 µg each; Antiserum dilutions of 1:125, 1:250, 1:500 (strips 1-3); Antibody concentrations of 1 µg/mL, 5 µg/mL, 10 µg/mL (strips 4-6). Signal detection was enabled a suitable anti Rabbit IgG Peroxidase conjugate followed by incubation with a colorimetric substrate.

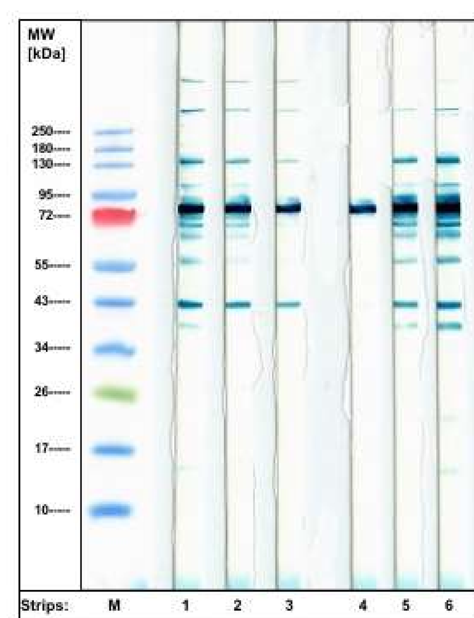

Figure 2: Western blot of HEK293-HCP (CL) immunostained with antiserum pool (CL) and HCP antibody (CL)
The molecular weight (MW) is indicated on the left (pre-stained protein standard, M). Protein amount of HCP per strip: 5 µg each; Antiserum dilutions of 1:125, 1:250, 1:500 (strips 1-3); Antibody concentrations of 1 µg/mL, 5 µg/mL, 10 µg/mL (strips 4-6). Signal detection was enabled a suitable anti Rabbit IgG Peroxidase conjugate followed by incubation with a colorimetric substrate.

HCP ELISA development

When setting up the HEK293|360-HCP ELISA Kit method for both variants (SN and CL) the following parameters were optimized: capture antibody concentration; detector antibody concentration; antigen incubation time; detector antibody incubation time. Subsequently, both generic ELISA kits were qualified, and the results obtained are presented in Table 2. Exemplaric standard curves are shown in Figure 3 and 4.

Table 2: Collective pre-validation results of HEK293 | 360-HCP ELISA Kit (SN and CL)

Pre-validation parameters	Results SN	Results CL
Accuracy (HCP recovery)	range: 81.9 – 97.0%	range: 81.4 – 88.0%
Repeatability (intra-assay precision)	CVs: 1.0 – 3.0%	CVs: 2.1 – 7.9%
Intermediate precision (inter-assay precision)	CVs: 1.1 – 2.7%	CVs: 1.7 – 4.0%
Limit of detection (LOD)	0.340 ng/mL	0.801 ng/mL
Limit of quantitation (LOQ)	1.021 ng/mL	2.404 ng/mL
Working Range	3.125 – 100 ng/mL	

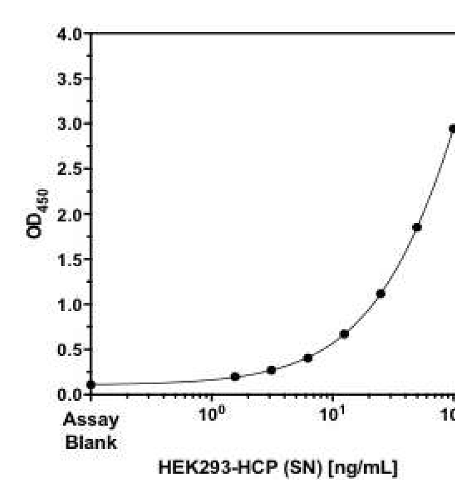

Figure 3: Standard curve of the optimized HEK293 | 360-HCP ELISA Kit (SN)
Each standard concentration was analyzed with 6 replicates.

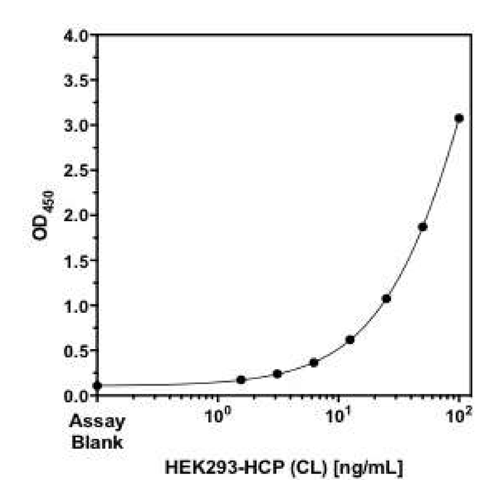

Figure 4: Standard curve of the optimized HEK293 | 360-HCP ELISA Kit (CL)
Each standard concentration was analyzed with 6 replicates.

Coverage analysis

HCP antibody coverage was assessed by Immunoaffinity Chromatography (IAC) followed by 2D difference gel electrophoresis (2D DIGE).
The different HCP antibodies (SN or CL) were immobilized on an affinity column and incubated with associated HEK293-HCP (SN or CL). Bound HCPs were eluted and comparatively analyzed with the corresponding HEK293-HCP (SN or CL) input sample by 2D DIGE. The results are presented in Figure 5a and Figure 5b.

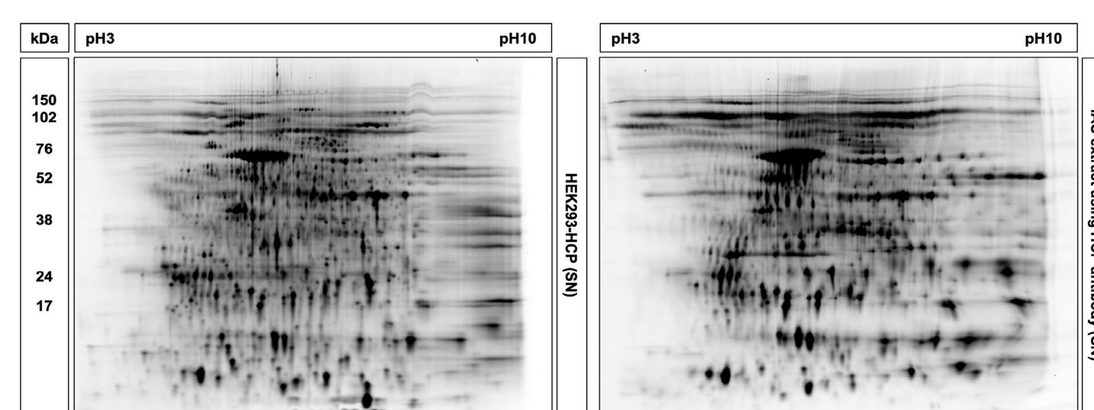

Figure 5a

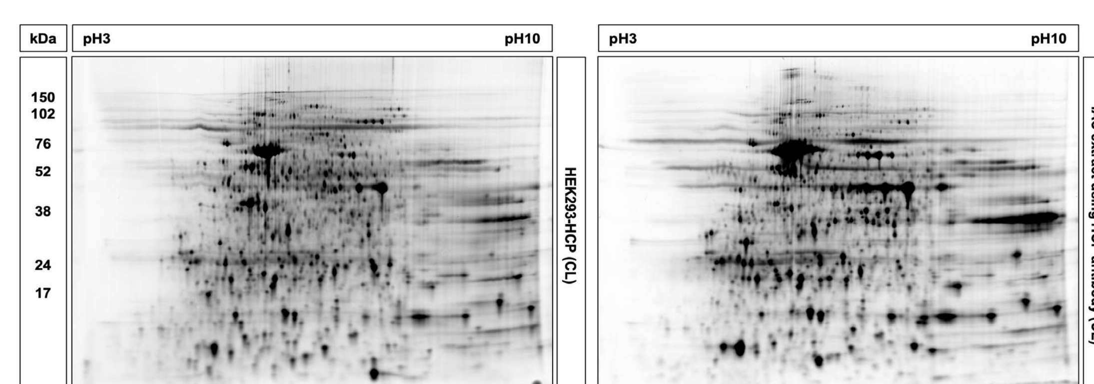

Figure 5b

Figure 5a/b: IAC was performed using immobilized HCP antibodies (SN or CL) and the associated HEK293-HCP samples (SN or CL)
The HEK293-HCP input samples and the corresponding IAC extracts were subjected to fluorescent minimal labeling and 2D DIGE for qualitative comparative analysis.

2D DIGE revealed highly comparable HCP patterns from a qualitative point of view when analyzing the HEK293-HCP input samples and the respective IAC extracts. The results indicate successful coverage of a very broad HCP spectrum from the HEK293-HCP samples for both HCP antibodies tested. Detailed spot-wise evaluation of IAC/2D DIGE data sets was performed with the help of Delta2D (DECODON) analysis software. The collective results are presented in Table 3.

Table 3: Collective coverage results from IAC/2D DIGE

HCP antibody	IAC/2D DIGE Coverage
SN	74%
CL	85%

Summary
Two high performance specific HEK293-HCP ELISA Kits (Type SN and Type CL) with a pre-validated working rangeof 3.125 – 100 ng/mL were developed. The immune-adsorbed HCP-specific antibodies yielded HCP coverages of 74% (Type SN) and 85% (Type CL) in IAC/2D DIGE analyses. The development of the two different ELISA types resulted in a great tool to monitor HCP impurities and their optimal removal during process improvement.

Literature:
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