BioGenes ASSAYS

CHO | 360-HCP ELISA

Development of an Enhanced Generic HCP Assay in CHO Cells

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Host cell protein (HCP) analysis is a big issue when it comes to early clinical testing, design of downstream processes and quality control of biopharmaceuticals. The decision of using an off-the shelf generic HCP assay or spending a considerable amount of money for the development of a specific HCP assay is often not an easy one. In theory, generic HCP assays should be suitable for all HCP determinations of a specific cell line – independent of cell line modifications, fermentation conditions and the design of the purification process. But in most cases generic HCP assays do not show the desired specificity and sensitivity. The new CHO|360-HCP ELISA could be a valuable alternative to currently used HCP assays as it is designed to cover a broader spectrum of CHO-HCPs

Antibody and Assay Development for CHO|360-HCP ELISA

Polyclonal HCP antiserum was generated by immunizing rabbits and goats with equal amounts of HCP derived from mock transfected CHO K1 and CHO S cells. We used differently prepared antigens: total HCP or fractionated HCP (table 1). Each HCP fraction (low molecular weight, middle molecular weight and high molecular weight) was used individually for immunization.

Monospecific polyclonal antibodies were obtained by using an optimized purification strategy against total HCP. Antisera of the different HCP fractions were pooled before purification. This results in a panel of four different HCP assays (Type A to D) that together build up the enhanced generic CHO|360-HCP ELISA kit (see table 1). For all four assay types the lower limits of detection (LOD) is between 0.5–1 ng/mL and the lower limits of quantification (LOQ) is 2–3 ng/mL with a working range between 2–100 ng/mL.

Assay Type	Animal Species	Antigen
Type A	*Goat*	*Total HCP*
Type B	*Goat*	*Fractionated HCP*
Type C	*Rabbit*	*Total HCP*
Type D	*Rabbit*	*Fractionated HCP*

Table 1: *The four assay types of generic CHO|360-HCP ELISA*

Assay specificity and HCP Recovery

The high specificity for the antigen in all four assays was determined by 2-dimensional (2 D) gel electrophoresis of the CHO-HCP standard and immunoblotting with the respective antibodies of the corresponding ELISAs type A – D (figure 1). As reference the 2-D protein pattern of the HCP was transferred to nitrocellulose membrane and stained with colloidal gold (see figure 2b)

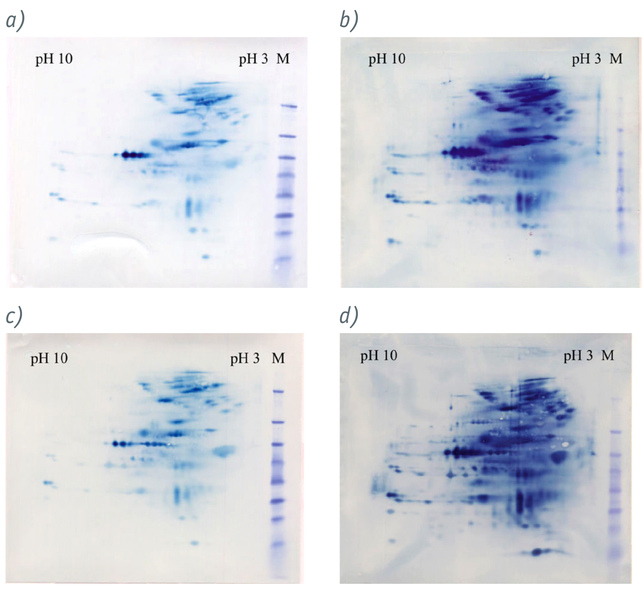

Figure 1a – d: *2-D gel electrophoresis of the CHO-HCP standard after immunoblotting with the detector antibodies type A – D.*

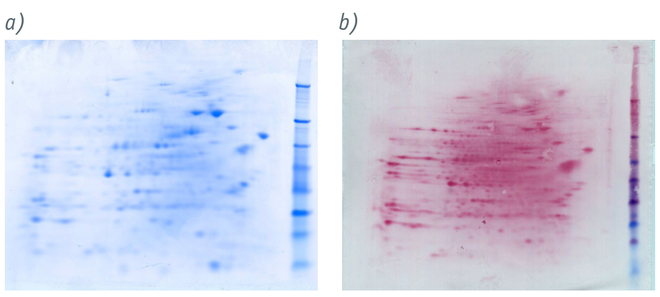

Figure 2a – b: *show the 2-D electrophoresis of the CHO-HCP standard with M indicating the molecular weight marker. 2a shows the Coomassie staining of the CHO-HCP standard after 2-D electrophoresis. 2b shows the colloidal gold staining of CHO HCP standard after blotting to a nitrocellulose membrane.*

HCP Determination in Different Mock HCP Samples and In-process Controls

The CHO|360-HCP ELISA has been widely tested on the basis of a great number of mock CHO-HCP samples. All samples originate from mock fermentations of CHO cells corresponding to production processes of certain biologicals. For each HCP sample the protein amount was determined by Bradford first. Additionally, each sample was analysed using five different CHO-HCP assays: the BioGenes generic CHO|360-HCP ELISAs type A – D and a commonly used, commercially available, generic CHO-HCP assay (figure 3). The protein recovery was calculated in percentage of the Bradford value determined for the sample.

The recovery for each sample depends strongly on the assay used (figure 3). In case of sample 4 (x) a recovery of > 90% was estimated using assay type D. The recovery with assay types A – C was much lower and the recovery with the commercial assay was only 20%. In most cases the best recovery was estimated with one of our four generic CHO|360-HCP ELISAs (type A – D). Recoveries higher than 100% are based overestimation in the respective ELISA.

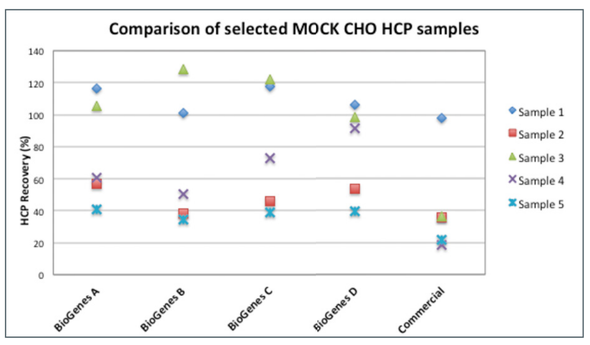

Figure 3: *Comparison of selected mock CHO-HCP samples. Each sample was analysed using five different CHO-HCP assays.*

Estimation of Protein Recovery in Sample 4

In order to estimate the protein recovery in sample 4, Coomassie staining and 2-D immunoblotting using the detector antibodies type A – D were used. Although all four blots show differences in the protein pattern illustrated by red circles, the comparison shows a good protein coverage (figure 4).

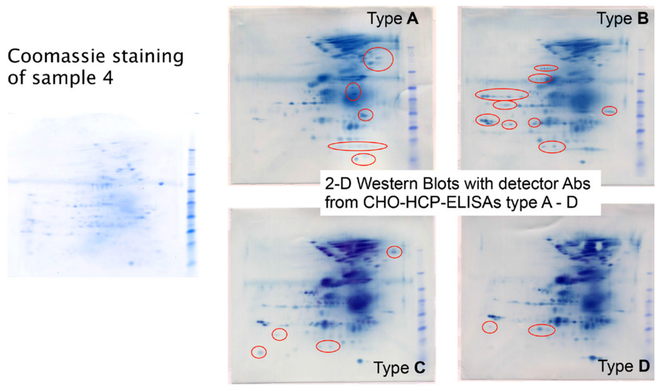

Figure 4: *Comparison of Coomassie staining and immunoblotting with detector antibodies type A – D of sample 4.*

Decreasing HCP Concentrations Within Different ICPs and DSPs

To show the ability of the CHO|360-HCP ELISA to cover a broad spectrum of CHO-HCP, in-process controls (ICP) from three downstream production processes of certain biological were investigated. Table 2 shows that with all four ELISA types A – D HCP concentrations decreased during purification.

	CHO|360-HCP ELISA			
	Type A	Type B	Type C	Type D
IPC	*c [µg/ml]*	*c [µg/ml]*	*c [µg/ml]*	*c [µg/ml]*
1-a	*283,77*	*189,84*	*290,37*	*343,23*
1-b	*6,58*	*3,12*	*2,30*	*2,26*
1-c	*0,04*	*0,03*	*0,035*	*0,106*
2-a	*265,16*	*176,51*	*236,44*	*286,87*
2-b	*4,40*	*2,08*	*1,57*	*1,57*
2-c	*0,03*	*0,11*	*0,006*	*0,019*
3-a	*846,18*	*518,10*	*504,90*	*540,52*
3-b	*149,99*	*47,86*	*97,23*	*108,34*
3-c	*5,77*	*4,50*	*5,22*	*5,73*
3-d	*1,51*	*1,06*	*1,00*	*0,97*

Table 2: *HCP concentrations determined by CHO|360-HCP ELISA type A – D within different ICPs corresponding to three different DPSs (1 – 3). Following IPCs correspond to one downstream process: DSP 1) 1-a, 1-b, 1-c; DSP 2) 2-a, 2-b, 2-c; DSP 3) 3-a, 3-b, 3-c, 3-d*

→ Conlusion

Based on the data shown above we conclude that there is not the one generic HCP assay suitable for all samples. The most suitable antibodies can be identified by testing the mock CHO-HCP of the respective process together with a number of in-process controls using the four ELISAs of CHO|360-HCP ELISAs (type A – D) and concomitant 2-D analysis. This 'feasibility test' does greatly increase the HCP recovery for most samples.

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