BioGenes ASSAYS

E.coli|360-HCP ELISA

Development of an Enhanced Generic HCP Assay for the Determination of HCP from E.coli Cell Lines

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E.coli is widely used for the manufacturing of biopharmaceuticals and the analysis of residual HCPs (Host Cell Protein) is a big issue when it comes to clinical testing, design of downstream processes and finally the quality control of biopharmaceuticals. The HCPs of E.coli vary with the sub cell line used and also cell line modifications and fermentation conditions have an impact on the HCP pattern. A 'one for all assay' does not sufficiently represent this diversity in a suitable manner, which is one of the reasons why generic HCP assays often do not show the desired specificity and sensitivity.

The new E.coli|360-HCP assay could be a valuable alternative to currently used generic HCP assays as it is designed to cover a broader spectrum of E.coli HCPs. This offers the possibility to postpone the development of a time-consuming specific HCP assay until a more informed decision on the success of a biologic in development can be made.

Antibody and Assay Development

E.coli|360-HCP assay is based on W3110 and BL21 (DE3) cell lines which were fermented in two different culture media and conditions resulting in four antigen preparations with distinct HCP patterns (figure 1).

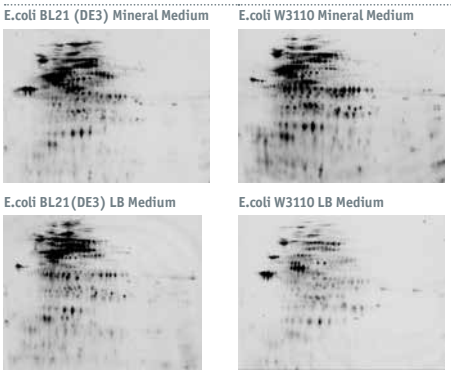

Figure 1: 2D Western Blots of Cy5-labeled E.coli HCP antigens fermented in Mineral Medium and LB Medium.

By immunizing goats with these antigens, a panel of four different HCP ELISA kits (types A to D) were developed which together build up the enhanced generic E.coli|360-HCP ELISA (table 1).

Mineral Medium	LB Medium
Type A: BL21 (DE3)	**Type D:** BL21 (DE3)
Type B: W3110	**Type C:** W3110

Table 1: E.coli|360-HCP ELISA consists of four kit types, based on different E.coli sub cell lines and culture media.

Sensitivity of E.coli|360-HCP ELISA kits (type A to D)

For all four assay types the lower limit of detection (LOD) is between 0.2–0.5 ng/mL and the lower limit of quantification (LOQ) is 0.6–1.6 ng/mL with a working range between 2–100 ng/mL (figure 2).

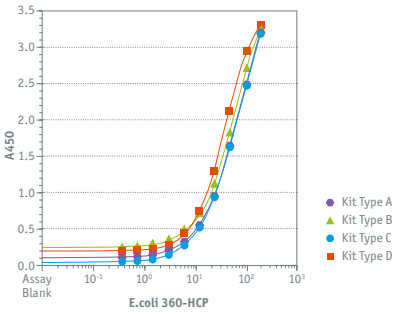

Figure 2: Standard curves for all four E.coli|360-HCP Elisa kit types were obtained by non-linear regression of measured OD values (A450) for different E.coli HCP standard concentrations.

Specificity and HCP Recovery

The E.coli|360-HCP ELISA antibodies (type A to D) have a high specificity for the E.coli HCP antigen. The specificity was measured by a 2D DIGE Western Blot with Cy5-labeled E.coli HCP standard and the corresponding anti-HCP antibodies (detected with anti-goat-IgG-Cy3 conjugate) (figure 3) and is illustrated as coverage in percent (table 2).

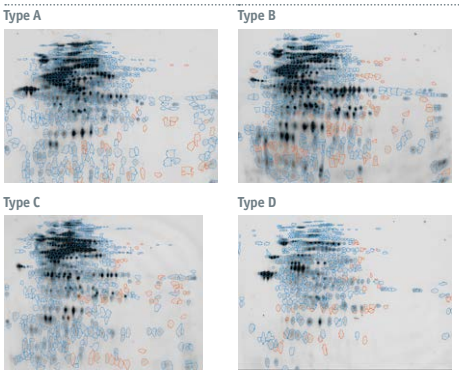

Figure 3: 2D DIGE Western Blots and overlay scans of Cy5-labeled E.coli HCP standards (A to D) and corresponding anti-E.coli-HCP antibodies (Cy3 detection framed in blue). Anti-E.coli-HCP antibodies type A detect 662 spots from 696 spots in total (95.1%), anti-E.coli-HCP antibodies type B detect 637 spots from 717 spots in total (81.1%), anti-E.coli-HCP antibodies type C detect 656 spots from 713 spots in total (92.0%) and anti-E.coli-HCP antibodies type D detect 481 spots from 524 spots in total (91.8%) as determined by ImageMaster™ 2D Platinum 7.0 (GE Healthcare).

E.coli|360-HCP Antibodies	Coverage
Type A	95,1%
Type B	81,1%
Type C	92,0%
Type D	91,8%

Table 2: Evaluation of HCP coverage of E.coli|360-HCP antibodies (type A to D).

HCP Determination in Different Mock HCP Samples

The E.coli|360-HCP ELISA has been widely tested on the basis of a great number of mock E.coli HCP samples. All samples originate from mock fermentations of E.coli 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 E.coli HCP ELISAs: the four enhanced generic E.coli|360-HCP kits A to D and a commonly used, commercially available competitor assay (figure 4). The protein recovery was calculated in percentage of the Bradford value determined for the sample.

The recovery for each sample depends strongly on the ELISA kit used (figure 4). In case of sample 1, a recovery of 80% was estimated using kit type B. For samples 2 the best recovery was determined using kit type A, using kit type B and C results in a recovery of 63% and 64%, respectively. With kit type D a recovery of only 6% was estimated. For sample 5, a sufficient recovery was only determined using kit type D, recovery rates determined with the other three kit types were below 30%. The competitor kit shows recoveries below 30% for all samples. Recoveries higher than 100% are based on overestimation.

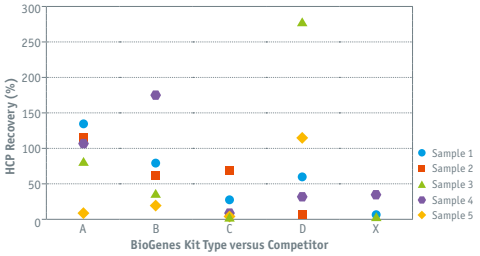

Figure 4: Comparison of selected mock E.coli HCP samples. Each sample was analysed using five different E.coli HCP ELISA kits.

Estimation of Protein Coverage in Sample 2

Since the information about the recovery alone is not sufficient for a qualified decision for the best suiting kit or antibodies alternative methods should be consulted. Since sample 2 showed almost similar recoveries using kit type B and C but almost no recovery using type D this sample was further investigated.

In order to estimate the protein coverage in sample 2 (E.coli mock HCP), 2D DIGE Western Blots using Cy5-labeled sample 2 and the four detector antibodies type A to D (detected with anti-goat-IgG-Cy3 conjugate) were performed. (figure 5).

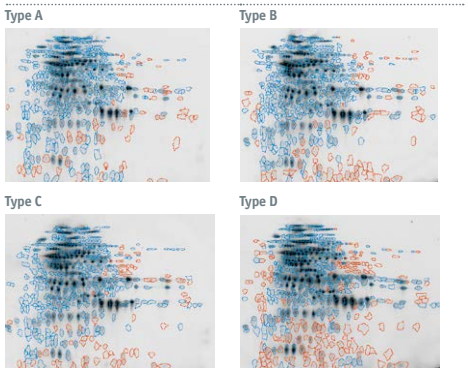

Figure 5: Comparison of coverage in sample 2: 2D DIGE Western Blots with Cy5-labeled E.coli HCP (red) and its detection by anti-HCP antibodies (Cy3 detection framed in blue).

E.coli|360-HCP Antibodies	Coverage of sample 2
Type A	88,9%
Type B	84,3%
Type C	87,3%
Type D	71,9%

Table 3: Evaluation of HCP coverage with the E.coli|360-HCP antibodies (type A to D). (sample 2)

For all four antibody types coverages above 70% were estimated. For antibody type A the coverage was almost 90%. But even for antibody type D an acceptable coverage of 72% was determined. The comparison of the estimated recovery of kit type D for sample 2 and the calculated coverage of 72% using antibody type D shows that there is no correlation between these two information. Coverage analysis provide information about the ability of HCP antibody pool to detect certain HCP specifies but do not give information about the quantitative relation between a certain HCP species and the respective antibody. We therefore strongly recommend to verify the data obtained by ELISA with orthogonal methods like 2D analytics.

Conclusion

Based on the data shown above we conclude that there is no 'one for all assay' suitable for all samples. The new generic E.coli|360-HCP ELISA provides four different antibody types and the scientist is given the possibility to fast and easily select the most suitable antibodies for a specific process.

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