2D DIGE Western Blot
HCP Detection and Coverage Determination: Advantages of Fluorescence over Colorimetric Detection
Claudia Geserick, Jamina Fiedler, Nicole Gliese

Introduction
Biopharmaceutical products must be proved to be minimized as much as possible of contaminating host cell proteins (HCPs) prior to approval by the regulatory authorities. Commonly, ELISAs are used for HCP determination and authorities ask for clear and manifest proof that the antibodies used in the HCP ELISA are fit for purpose. Their suitability needs to be shown, not only by the obtained ELISA data, but also by determination of HCP coverage in %. So far a colorimetric 2D Western immunoblotting method has been used. But fluorescence detection using a 2D DIGE Western Blot has several advantages over the colorimetric detection while the HCP coverage of the two methods is comparable.

Advantages of 2D DIGE Western Blotting in comparison to colorimetric detection:
- Higher sensitivity: Less than 30 % of usual protein amount is needed
- Higher dynamic range: Only one blot is necessary
- No inter-gel variation: No gel/membrane matching is needed
- Electronic evaluation of coverage

Practical Differences
For 2D DIGE Western Blot method a protein sample is labeled with Cy5 before its application. The proteins are separated in two dimensions according to their isoelectric points (IEF) and to their molecular weights (SDS-PAGE) with subsequent Western blotting and immunostaining using Cy3-labeled antibodies. This results in one 2D DIGE Western Blot membrane on which both, the total HCPs and the anti-HCP antibodies can be detected.

High Sensitivity
The 2D DIGE Western Blot requires less than 10% of the HCP amount needed for the preparation of a 2D Coomassie BlueSilver gel (Fig. 1).

High Dynamic Range
The high sensitivity and broad dynamic range of the fluorescent dyes enable detection of both abundant and scarce proteins. The high dynamic range applies to the patterns of total HCP (Cy5, Fig. 2) and anti-HCP antibodies (Cy3) detection.

Overcoming Inter-gel Variation and Complete Electronic Evaluation
2D Western Blot (colorimetric based)
For the commonly used colorimetric procedure, two gels are run. One gel is used for total protein staining by Coomassie or silver, and the other is used for Western blotting followed by immunostaining. Furthermore, for the detection of both abundant and scarce proteins two blots with low and high antibody concentrations are necessary. For coverage determination, the matching of the protein spots stained in the gel with the immunodetected proteins on the membrane can be difficult due to inter-gel variation (Fig. 3).

2D DIGE Western Blot (fluorescence based)
As total HCP and anti-HCP antibodies are detected on the same membrane there is no inter-gel matching required, thus allowing a complete electronic evaluation (Fig. 5). The transfer efficiency to the membrane has no influence on the coverage determination. The 2D DIGE Western Blots and the colorimetric based 2D Western Blots show a comparable coverage (Fig. 4, 6).

Summary
The fluorescence based 2D DIGE Western blot method has considerable advantages towards the colorimetric methods: It is more sensitive, has a higher dynamic range and needs about a tenth lesser amount of HCP. Another significant advantage is that for evaluation the spot detection and coverage determination can be performed fully electronically.

*Contact: BioGenes GmbH · Köpenicker Str. 325 · 12555 Berlin, Germany · nicole.gliese@biogenes.de · Tel.: +49 (0)30 65 76 20 22