Enhanced HCP Coverage Analysis utilizing Multiplexed 2D Electrophoresis

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Introduction

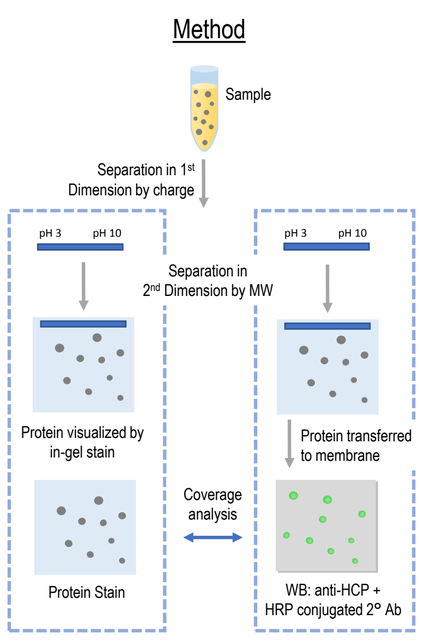
A crucial prerequisite in the drug manufacturing process is an efficient analysis of Host Cell Protein (HCP) impurities that result from process specific expression conditions as well as downstream purification procedures. Present guidelines call for minimum levels of HCP contaminants left behind during the purification process from the expression hosts. To investigate the presence of residual contamination of the final biopharmaceutical product, the development of polyclonal antibodies with maximum coverage against native HCP lysate provides a valuable tool to demonstrate product purity.

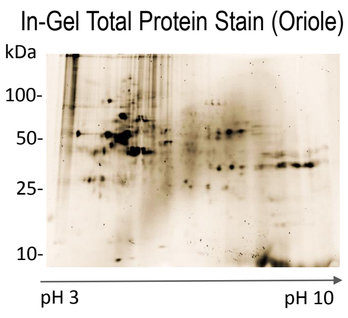
Quality of the HCP specific antibody is most commonly analyzed via ELISA and orthogonal methods like 1D and 2D-PAGE assays where the coverage of the antibody is confirmed. Determining anti-HCP antibody coverage is essential to ascertaining the robustness of the antibody in comprehensive reactivity toward HCPs during process points.

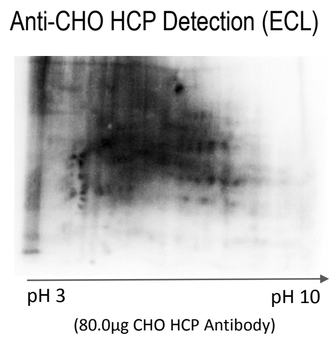
Here we evaluate the coverage of a generic anti-CHO-HCP antibody (Rockland Inc.) by 2D-PAGE separation using two experimental methods for the analysis: (A) chemiluminescent based detection method; and (B) Two-dimensional Differential In Blot Electrophoresis (2D-DIBE). For (A): the proteins detected by the antibody on a Western Blot (WB) via a chemiluminescent detection system, are equated to the proteins separated on a separate SDS-gel and visualized by an in-gel protein stain. For (B): 2D-DIBE utilizes a fluorescent multiplexing approach where the proteins and antibodies are tagged with different CyDyes™. Proteins and antibody coverage can be analyzed on the same membrane.

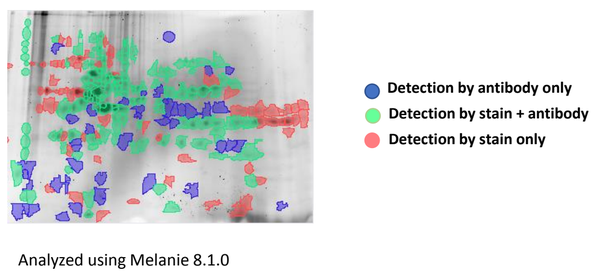
Sensitivity and antibody reactivity of the two methods are compared and analyzed.

2D-Chemiluminescence coverage detection

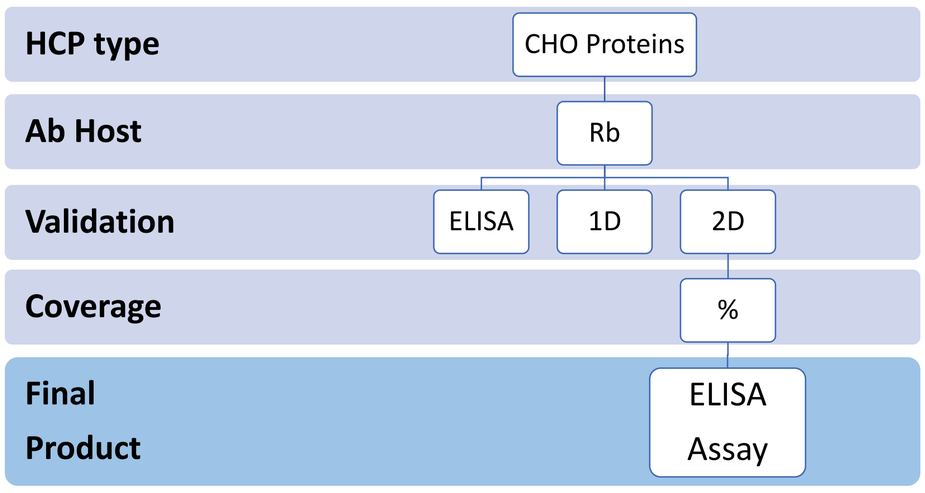








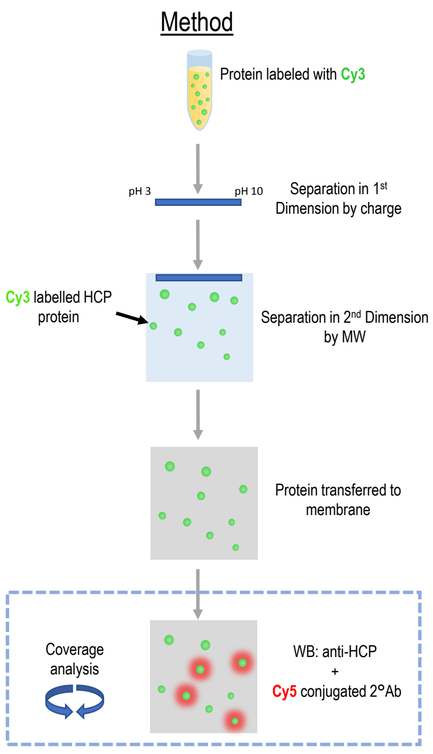
Antibody development

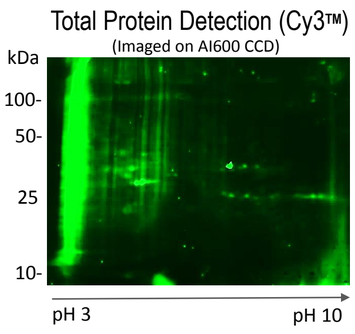


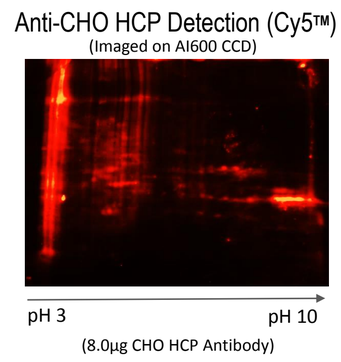
HCP antibody development entails immunization using a representative sample of the HCP extract. The selection of this sample is critical, and can be taken from various stages of the bioprocess workflow. Generation of HCP antibodies requires the use of a variety of immunization protocols to obtain acceptable detection of low abundant or poorly immunogenic proteins. Quality of the antibody is validated by 1D and 2D-SDS-PAGE for immunocoverage in addition to ELISA based assays.

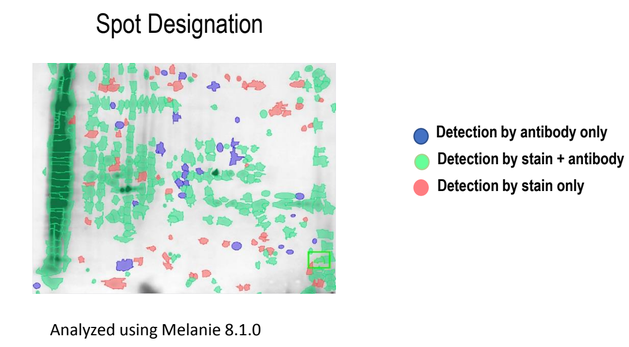
Here we analyze the coverage of a generic anti-CHO HCP polyclonal antibody (Rockland Inc.) as an alternative to process specific reagents that are functional for HCP detection in 2D WB and ELISA.

2D-DIBE coverage detection



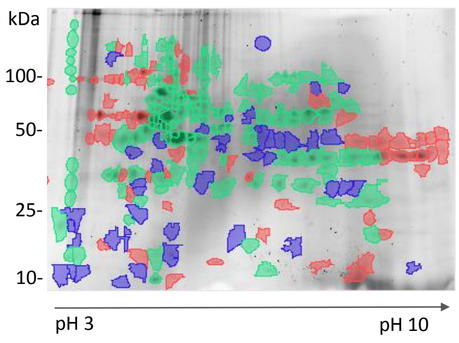


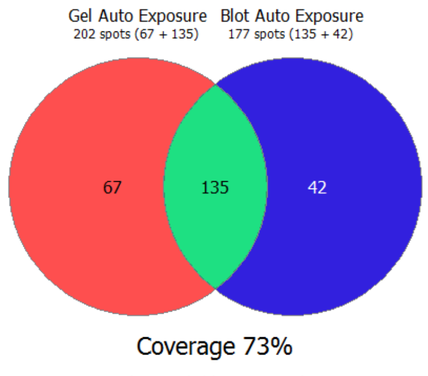




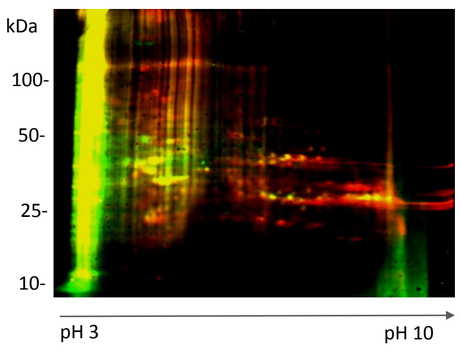
Comparative Results & Conclusions

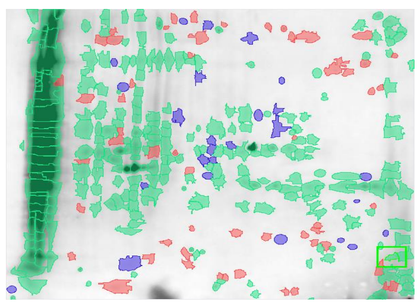
2D-Chemi Coverage Results

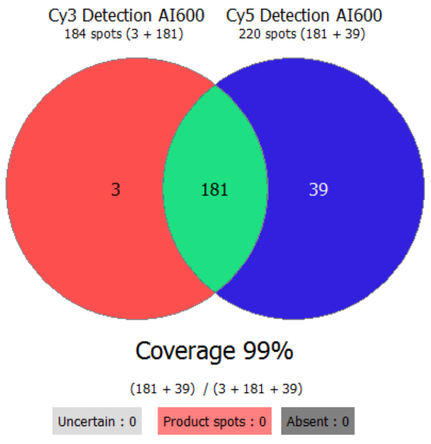




2D-DIBE Coverage Results







Anti-CHO HCP antibody coverage:

- Sensitivity and immunoreactivity of the antibody is robust for proteins in all 4 quadrants of the 2D protein map (data not shown).
- Difficult to detect proteins in the lower molecular weight regions are identified by the CHO HCP-antibody presented here.
- Low concentrations of anti-CHO HCP antibody are sufficient to obtain good signal in both assays.

Chemiluminescence assay:

- Parallel detection of immunoreactive high molecular weight proteins and low abundant low molecular weight proteins requires imaging of the WB at several exposure times. Shown here, a single exposure time was analyzed. Multiple exposure times (often 3-4 exposures) need to be evaluated to determine accurate coverage.
- Detection of high molecular weight protein in overexposed regions of the blot can be challenging with chemiluminescence.

2D-DIBE assay:

- Bypasses the need of the alignment step (gel-WB) in coverage analysis.
- Simultaneous visualization of total protein and antibody coverage allows for overall accuracy in spot alignment and reduces user error in the analysis step.
- Well-defined, resolved spots in 2D-DIBE make manual spot review and designation easy, quick and free of user bias.
- 2D-DIBE assay allows for an approximate 10-fold increase in sensitivity compared to the chemiluminescent assay.

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