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Robust, streamlined 2-D western blot workflow for evaluation of antibodies developed for: Detection of host cell proteins (HCP)

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Abstract

Biologics and biosimilars can contain traces of proteins from the host cell line, which need to be minimized to prevent adverse immune reactions. The presence of host-cell proteins (HCP) is usually assayed by ELISA using a polyclonal antibody mixture raised against a population of proteins derived from the host cell background. This antibody should recognize as high a proportion as possible of the potential HCP's in a given sample. A recommended method for evaluating the assay involves two-dimensional electrophoretic separation followed by Western blotting. Although this method allows a straightforward determination of the range of reactivity of the antibody mixture, adoption of this methodology has been hindered by the perceived lack of sensitivity, lack of reproducibility and overall laborious nature of the 2-D electrophoresis and blotting workflow.

Tools that simplify, standardize and accelerate 2-D electrophoresis and blotting have become available. Advanced electrophoresis and blotting instrumentation, stable and reproducible pre-cast gels, next-generation imaging and image analysis software all allow this assay to be performed in a reproducible manner in a relatively short time. We have undertaken to demonstrate this using a commercial anti-HCP antibody and samples derived from Chinese Hamster Ovary (CHO) cells. The 2-D electrophoresis procedure resulted in highly reproducible spot patterns and entire procedure was completed in less than two days. The software used (PDQuest) enabled the straightforward generation of percent coverage values for the antibody when used to probe both CHO cell lysate and CHO cell secreted protein.