

## Western Blot vs. ELISA: Sensitivity & Specificity Differences

Western blot is very rarely acceptable for detection of HCPs in your drug substance or drug product samples. Samples downstream in your purification process typically contain HCPs below the sensitivity of western blot. For western blot, you are limited in the amount of total protein you can load and still get good PAGE resolution. When you load final product or samples from downstream in the purification process the vast majority of protein will be the product itself. For example, the maximal load of protein for a PAGE run on a mini gel is on the order of 10  $\mu\text{g}/\text{lane}$ . If HCP contamination is 100 ppm, a level typical of many final drug products, then the amount of total HCP in that 10 $\mu\text{g}$  of drug would be 1ng. With the sensitivity of western blot on the order of 1 ng/band it could in theory detect HCP contamination down to 100ppm if the 100ppm were a single HCP and not a mixture of several different HCPs. As it turns out there are usually several HCPs that contaminate final product and for this reason western blot is almost always negative for HCP on downstream and final product samples. ELISA demonstrates less interference from drug product and shows sensitivity more than 100 fold lower than western blot. As such, ELISA will typically allow for the detection of total HCP contamination to less than 1ppm. There are many other fundamental reasons why the sensitivity of western blot is inferior to ELISA. For example, western blot often requires that the PAGE step be carried out under reducing conditions (DTT or BME followed by boiling) and in the presence of high concentrations of SDS detergent. These procedural components may actually denature or block some of the native HCP epitopes that would be detectable in an ELISA. Incomplete transfer of the proteins out of the PAGE and onto the membrane and adsorption on the membrane at or near antigenic sites will also limit the amount of binding seen by western blot.

As you try to increase the sensitivity of western blot it is very common that the specificity of the method is also compromised. What is typically seen is that a non-immunoreactive protein present in very high concentration (e.g. your drug substance) will invariably adsorb some of the excess anti-HCP antibody non-specifically leading to the erroneous conclusion that the anti-HCP antibody seems to "cross react" with your product. The way to confirm this non-specific binding to your product is to use a non-immune immunoglobulin of the same species and at the same concentration as the anti-HCP antibody. If the intensity of the drug substance band is the same with both the normal goat IgG and the anti-HCP antibody, you can conclude the band is non-specific. Beyond that experiment it should be understood that the specificity of the ELISA method is typically orders of magnitude better than western blot owing in large part to the fact that any protein must be bound simultaneously by both the capture antibody and the detection antibody. For this reason most artifactual product bands in the western will not yield apparent HCP activity in the ELISA method.

While western blot is of little value for detecting HCP in all but very upstream samples, it is an important method for demonstrating that the antibodies in the ELISA kit react with the majority of the HCPs from your cell line. This is typically a one time experiment where you lyse some cells or use conditioned media from your cell line in a western and compare the western to a protein stain from PAGE such as colloidal gold or silver stain. If the homology is adequate one can conclude that the antibody has adequate reactivity to be useful in the ELISA kit.