Non-specific Binding in Western Blots

A common and unfortunate artifact of western blot is that some proteins particularly those at very high loads will non-specifically bind to a western blot antibody. Attempts to increase the sensitivity of western blot for HCP contaminants in the presence of a very large excess of product often involve use of higher concentrations of HCP antibody and loading of more sample. Increasing the sensitivity by these approaches will also tend to compromise specificity. Almost all non-immunoreactive proteins if present in very high concentration (e.g. your drug substance) will 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. There are a number of approaches to confirm the specificity of your western blot as discussed below.

1. You can often demonstrate that binding to your product protein at its chosen load is non-specific by performing a negative control blot at the same time you blot with the HCP antibody. The control blot uses a non-immune or normal immunoglobulin conjugate at the same concentration and from the same animal species as the anti-HCP antibody. If the intensity of the drug substance band is the same with both the normal IgG and the HCP antibody, you can conclude the band is nonspecific.

2. Loading of much lower quantities of your product protein will also help to confirm specificity. The specific sensitivity of western blot is on the order of 1ng/band. If you fail to detect a product band at product loads in this range it is reasonable to suspect that bands seen at much greater loads are non-specific in nature.

3. Specificity can also be confirmed by ELISA analysis. Our ELISA kits use the same antibody as the respective western blot kit. The specificity of ELISA is in orders of magnitude, better than western blot, owing in largest part to the fact that any protein must be bound simultaneously by both the capture antibody and the enzyme labeled antibody. For this reason, artifactual product bands in the western blot will not yield apparent HCP activity in the ELISA method. The ELISA experiment is performed by diluting your product protein to concentrations within the analytical range of the ELISA (typically from 1 to 200ng/mL). Assuming that your product protein is not heavily contaminated with HCP (has less than parts per hundred HCP), you should not see any apparent HCP activity unless your product cross-reacts with both the capture and enzyme-labeled antibody. Some products, in particular therapeutic antibodies, can non-specifically bridge the anti-HCP antibodies in the kit, but in most cases we have been able to design our kits to overcome this problem. Contact our Technical Service Department if you are having difficulty demonstrating specificity of the HCP antibodies.