

CHO HCP Kits...Which kit to use, #F015 or #CM015?

Cygnus Technologies offers 2 ELISA kits to measure CHO HCPs. Kit # F015 was the first kit developed and utilizes a lysate of washed CHO cells for the immunogen as well as the for affinity purification of the antibody and for kit standards. Kit #CM015 was developed about 4 years later with the rationale that since most CHO expressed products are released into the growth media (conditioned media) then an antibody and assay made to those HCPs found in conditioned media should be more specific than a cell lysate assay.

Testing of both the lysate and conditioned media antibodies by western blot showed that the vast majority of proteins found in cell lysate could also be found in conditioned media and that both antibodies had very similar reactivity. While the antisera are qualitatively similar, the assays themselves may not give the same values for some sample types. There are many reasons for this but perhaps the most significant is that the relative abundance of certain HCPs will be different in lysate versus conditioned media. Since different HCP preparations are used to make the standards, generate the antibody and affinity purify the antibody, then it is not unexpected that various samples types which contain yet another array of purification process specific HCPs, will be differentially detected between the two assays. **Any assay which attempts to detect simultaneously multiple antigens that can vary in their relative proportions from the calibrators used, will be at best a semi-quantitative estimate of HCP content.** Such assays may underestimate or overestimate the true HCP content and thus it is important to appreciate the uncertainty in any value reported for a sample. This is why we recommend reporting of HCP contamination not in absolute concentration units like ng/mL but rather in the arbitrary units such as ng of total immunoreactive HCP equivalents/mL. Such qualified units acknowledge the inherent limitations for absolute quantitation in this type of assay.

Cygnus cannot predict which assay will be best suited for a given product or sample type. Our survey of customers who have evaluated both kits, shows that about half believe that the F015 assay gives more accurate results even though their product is expressed into the culture media while others prefer the CM015 assay. There are no hard factors to recommend one assay over the other. It is up to the user to validate whatever method they select to determine that it provides adequate sensitivity, accuracy, and specificity. That validation study should first determine the specificity of the assay. Nothing in the samples such as additives like detergent, buffer salts, pH or the presence of product protein itself should cross react with the antibody or otherwise cause an increase in non-specific binding that could result in an overestimation of HCP. With the specificity established, the user must then determine that nothing in the samples inhibits the detection of true HCP. This is determined by performing spike and recovery experiments in the various samples types to be tested as well as dilutional recovery/linearity experiments in samples containing elevated levels of HCPs such as those typically found in upstream purification process

samples. With the results of these validation studies in hand it is usually possible to select one assay over the other. Assuming both assays are equally specific and accurate we normally recommend that assay which yields the best sensitivity for HCP detection in your specific downstream or final product samples.

Cygnus Technologies appreciates the challenges in detection of HCPs and is pleased to offer close customer support in validating and troubleshooting product and sample specific problems. Please contact our highly experienced Technical Services Department if you have any questions.