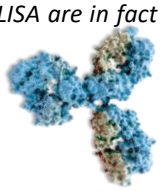
HCP Risk Management

Is the commercial HCP ELISA kit I use suitable to secure my Bioprocess?

*Many **commercial Host cell protein ELISA kits** (also called generic HCP ELISA) are nowadays available on the market. Although an easy first selection can be made depending on the expression cell line (CHO, E.coli, Pichia pastoris, …), the final selection of the most appropriate one for a peculiar bioprocess is less straightforward than most people believe.*
The nature / concentration of the drug substance (DS) and the USP and DSP processes can highly impact on the HCP expressed by the cell line during the bioprocess. The performances of a generic HCP ELISA are in fact strongly dependent on the two following critical reagents :



Anti-HCP antibodies used in the generic ELISA

An imbalance between some of the HCP produced during the bioprocess and the ELISA antibodies available for these HCP can lead to **dilutional linearity concerns**. This result in the fact that the more you dilute your sample the more the HCP concentration increases, leading to **unreliable or even impossible quantification of the HCP in your sample.**

Limited recognition of the bioprocess' HCP by the antibodies from the ELISA is a huge concern since **some HCP can be present in the DS, but not detected by the ELISA because of a lack of specific antibodies**. It is consequently of the utmost importance to verify that the antibodies form the ELISA recognizes as must as HCP possible, with an equal distribution on all of the bioprocess' HCP population. This point is a regulatory requirement and is achieved by **2D-DIBE coverage assessment on large gels**.

HCP standards of the kit

The HCP used as standards in the ELISA kits are produced in a different way and concentration than the HCP generated by the bioprocess. These differences in HCP population leads to **miss quantification of the HCP** in the bioprocess samples and DS because the calibration curve of the ELISA kit is not well adapted.

If these 2 critical reagents are not adapted to your bioprocess, an over-quantification or, most often and more risky, under-quantification of the HCP can easily be made with potential harmful effects on validation of the DSP protocol and on your whole project.

IDBiotech has implemented stepwise and fully customized services for the characterization of generic HCP ELISA fully aligned with the regulatory authorities' requirements

*The expert team of IDBiotech can assist you to select the most appropriate HCP ELISA kit for your Bioprocess. We advise to **compare the performance of 2 to 4 HCP ELISA kits** for an optimal selection. After selection, we advise to validate the reliability of the antibodies from the generic ELISA by **2D-DIBE Coverage assessment** and also to **validate the HCP ELISA kit selected**.*

As IDBiotech does not commercialize any generic ELISA, a completely objective evaluation of generic ELISA can be ensured

HCP Risk Management

1. Selection and suitability study of generic HCP ELISA kits

- Evaluation of the Dilutional Linearity on HCCF, in-process samples and DS
- Control of the LOD & LOQ
- Determination of the accuracy by spike recoveries experiment on the DS. Ideally this experiment should be carried out using the HCP issued from the bioprocess.
- Verification of the specificity: absence of cross-reactivity with the DS
- Comparison of the HCP concentrations with the total protein concentrations in the samples
- Comparison of the HCP pattern used as standard in the ELISA kit with the HCP from the bioprocess by 2D-DIGE.

2. 2D-DIBE Coverage assessment of anti-HCP antibodies from generic ELISA kits

The determination of the percentage of coverage of the anti-HCP antibodies from the generic HCP ELISA over your process-specific HCP is determined by 2 Dimensional Western-Blot analysis (**2D-DIBE**). This coverage assessment is required by the FDA and EMA authorities. Ideally, a coverage of > 70 % equally distributed on the 4 quadrants of the gel is required.

IDBiotech has a strong expertise for 2D-DIBE coverage assessment combined with a state-of-the art protocols and equipment:

- Optimized protocols for fluorochrome labelling of the HCP from the bioprocess.
- Optimized 2D-DIBE protocols for generation of high-resolution HCP patterns using horizontal electrophoresis on large gels (20 x25 cm).
- Optimized protocols for the determination of the western-blotting experimental conditions.
- High-resolution scans of the membranes using the Typhoon RGB scanner
- Use of the Melanie 9 Software for spot detection, count and coverage determination. The results are expressed as total coverage percent and also as percent of coverage on the 4 quadrants of the membrane, as requested by the health authorities.

3. ICHQ2R1 validation of the generic ELISA kit

If the generic ELISA is used for **R&D purposes** and bioprocess development (USP & DSP), the above-mentioned characterizations are sufficient to qualify the use of a generic ELISA.

Once the generic ELISA kit is used for **drug release testing for clinical phases**, the authorities will require a ICHQ2R1 validation of the ELISA. IDBiotech has validated many of generic HCP ELISA kits and can perform this validation for your projet.

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*If no generic ELISA is adapted to your production process, IDBiotech can implement some **fine-tuning modifications of the generic ELISA** to better reliability or, if not possible, can propose the **development of a process-specific HCP ELISA kit***
(see one of the upcoming newsletters)

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