HOST CELL PROTEIN ANALYSIS
ELISA or LC-MS/MS

1 HOST CELL PROTEIN

Host cell protein (HCP) is a protein produced or encoded by a host cell during the synthesis of recombinant therapeutic proteins. Recombinant therapeutic proteins are usually produced by genetically modified prokaryotic or eukaryotic host cells using cell culture or fermentation techniques. Genetic engineering enables host cells to be transformed to selectively express the target protein. In the process of recombinant protein production, host cells also jointly produce proteins related to normal cell functions, such as cell growth, proliferation, survival, gene transcription, protein synthesis, and so on. Due to cell apoptosis, death, and lysis, other non-essential proteins can also be released into the cell culture medium.

As a foreign protein, residual HCP in biological products is low in content, but could still trigger the body's immune response to varying degrees, and ultimately lead to allergic reactions or other adverse reactions. HCP constitutes the main part of process-related impurities in the production process of biological agents. The amount of residual HCP in a drug is usually considered a critical quality attribute (CQA) because it may affect product safety and efficacy. Therefore, the regulatory requirement is to monitor the removal of HCP in biopharmaceuticals during the development of bioprocesses.

2 METHOD OF HOST CELL PROTEIN ANALYSIS

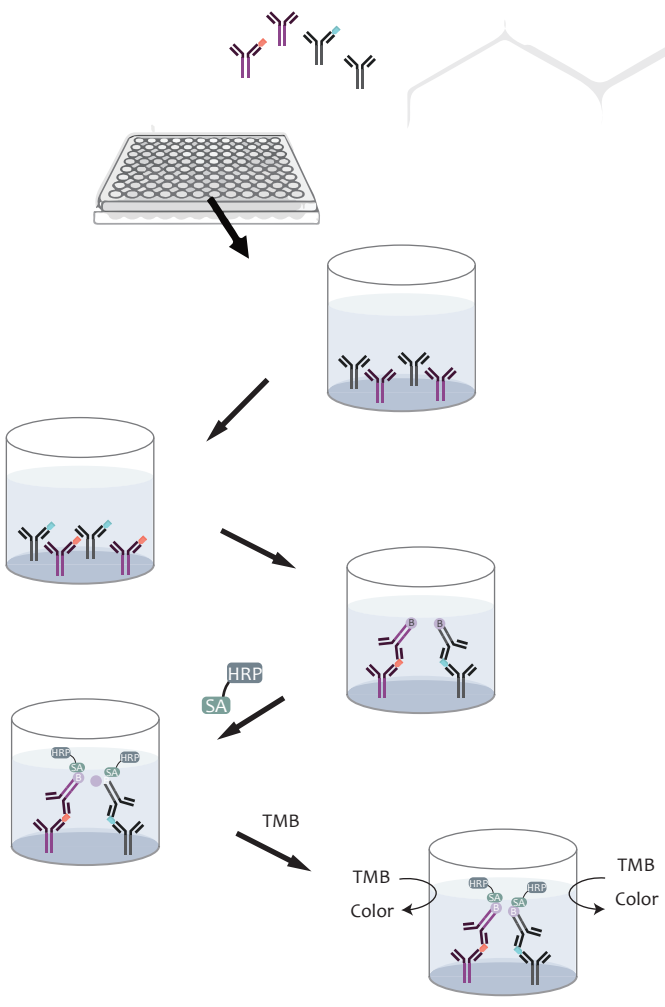
ELISA **LC-MS/MS**

Enzyme-Linked ImmunoSorbent Assay (ELISA)

ELISA developed using polyclonal antibodies produced against parental cell lysates or cell culture supernatants can detect most HCP species.

Enzyme-Linked ImmunoSorbent Assay (ELISA) is the most widely used HCP detection method, but this technique has limitations.

- It does not guarantee the detection of every potential HCP impurity, can only be semi-quantitative, and cannot determine whether it is multiple HCP residues or a high concentration residue of HCP.
- It takes a long time and costs much to develop a dedicated ELISA kit for a specific process.
- It is not suitable to comprehensively evaluate biopharmaceutical products made with different cell types or purification schemes.

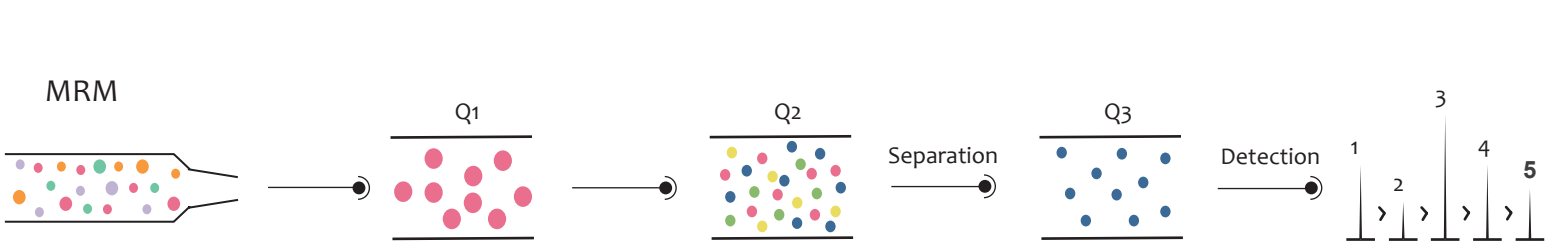


To solve these problems, LC-MS/MS emerged as an orthogonal and complementary means of the ELISA method, mainly for the identification and quantification of trace HCP.

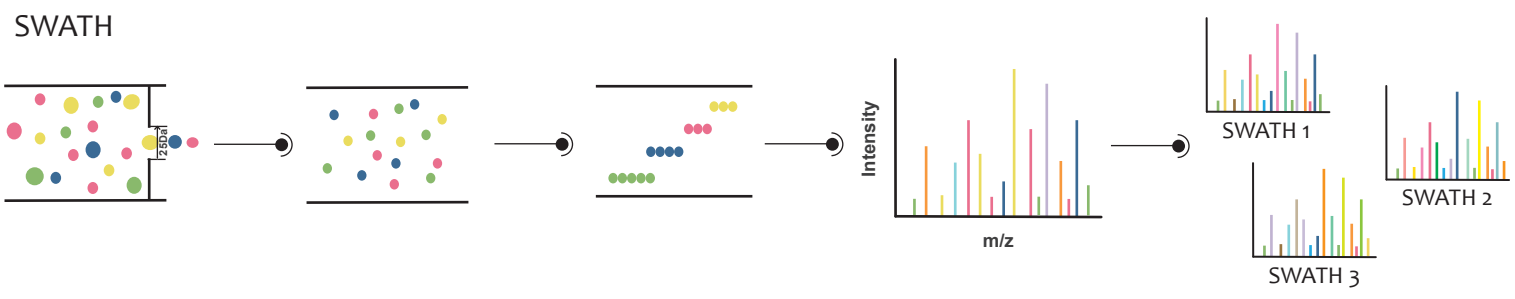
Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

According to different research purposes, there are two main detection strategies when using LC-MS/MS for HCP:

- **Target HCP determination:** it is applicable to the quality control of biological drugs whose production process has been determined. The MRM or high resolution MRM (MRMHR) scanning mode of conventional resolution mass spectrometry is usually used to obtain sub-μg/mL or μg/mL sensitivity.



- **Unknown HCP determination:** During process research and development, in order to design and optimize the purification process, it is necessary to conduct a high-throughput and stable evaluation of HCP for different purification steps. Since the purification process of the product is often carried out through a specific combination or reaction, this indicates that the residual HCP is often unknown. Therefore, in this type of research, High-Resolution Mass Spectrometer (HRMS) is widely used.



The selection of high-resolution mass spectrometry data depends on the acquisition mode, and thousands of proteins can be identified. When using SWATH acquisition technology for relative or absolute quantification, the sensitivity can reach 10μg/mL. The reproducibility is excellent, the method is simple, and the measurement throughput is high.

Contact Us

Creative Proteomics