



BIOLOGICS TESTING SOLUTIONS

## Host Cell Protein Assays

Charles River works with clients to develop project-specific and platform-specific host cell protein (HCP) assays. Additionally, we serve clients with the characterization of commercially available kits and coverage determination. The type of assay required to determine HCP content is dependent upon the phase of product development. In early process development and early clinical phases, generic assays are normally acceptable. However, once the biopharmaceutical is used in Phase III clinical studies, a validated, product-specific HCP assay is usually required. We can provide clients with assay development and validation, regardless of whether they are in preclinical development or embarking on Phase III studies.

### What Are Host Cell Proteins?

Host cell proteins (HCP) represent a heterogeneous pool of contaminant proteins that are an inevitable impurity of biopharmaceuticals, regardless of whether they are produced by recombinant fermentation or extracted from natural sources. Even after multiple sophisticated purification steps, HCPs may co-purify, or “hitchhike,” with the product, and thus need to be characterized and quantified in drug substance and in downstream purification process (DSP) intermediates. The risk for adverse effects such as immunogenic reaction does not necessarily correlate with the amount of certain host cell proteins, and even traces of an HCP can be highly immunogenic, or may adversely impact product quality over its shelf life. Traditional protein detection methods such as HPLC and total protein stains are not suitable for HCP detection due to insufficient sensitivity and specificity. Consequently, optimized immunoassays (ELISA) and mass spectrometry (MS) methods have established themselves as the methods of choice for the measurement and characterization of HCPs.

Currently, we are the only provider offering best-in-class service for HCP immunoassays and HCP mass spectrometry under one roof. Leveraging both technologies, we are able to generate a near-perfect HCP assay by applying our HCP-GAPex strategy.

EVERY STEP OF THE WAY



## Development of a Customized Quantitative Immunoassay HCP Assay

Arriving at a product-specific, quantitative HCP assay involves several steps:

- Conditioning of host cell protein antigens (fractionation and concentration)
- Production of antisera using rabbits, goats, and chicken
- Affinity purification of antibodies from crude serum
- Quality control of the antibodies with respect to specificity and sensitivity (linear epitope coverage by 2D SDS-PAGE and western blot)
- Host cell protein assay setup and optimization
- Validation for the assay in compliance with ICHQ2 (R1) Guidance
- Characterization of the host cell antigen by shotgun proteomics (LC-MS/MS)
- Conformational coverage: native protein epitope coverage determination using immunoaffinity with LC-MS/MS readout to identify immunogenic HCP antigens

Our team supports clients in all of the above processes to ensure their biopharmaceutical product is provided with a well-characterized and well-controlled impurity profile ready for use in each stage of their drug development pipeline.

## Antisera Production for Customized HCP Assays

In contrast to single molecule impurities, contaminants, and leachables, such as trypsin or Protein A, which can be approached with a monoclonal antibody, the complexity and heterogeneity of HCPs always requires a polyclonal antiserum for assay development. Our polyclonal antisera production is available in a variety of species including rabbits, goats, chickens, and rodents. Rabbit and chicken immunization leverages Charles River's specific pathogen free (SPF) animals, which typically results in increased specificity and no anti-product cross-reactivity.

To maximize the knowledge obtained from immunization experiments, the following measures are an additional component of our offerings:

- Concentration of antigens
- Fractionation of antigens
- Innovative immunization strategies
- Antibody purification and labelling
- Coverage determination by high resolution 2D-SDS-PAGE and Western Blot
- Coverage determination by LC-MS/MS

### Custom LC-MS/MS HCP Assays for Identification and Quantitation

Mass spectrometry is an extremely powerful tool for identification of HCP contaminants, and LC-MS/MS can be used as an orthogonal tool for the identification and quantitation of specific HCP impurities alongside immunoassay development. A smart combination employing mass spectrometry for direct protein identifications in both product and manufacturing samples, along with MS-based immunoaffinity experiments, provide full intelligence about the downstream process (DSP) performance as well as the immunoassay reagents used to control and develop the DSP. These work packages comprise:

- characterization of the HCP proteome/secretome in the mock cell line and the transfected cell line.
- characterization and quantification of HCPs through the DSP by LC-MS/MS using the label free quantification (LFQ) method.
- identification of specific HCPs in drug substance and drug product.
- MS-based qualification of the ELISA reagent to determine its coverage against conformational (native) protein epitopes.
- identification and closure of detection gaps in ELISA reagents to achieve maximum coverage of the immunoassay by HCP-GAPex.

### Service Areas:

- Immunoassays (ELISA, ILA, and Immuno-PCR)
- Proteomic LC-MS/MS assays
- Multi-Expression-System experience, including:
  - mammalian cells (e.g., MRC-5, Vero, A549, BHK, CHO, 293)
  - insect cells (e.g., Sf9)
  - bacteria (e.g., *E. coli*, *S. aureus*)
  - yeast (e.g., *P. pastoris*, *S. cerevisiae*)
- Rapid assay setup, qualification, and validation
- Lifecycle management of assay reagents including long-term storage, purification, and re-qualification of assay reagents. In-house production of antisera available to support late-phase product development.

### HCP-GAPex<sup>SM</sup> A new holistic approach for the control of HCP impurities

Charles River is currently the only provider offering best-in-class service for HCP immunoassays and mass spectrometry under one roof. Leveraging both technologies, we are able to generate a near-perfect HCP assay by applying the HCP-GAPex strategy.

## What is the best assay to test HCP in your product?

	Generic Kit-Based Assays	Project Specific Assays	Platform Assays
<b>Availability</b>	Instantly available	Requires antigen preparation, including cloning of a mock cell line	Requires antigen preparation from a null or mock cell line
<b>Development</b>	No need for preparation work	Assay development including antigen characterization, immunization and qualification takes a minimum of six months	Assay development, including antigen characterization, immunization, and qualification, takes a minimum of nine months
<b>Diversity</b>	Availability is limited to the most common expression systems	Feasible for all expression systems	Feasible for all expression systems
<b>Specificity</b>	Specificity might not be suitable, high risk of undetected HCP species	Typically enhanced specificity, antibody coverage more customized to the specific HCP pattern	Improved specificity compared to commercial assays, improved understanding of assay performance, coverage on HCP species level
<b>Cost</b>	Initially fairly inexpensive, but for an entire DSP, development costs add up quickly	Initially costly, but the break-even point is typically at a value of 100 kits	Significant cost advantage due to multi-product usage
<b>Dependence</b>	Dependency to one vendor, black box for reagents and controls	Full control of all reagents and buffers, optimization/adaptation is always possible	Full ownership and control of all reagents, risk mitigation with supply chain and batch comparability
<b>Regulatory Needs</b>	Not suitable for supporting marketing authorization applications	Suitable to support from early development to final product release of authorized product	Suitable to support early development up to MAA/BLA (less risk for transition from early phase to Marketing Authorization Application)