ROCKLAND

HOST CELL PROTEIN & BIOPROCESSING REAGENT DEVELOPMENT

INTRODUCTION

Biopharmaceuticals require products to be free of residual host cell protein (HCP) contaminants from the bioprocessing workflow. To evaluate the presence of residual contamination during the bioprocessing process and final biopharmaceutical product, development of custom polyclonal antibody reagents with maximum coverage against contaminant HCP extracts is required.

Rockland provides multiple customizable options for HCP antibody development. These options allow flexibility for development of early stage through late stage HCP detection reagents.

SERVICES

Antigen Preparation
- Product Platform: *E. Coli*, Yeast Insect, Plant, & Mammalian
- Fractionation
- Ultrafiltration
- Crosslinking & Conjugation

Antisera Generation
- Standard Protocol
- Size Fractionated Immunogen
- Cascade Immunogen
- Rabbit, Goat, Chicken, Sheep

Reagent Characterization & Analysis
- ELISA
- 1D SDS & Western Blot
- 2D SDS & Western Blot
- Reagent Qualification
- Coverage Analysis & Characterization

Assay Development
- Scouting Study
- Bridging Study
- Process Specific HCP Assays
- Multiple Platform Support

HCP ANTIBODY GENERATION TIMELINE (MOS.)

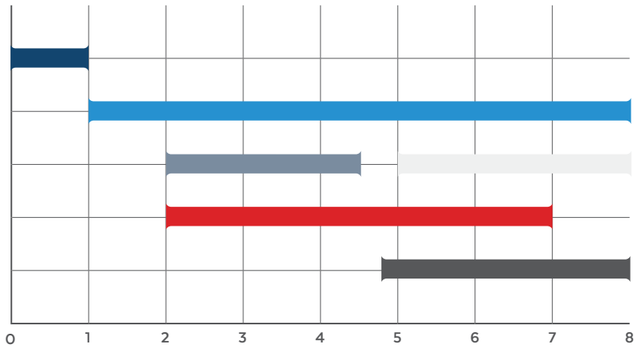
Setup
- Antigen Preparation

Immunization & Boost
- Repeat Boost Period

Reagent Generation
- Test Bleeds
- Production Bleeds

Sampling & Analysis
- ELISA
- Western Blot

Reagent Qualification
- 2D Western Blots



ANTIBODY GENERATION

Rockland scientists have performed HCP antibody reagent generation for more than 15 years. Rockland can do as much or as little as the client needs, fulfilling the project specifications under strict timelines. With every project, documentation and traceability is provided to satisfy FDA requirements.

ANTIGEN PREPARATION

As part of the strategy for a successful HCP antibody project, Rockland performs multiple HCP sample preparations.

Analytical sample preparation produces a robust and consistent HCP reference sample that can be used for all analytical work, including screening of antisera and qualification of the reagent in 2D Western blots and mass spectroscopy analysis.

HCP immunogen preparation produces a fractionated or modified version of the HCP immunogen (see Figure 1). Other strategies may call for chemical cross-linking or other HCP modifications.

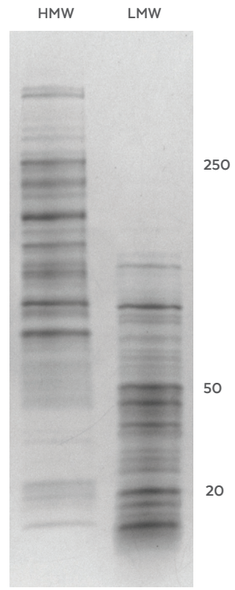


Figure 1. Typical enrichment of HCP sample into high, moderate, and low molecular weights. Low molecular weight enriched fraction shown on right.

ANTISERA GENERATION

HCP antisera generation can be a time consuming process and can require complex immunization strategies. Building on preparation of the HCP immunogen, one or more host animal cohorts are initiated to create a diversity of immune responses.

Rockland's goal is to create a broad immune response, which is measured by a high percent of coverage on a 2D Western blot.

Development strategies:

1. Standard HCP immunization
2. Fractionated HCP immunization
3. Chemically-modified HCP immunization

Multiple host species:

1. Rabbit
2. Goat
3. Chicken
4. Sheep

Analytical data is generated to measure coverage during the course of the project. Both 1D and 2D Western blots demonstrate basic and overall HCP protein coverage. The timeline for a HCP antibody project is composed of project preparation and antisera generation, generally requiring 7–8 months.

ANTIBODY QUALIFICATION BY ORTHOGONAL METHODS

HCP antisera and purified antibody require characterization and must be qualified for use in downstream validated assays. HCP reagent qualification is difficult due to the inherent complexity of the HCP sample. Accepted HCP qualification methods incorporate orthogonal techniques, which typically include:

Host-specific Enzyme-Linked Immunosorbent Assays (ELISAs)

One-Dimensional (1D) Western blot

Two-Dimensional (2D) Western blot (chemiluminescent or fluorescent)

Mass spectrophotometric

COVERAGE ANALYSIS BY 2D ELECTROPHORESIS

The quality of an HCP-specific antibody is most commonly analyzed via ELISA and associated orthogonal methods like 1D, 2D-PAGE, and Western blot assays. Rockland determines the coverage of any given anti-HCP antibody by 2D-PAGE separation using two methods for the analysis:

Conventional coverage analysis (see Figure 2a–2c)

Two-Dimensional Differential In Blot Electrophoresis (2D-DIBE) (see Figure 3)

The evaluation of the antibody coverage shown uses a combination of automated software and expertise of our scientists.

COVERAGE ANALYSIS & SPECIES IDENTIFICATION BY MASS SPECTROSCOPY

Mass Spectrometry (MS) identifies all possible HCPs present in a biopharmaceutical sample, giving it the advantage over current HCP methods. Liquid Chromatography Mass Spectrometry (LC-MS) HCP analysis determines the identity and quantity of individual host cell proteins. Sensitivity is in the ng/mg (ppm) range and MS analysis can be applied to final drug product samples.

Total HCP characterization

Coverage evaluation

HCP analysis of drug substance

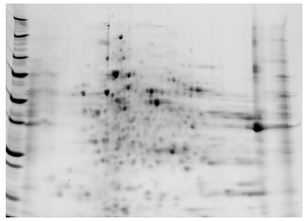


Figure 2a. CHO-HCP sample in-gel protein stain

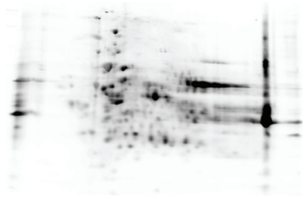


Figure 2b. CHO-HCP Western blot anti-CHO HCP

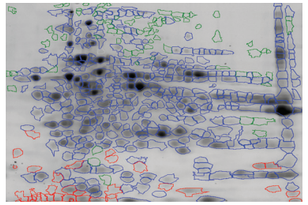


Figure 2c. Coverage analysis by 2D gel and Western blot overlay

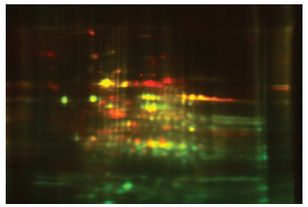


Figure 3. In-blot total Cy3™-labeled protein merged to anti-CHO HCP detection (Cy5™)

IMMUNOASSAY DEVELOPMENT

Analytical methods for measuring HCPs are challenging due to the five orders of magnitude and dynamic range of the HCP protein concentration. ELISA is a proven tool that examines the relative levels of residual HCP contaminants during bioprocessing and in the final biopharmaceutical product. Rockland can perform immunoassay development according to ICH and regulatory guidelines using a custom polyclonal HCP antibody, delivering maximum coverage and sensitivity against HCPs. Immunoassay development can be initiated as a standalone service or as part of an antibody development project.

Rockland provides a high degree of assurance that any custom HCP ELISA assay kit will consistently yield results that accurately reflect the quality characteristics of the product.

Key parameters:

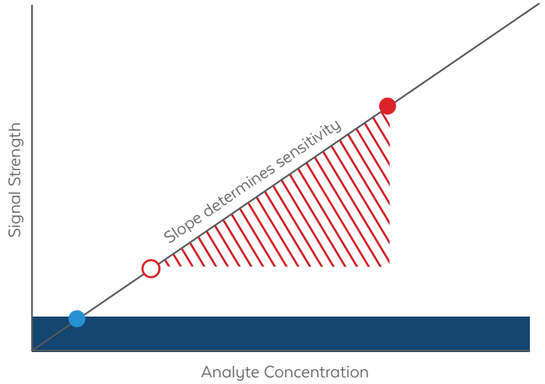
Matrix effect analysis

Assay accuracy and precision

Antigen Lower Limit of Detection (LLOD)

Full protocol and reporting

- **Lower Limit of Detection (LLOD)**
- **Lower Limit of Quantification (LLOQ)**
- **Upper Limit of Quantification (ULOQ)**
- **Dynamic Range**
- **Noise**



ASSAY DEVELOPMENT: WORKFLOW

Antibody Pair Screening
Testing capture and detection antibody pairs

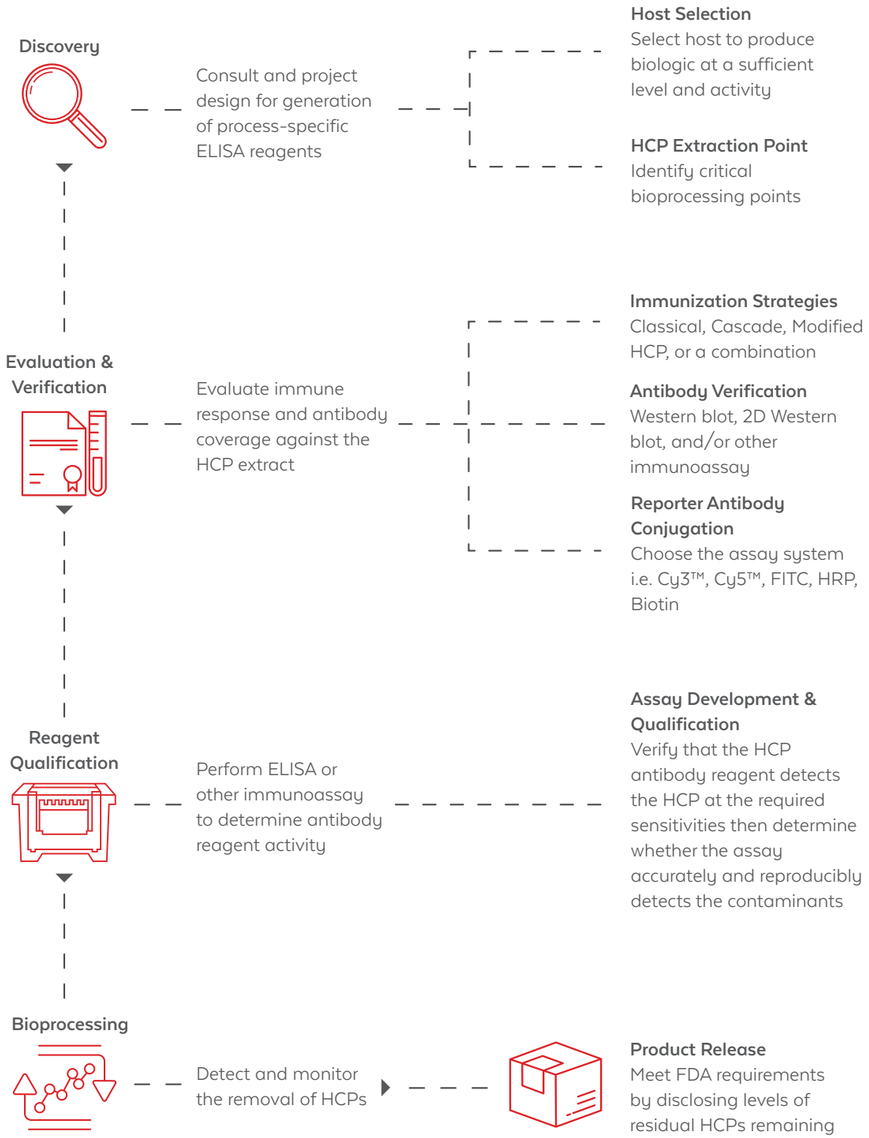
Assay Optimization
Optimizing assay conditions

Assay Validation
Validating assay performance

Manufacture & QC
Producing reagents or kits and managing long term supply chain

PRODUCING AN HCP ANTIBODY: WORKFLOW

Development of HCP antibody reagents—particularly anti-mammalian HCP—is a difficult and critical process for biopharmaceuticals. Many key decisions must be made early in the process to ensure the correct process-specific reagent is appropriately developed.



RELATED SERVICES

Rockland offers the following additional contract laboratory support services:

Antibody reagents
- Monoclonal and polyclonal antibody development
- Anti-idiotype development
- Anti-oligonucleotide development
- Anti-drug antibody development
- Single-domain (VHH) antibody development
- Conjugation and fragmentation
- Stability study design and execution
- Supply chain management

Molecular Biology
- Cloning
- Protein expression
- Protein purification
- Immunohistochemistry (IHC)
- Immunofluorescence microscopy (IF)
- Transient and stable cell line development

Cell Culture
- Cell storage
- Cell banking
- Cell line characterization
- Short Tandem Repeat (STR) profiling

Biomarker Discovery
- Proteome analysis
- Protein identification
- Peptide mapping
- Post-Translational Modification (PTM) identification

Assay Development
- Cell-based assay design and development
- Immunoassay design and development
- Assay qualification
- Assay validation

In addition to these related services, Rockland offers catalog reagents such as primary and secondary antibodies, controls, buffers, substrates, gamma globulins, and more. For a complete list, please visit **rockland.com.**

ROCKLAND IMMUNOCHEMICALS, INC.

For 55 years, Rockland has supported the research, diagnostic, and biopharma communities by providing the highest quality antibodies, assays, and research services including primary and secondary antibodies, chemiluminescent substrates, custom polyclonal and monoclonal antibody production, and assay development.

Rockland antibodies, substrates, buffers, and services adhere to QSR / cGMP with full reporting and traceability (CFR:21H part 820), as well as optional analysis options that include ELISA, WB, IF, IHC, HPLC, and SDS-PAGE (1-D, 2-D).

Protect your experiment with Rockland antibodies and services.

+1 484.791.3823
P.O. Box 5199
Limerick, PA 19468, USA
sales@rockland-inc.com
www.rockland.com