ROCKLAND

Host Cell Protein Antibody & Assay Development

Introduction

Biopharmaceutical, cell-based vaccines, and gene therapy products are required to be free of process related impurities. Residual Host Cell Protein (HCP) and other impurities must be monitored during the downstream bioprocessing process workflow and for finished drug product. Analytical strategies typically include orthogonal methods including ELISA, 2D immunoblot coverage assessment, and mass spectroscopy. A qualified custom polyclonal HCP antibody reagent with broad HCP coverage is critical to support a robust bioprocess control strategy.

Rockland provides customizable options for HCP antibody development and validation. These options allow flexibility for development of early-stage through late-stage HCP detection reagents and support supply chain security.

Services

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

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

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

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

HCP Antibody Generation Timeline

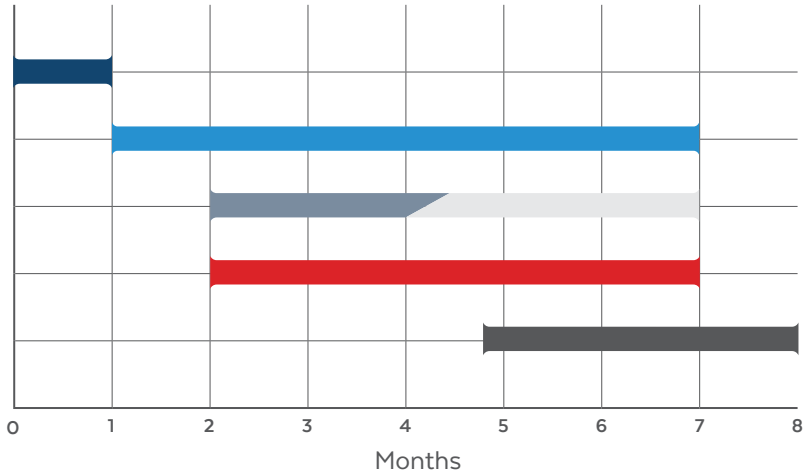
Setup
Antigen Preparation

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 over 20 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
This produces a robust and consistent HCP reference sample for use in any analytical work, including screening of antisera and qualification of the reagent in 2D Western blots and mass spectroscopy analysis.

HCP Immunogen Preparation
This 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.

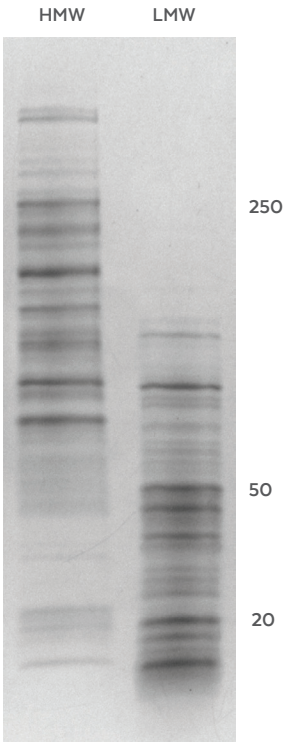


Figure 1. Typical enrichment of HCP sample into high and low molecular weights. Low molecular weight enriched fraction shown on right.

Antisera Generation

HCP antisera generation can be a time-consuming process requiring complex immunization strategies. Building on preparation of the HCP immunogen, one or more host animal cohorts and types can be initiated to create a highly diverse immune response.

Rockland technology and experience are used creates a broad HCP immune response, which results in high percent of coverage in 2D Western blot and orthogonal mass spectroscopy

Development strategies:

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

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 (large format also supported)

Mass spectroscopy (MS) and Immuno-MS (or ELISA-MS™)

Coverage Analysis by 2D Electrophoresis

2D coverage assessment is a mainstay analytical assay to characterize and qualify the HCP antibody as fit for purpose. Rockland performs coverage assessment by 2D immunoblot and can determine the percent HCP coverage of an anti-HCP antibody reagent using traditional or modern 2D-DIBE analytical methods.

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 (conventional or ELISA-MS) can provide additional information on the identity and quantity of residual HCPs present in a biopharmaceutical sample and thus provide additional support to other HCP analytical methods.

Total HCP characterization

Coverage evaluation

HCP analysis of drug substance

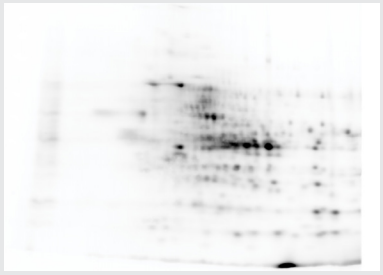


Figure 2a. Cy3-conjugated CHO-HCP protein resolved by 2D SDS-PAGE

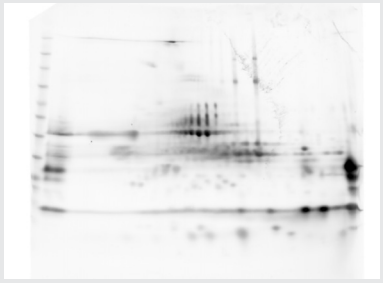
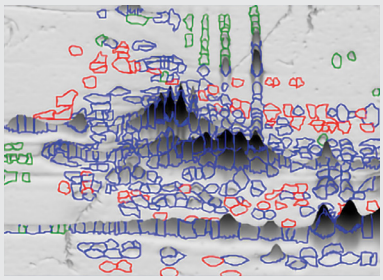


Figure 2b. CHO-HCP Western blot probed with Cy5-conjugated anti-CHO HCP antibody



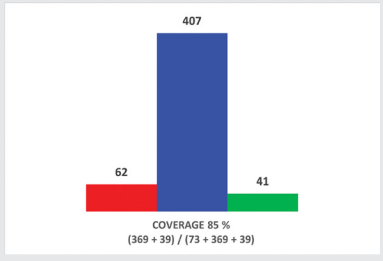


Fig 2c. DIBE coverage analysis of 7 cm 2D gel using Melanie software

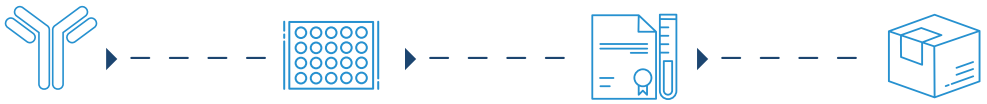
Immunoassay Development

Developing a robust analytical method for HCP monitoring is 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 also in the final drug substance biopharmaceutical product.

Using a qualified HCP antibody, Rockland can develop an HCP immunoassay that satisfies regulatory guidelines. Immunoassay development can be initiated as a standalone service or as part of a comprehensive 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.

Assay Development Workflow



Antibody Pair Screening
Testing capture and detection antibody pairs

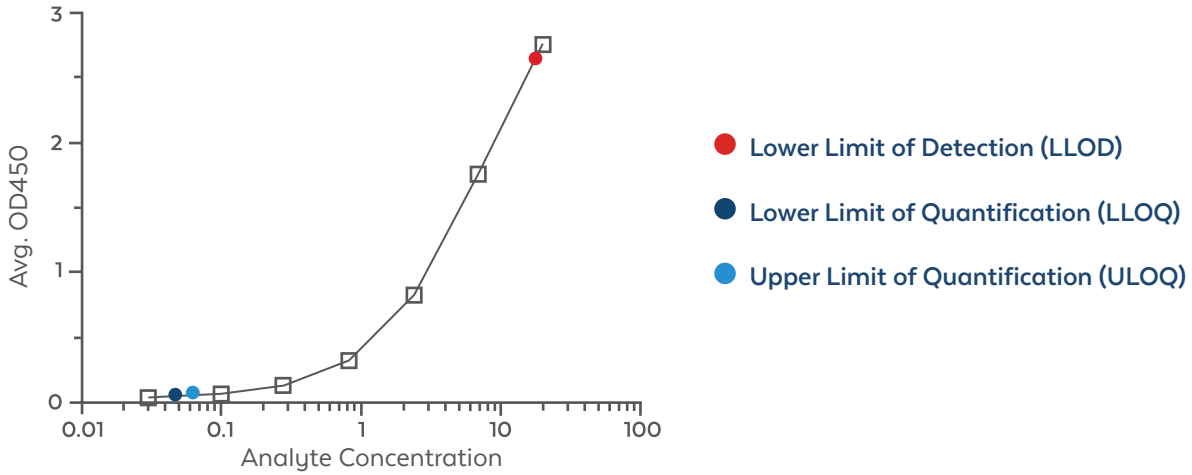
Assay Optimization
Optimizing assay conditions

Assay Validation
Validating assay performance

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

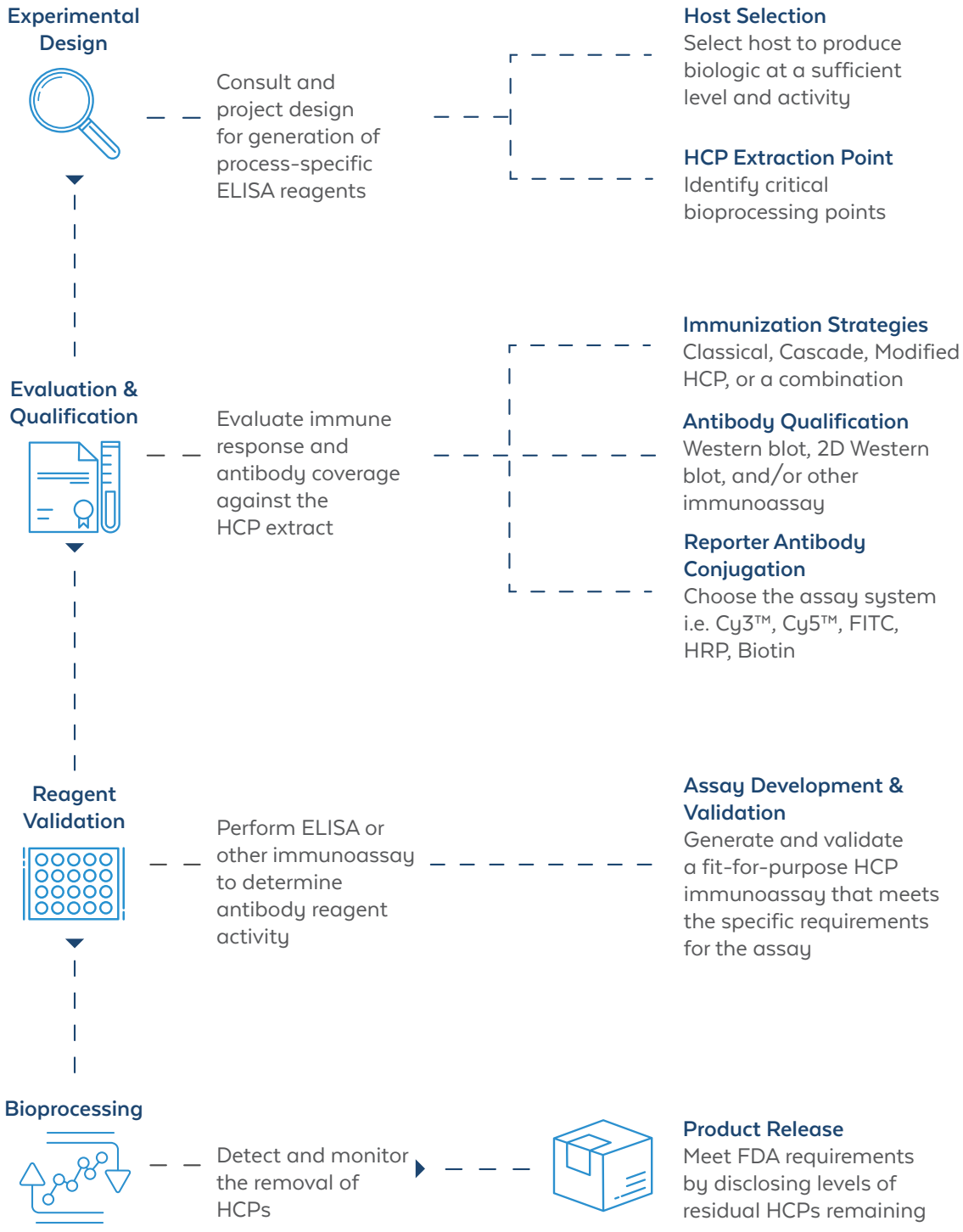
Key parameters:

Limits of quantification	Accuracy & precision	Dilutional linearity
Parallelism	Recovery	Robustness



HCP Immunoassay Development 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 Products & Services

Rockland offers the following additional contract laboratory support services and products:

Antibody Development
- Monoclonal and polyclonal
- Recombinant
- Anti-idiotype
- Anti-oligonucleotide
- Anti-drug
- Single-domain (VHH)

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 Design & Development
- Cell-based assay
- Immunoassay design
- Assay qualification
- Assay validation

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

Antibody Characterization
- Conjugation
- Fragmentation
- Antibody affinity assessment (OCTET)
- Stability program (study, design, and execution)

Impurity Detection Kits	Item No.
AccuSignal™ Nuclease Kit	KJE-4001
PrismA ELISA Kit	*Request information
Amersham™ HCPQuant™ CHO Kit (supernatant)	*Request information

Buffers & Substrates	Item No.
Blocking Buffer for Flourescent Western Blotting	MB-070
ELISA Microwell Blocking Buffer with Stabilizer	MB-064-0100
TMB ELISA Peroxidase Substrate	TMBE-100
Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate	FEMTOMAX-110

Supporting Reagents	Whole Molecule	Peroxidase	Biotin	Cy3	Cy5
Goat IgG	005-0102	-	-	-	-
Human IgG	009-0102	009-0302	-	-	009-010-002
Rabbit IgG	011-0102	011-0302	011-0602	-	-
Streptavidin	-	S000-03		S000-04	S000-06

Secondary Antibodies	Cy5	Cy3	Peroxidase	DyLight 680	DyLight 649
Anti-Goat IgG (H&L) Pre-Adsorbed	605-710-125	605-704-125	605-4313	605-444-013	605-743-125
Anti-Human IgG (H&L) Pre-Adsorbed	609-713-123	609-704-123	609-4317	609-144-123	609-743-123
Anti-Rabbit IgG (H&L) Pre-adsorbed	611-110-122	611-104-122	611-103-122	611-144-122	611-743-127

Rockland Immunochemicals, Inc.

For over 60 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.

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