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# Evaluation of novel high coverage generic CHO-HCP reagents

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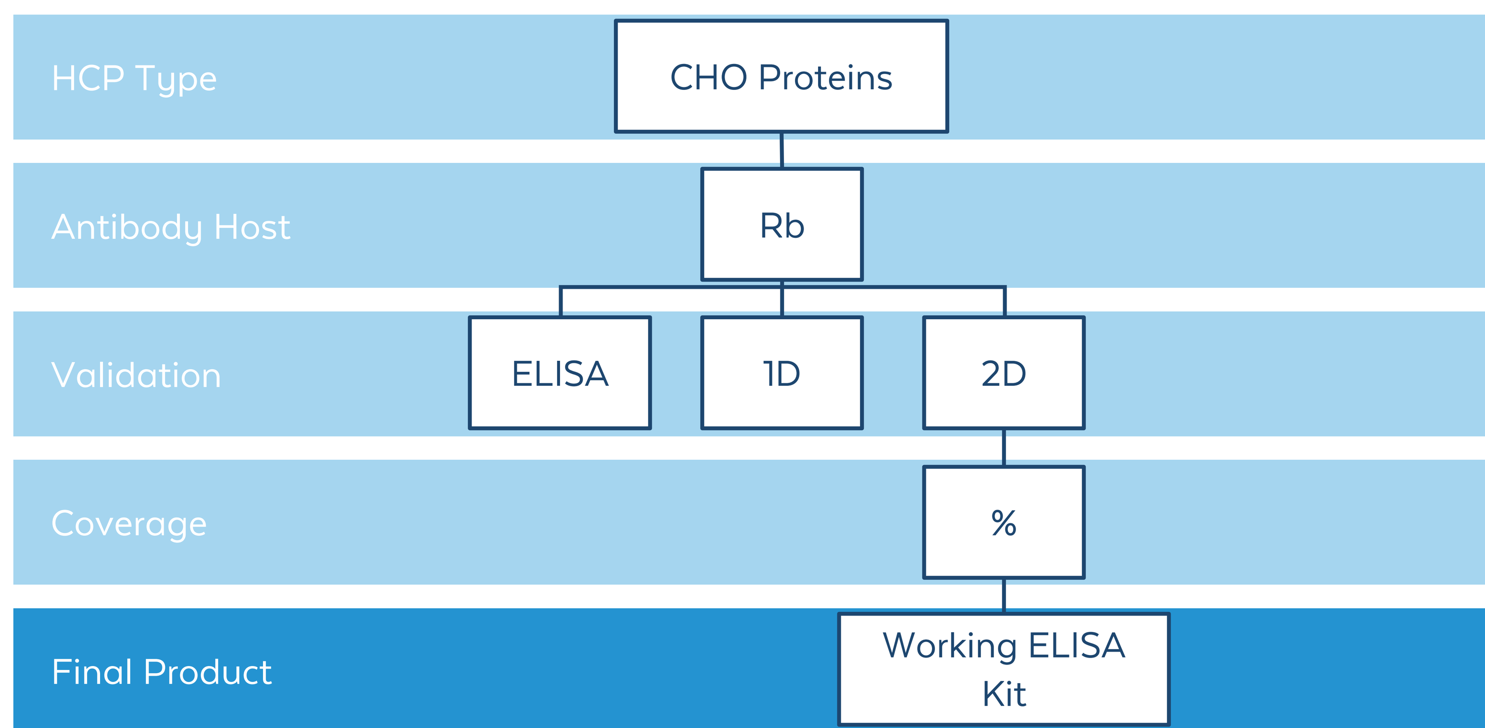
## INTRODUCTION

Efficient analysis of Host Cell Protein (HCP) impurities is a crucial prerequisite for testing products in the drug manufacturing process. Generic CHO HCP assays are frequently employed early in biologic development when the process is still poorly defined, biologic manufacturers often migrate to a process-specific HCP assay in later stages of development. The generic HCP assay has utility supporting early process development workflow.

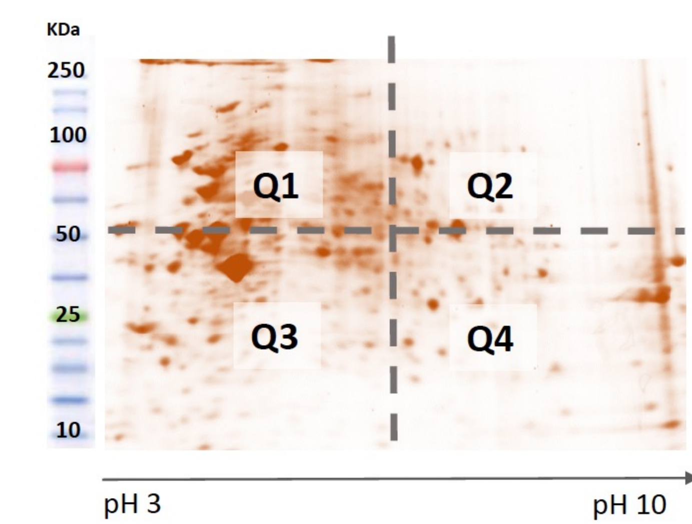
The quality of HCP antibody reagents can vary depending on antibody manufacturing conditions and robust characterization is therefore needed to ensure quality. HCP antibody reagents are most commonly evaluated via ELISA and orthogonal methods like one- and two-dimensional Western blot assays (1D and 2D) where the sensitivity and coverage of the antibody are established. Appropriate coverage across a 2D-blot is demonstrated using 2 criteria. First the percent coverage is determined by identifying the number of detected protein species compared to the number of existing species shown on a corresponding 2D SDS-PAGE. The second criteria is quadrant analysis, that is used to demonstrate the proper detection of proteins having diverse sizes and isoelectric points. Well-developed HCP reagents show broad coverage that includes the low molecular weight (LMW) proteins. Here we evaluate and demonstrate improved coverage of a new generic CHO-HCP reagent developed at Rockland. Comparison of coverage to a leading commercial reagent was performed. We observed differences in both percent total coverage between each reagent, with a significant variance in the lower quadrants.

## ANTIBODY DEVELOPMENT




HCP antibody development entails immunization of a representative sample of the HCP extract. The selection of this sample is critical, and can be taken from various stages of the bioprocess. In general, HCP-antibody generation requires the a variety of immunization protocols to obtain detection of low abundant or poorly-immunogenic proteins. Quality of the generated CHO-HCP antibody is validated by 1D and 2D-SDS-PAGE and western blot immunocoverage prior to development of ELISA based assays.

Here we analyze the coverage of a generic anti-HCP polyclonal antibody (generated for CHO proteins) as an alternative to customized reagents that are functional for HCP detection in 2D WB and ELISA.




**Quadrant analysis**:

Review of antibody coverage on each quadrant of the 2D gel. Appropriate coverage across a 2D-blot showing that proteins of all sizes and pI's are properly detected. Well-developed HCP reagents show broad coverage that includes the difficult-to-detect low molecular weight (LMW) proteins.

Q1: High molecular weight (HMW) proteins – above 50 kDa – in low pH region – below pH 6.5

Q2: HMW proteins in high pH region – above pH 6.5

Q3: Low molecular weight (LMW) proteins – below 50 kDa – in low pH region

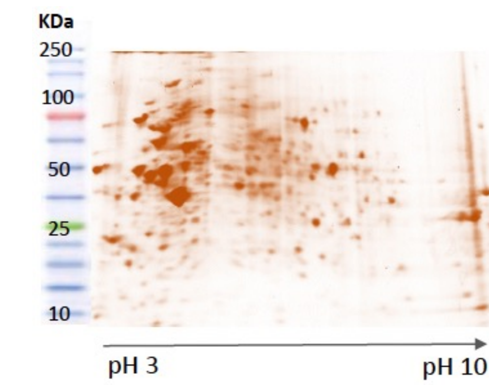
Q4: LMW proteins in high pH region

## 2D-WESTERN BLOT COVERAGE

CHO HCP proteins detected by a commercial and Rockland's anti-CHO HCP antibody on a membrane (WB) via HRP detection. Proteins are equated to the proteins separated on a separate SDS-gel and visualized by an high sensitivity in-gel protein stain.

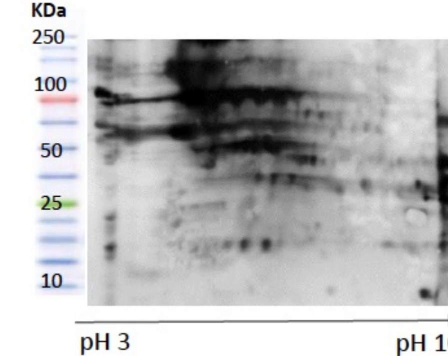
**A. Commercial Generic Antibody**

In-Gel Total Protein Stain (Oriole)



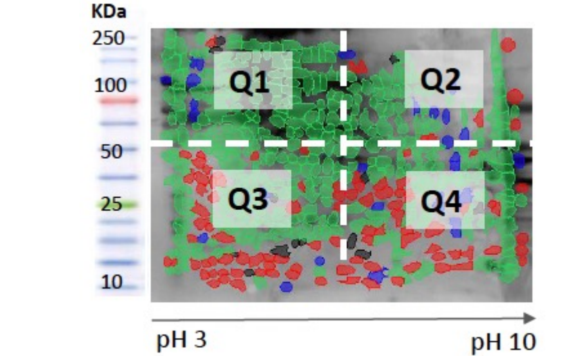

Sample and load: Total CHO HCP, 50 µg
Detection: Oriole
Exposure Time: Auto Exposure

Commercial Anti-CHO HCP Antibody Detection



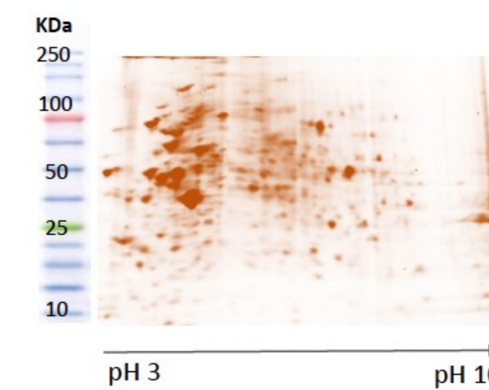

Sample and load: Total CHO HCP, 50 µg
Membrane: PVDF
1°Antibody: Commercial rb Anti-CHO HCP
1°Ab Dilution: pre-diluted
Detection: provided AlkPhosSubstrate
Exposure Time: Auto Exposure

Commercial Anti-CHO HCP Antibody Spot Designation in Quadrants

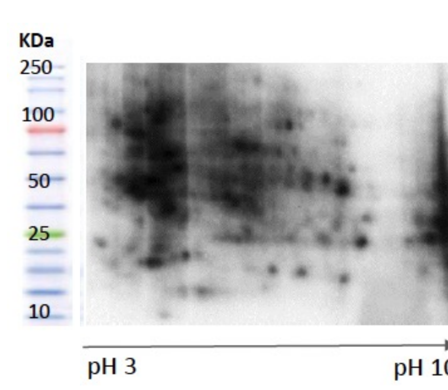



Coverage analysis performed using Melanie 9 software (GE Life Sciences)

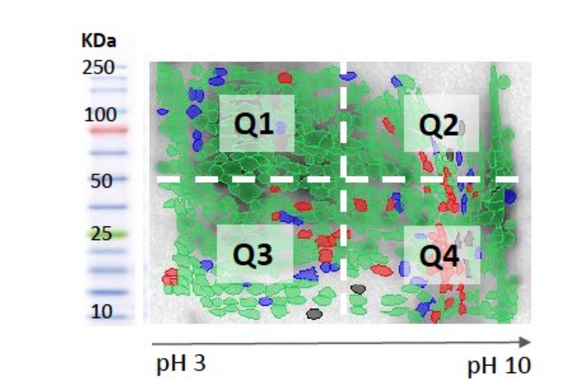
**B. Rockland Generic Antibody**




Sample and load: Total CHO HCP, 50 µg
Detection: Oriole
Exposure Time: Auto Exposure

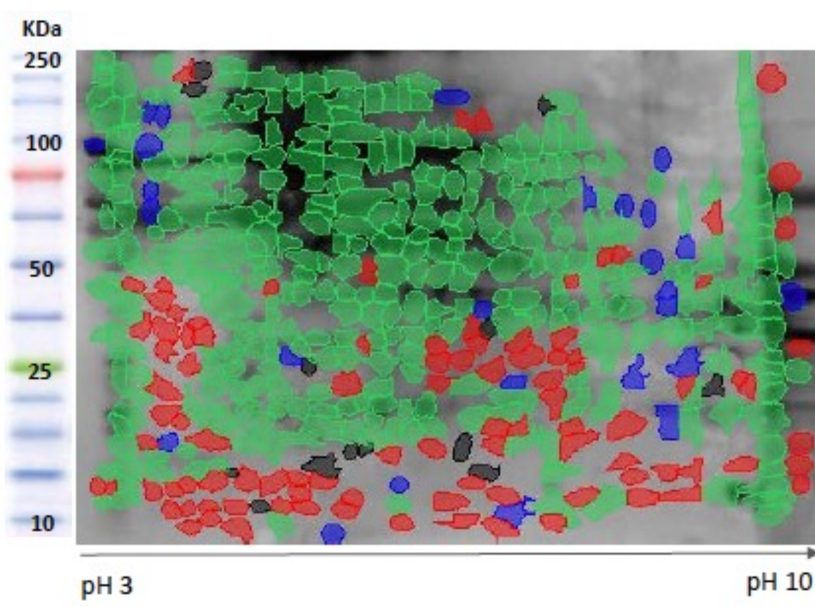


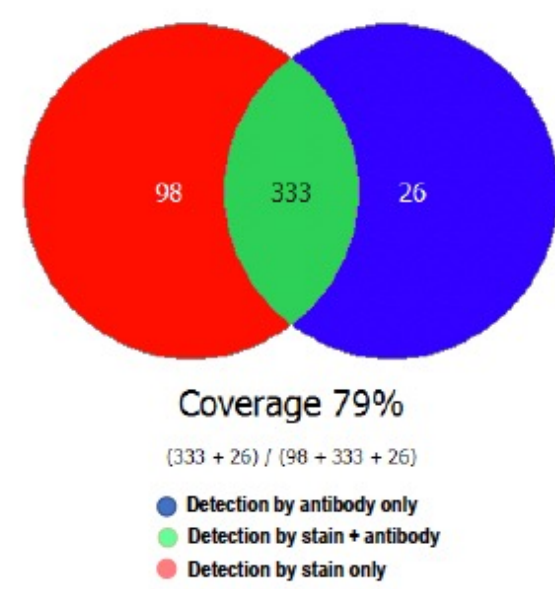

Sample and load: Total CHO HCP, 50 µg
Membrane: PVDF
1°Antibody: Rockland rbAnti-CHO HCP
1°Ab Dilution: 1:50000
2°Ab: Rabbit IgG (H&L) Antibody HRP Conjugated (611-703-127)
2°Ab Dilution: 1:40000
Detection: FemtoMax™ (FEMTOMAX-110)
Exposure Time: Auto Exposure




Coverage analysis performed using Melanie 9 software (GE Life Sciences)

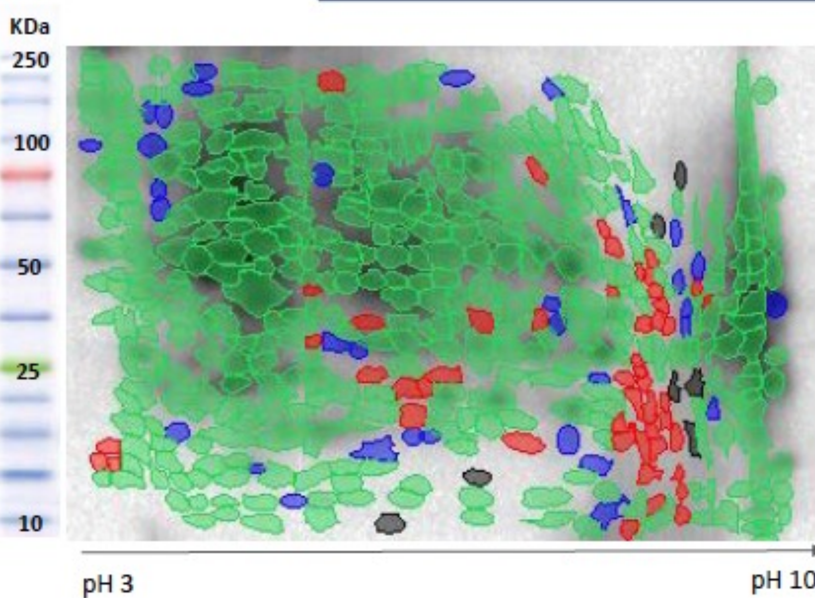
## COMPARATIVE RESULTS & CONCLUSIONS




Coverage 79%
(333 + 26) / (98 + 333 + 26)
Detection by antibody only
Detection by stain + antibody
Detection by stain only

**Rockland Anti-CHO HCP Antibody Coverage**




49 382 33

Coverage 89%
(382 + 33) / (49 + 382 + 33)
Detection by antibody only
Detection by stain + antibody
Detection by stain only

**Commercial Anti-CHO HCP antibody**:

- Sensitivity and immunoreactivity of the antibody is robust for proteins in the top 2 quadrants (Q1 and Q2) of the 2D protein map.
- Commonly difficult to detect proteins in the lower molecular weight regions (Q3 and Q4) are identified by the commercial anti-CHO HCP-antibody. Coverage in these 2 quadrants is between 50% and 70%.
- Total coverage of the antibody is 79%

| Coverage Analysis | Total Coverage | Q1 | Q2 | Q3 | Q4 |
|---|---|---|---|---|---|
| Commercial Antibody | 79% | 96% | 92% | 67% | 59% |
| Rockland Antibody | 89% | 99% | 91% | 92% | 76% |

**Rockland Anti-CHO HCP antibody**:

- Sensitivity and immunoreactivity of the antibody is consistent for proteins in all 4 quadrants of the 2D protein map.
- Commonly difficult to detect low molecular weight proteins, regions (Q3 and Q4) are identified by the Rockland anti-CHO HCP-antibody presented here. The coverage of the Q3 and Q4 quadrants is between 70% and 90%.
- Low concentrations (1:50000 dilution) of anti-CHO-HCP antibody are sufficient to obtain good signal in 2D type experiments.
- Total coverage of the antibody across all quadrants is 89%.
- Development of a generic ELISA for this CHO-HCP reagent is presently ongoing

## REFERENCES

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