# Production of Anti-CHO HCP Antibody for quantification of CHO Host Cell Proteins

Project Code: CHO HCP polyclonal antibody

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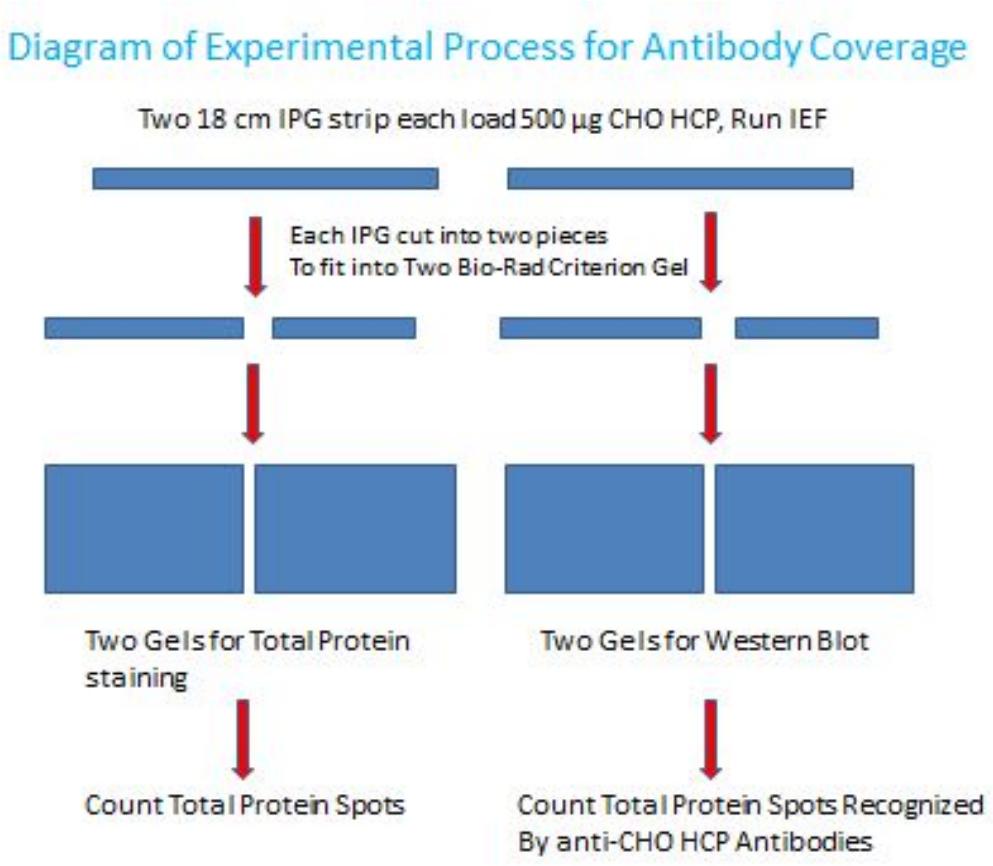
# 2-D GEL ELECTROPHORESIS AND 2-D WESTERN BLOT ANALYSIS OF CHO HCP IMMUNOGEN AND ANTI-CHO HCP ANTIBODY COVERAGE

The total protein detection from the CHO HCP preparation was carried out with two different lengths of Isoelectric Focusing (IEF) gels from Bio-Rad, the 11 cm and 18 cm IPG strips respectively. The goal of the evaluation is to find an optimum condition so that as many CHO HCP proteins as possible could be detected, at the same time, the gel resolution and repeatability should meet the requirement of a 2-D Western Blot.

Figure. 1. Diagram of the 2-D Western Blot Antibody Coverage Analysis



## Melanie 7 Analysis of Antibody Coverage

- All the images (total protein staining and Western Blot) were analyzed using the following same parameters: Smooth: 4; Saliency: 1; Minimum Area: 18.
- Artifact spots were deleted manually.
- Spot account performed by the software.

Figure. 2. Total protein detection of acidic to neutral pH CHO HCPs stained with Sypro Ruby

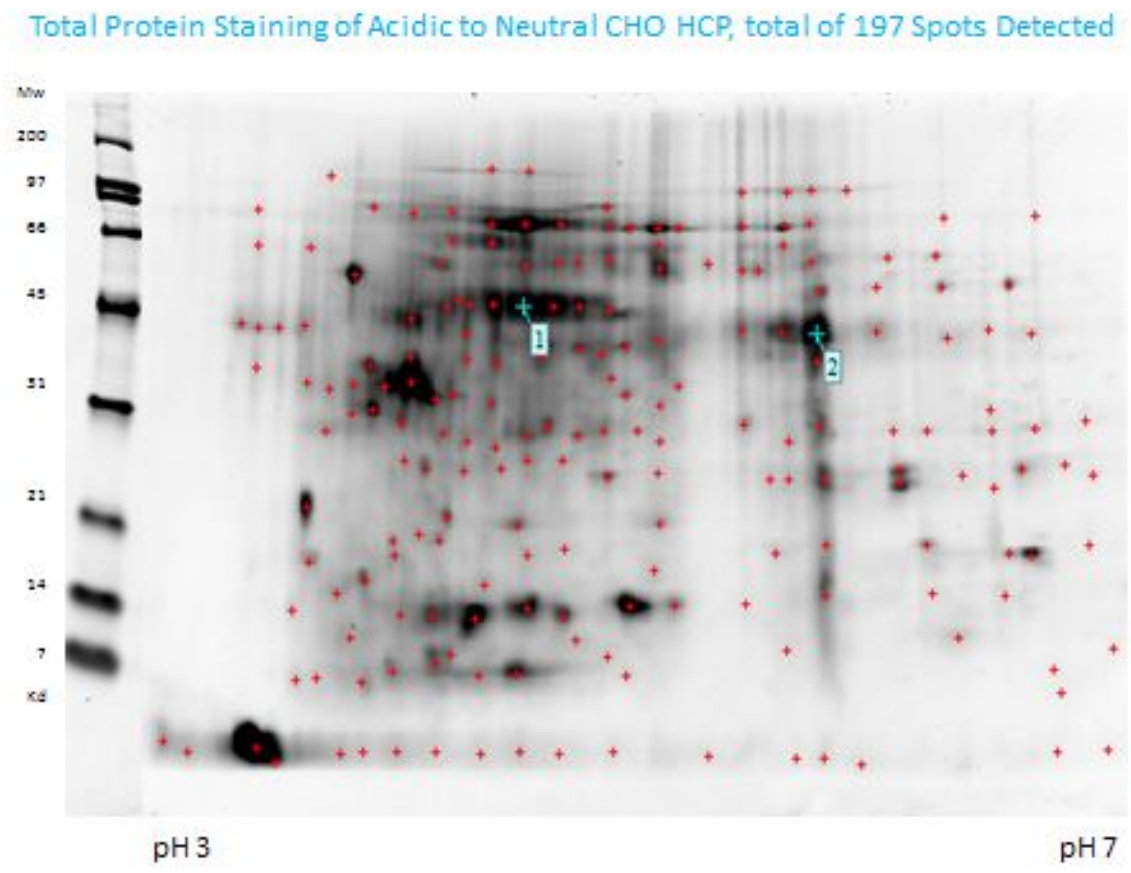
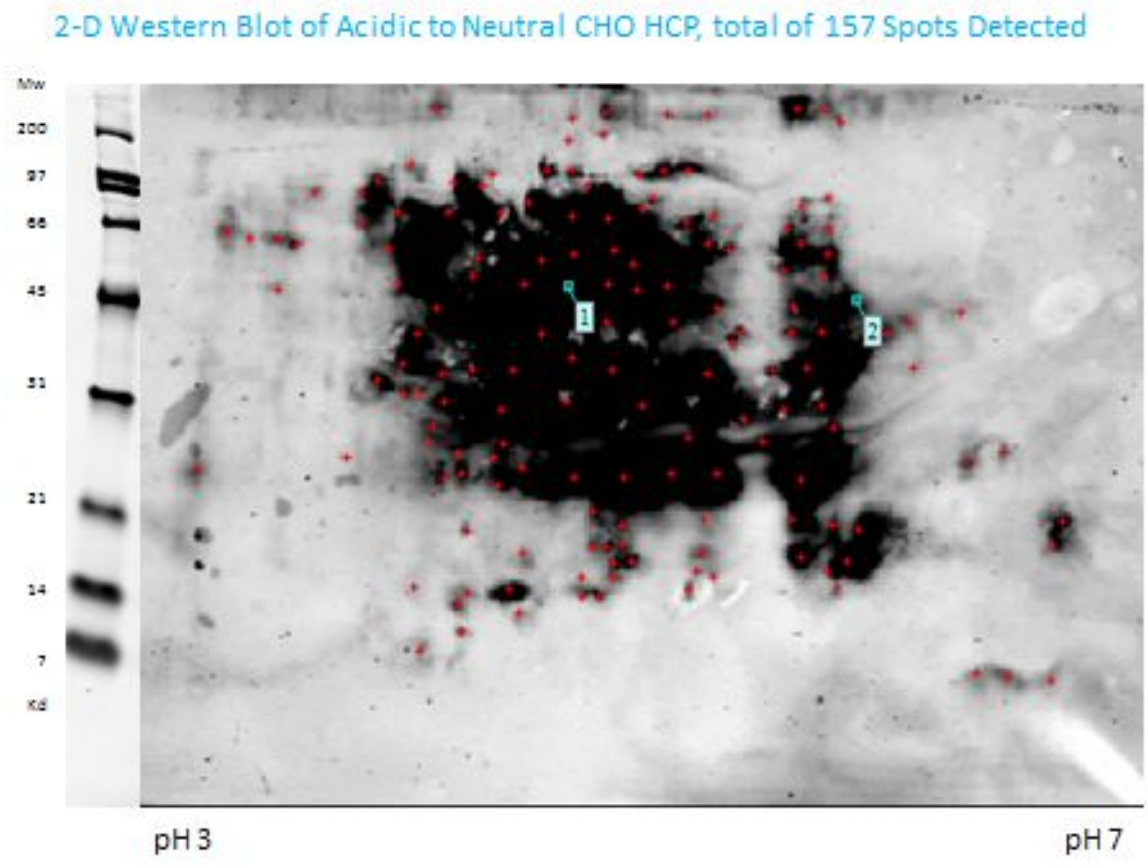
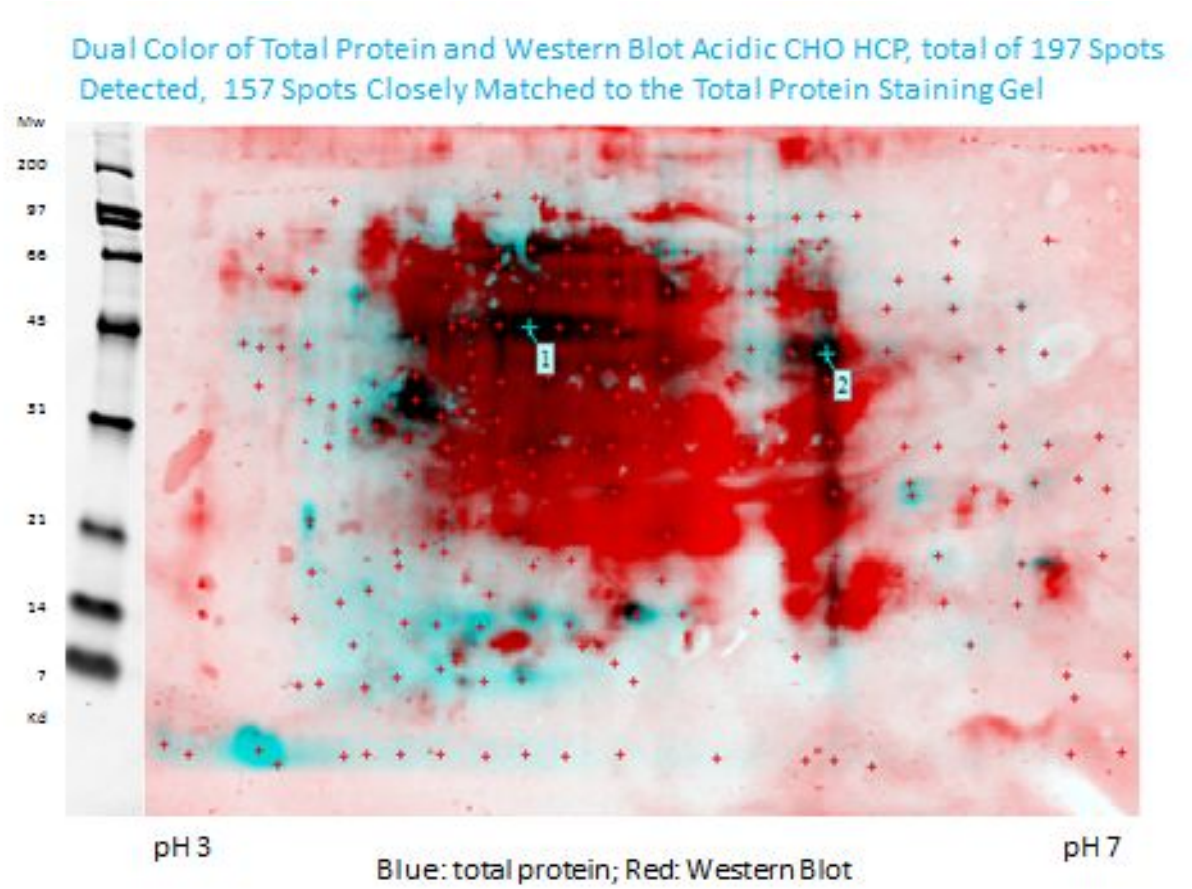


Figure. 3. Western Blot analysis of anti-CHO HCP antibody coverage for acidic to neutral pH HCPs




The artifact spots detected along the edge of the gel were deleted before total spot count.

Figure. 4. Total protein and Western Blot overlay for antibody coverage analysis




The analysis for the anti-CHO HCP antibody coverage was conducted with different scan sensitivity for the 2-D Western Blot. The graph above is the most sensitive 2-D Western Blot scan over the total protein spots. The reason for this approach is to detect as many protein spots as possible in the 2-D Western Blot analysis so that an objective coverage analysis can be conducted. If only less sensitive scan was used, the number of protein spots detected will be under estimated thus missing many of the minor spots even if they are detected by the corresponding antibodies.

Figure. 5. Total protein staining of neutral to basic CHO HCPs detected with Sypro Ruby staining

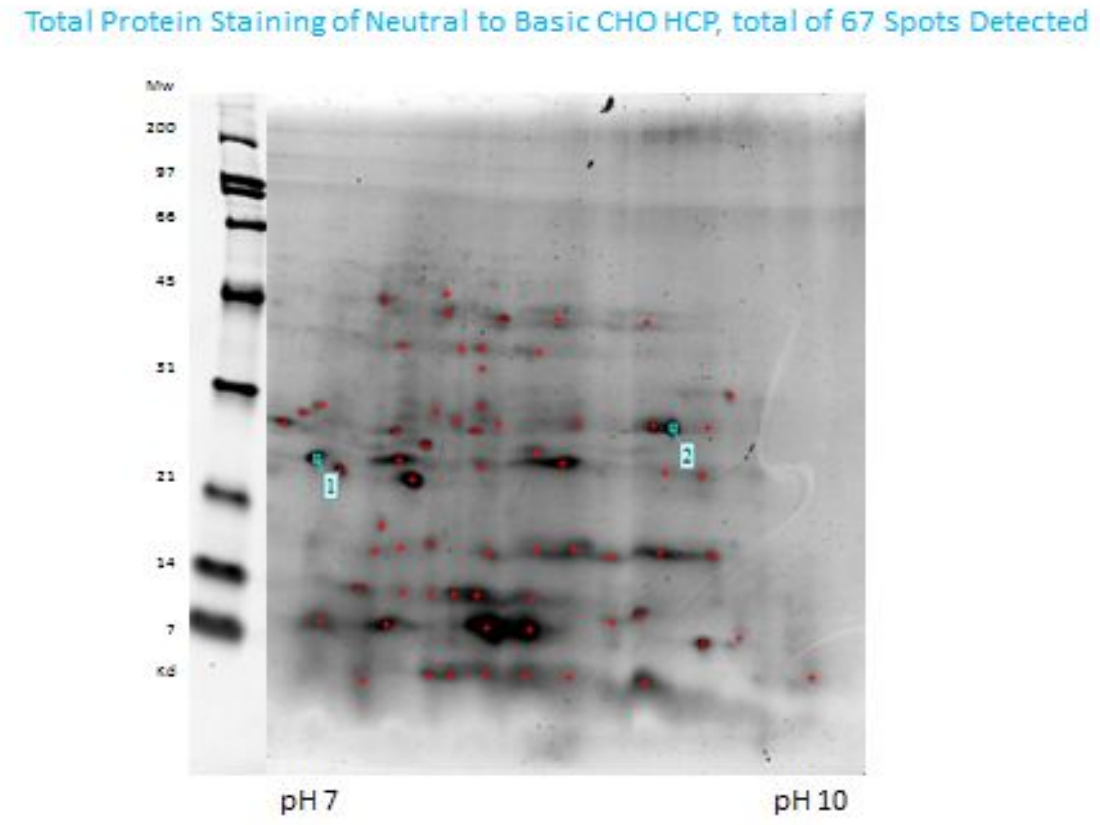


Figure. 6. 2-D Western Blot analysis of neutral to basic CHO HCPs

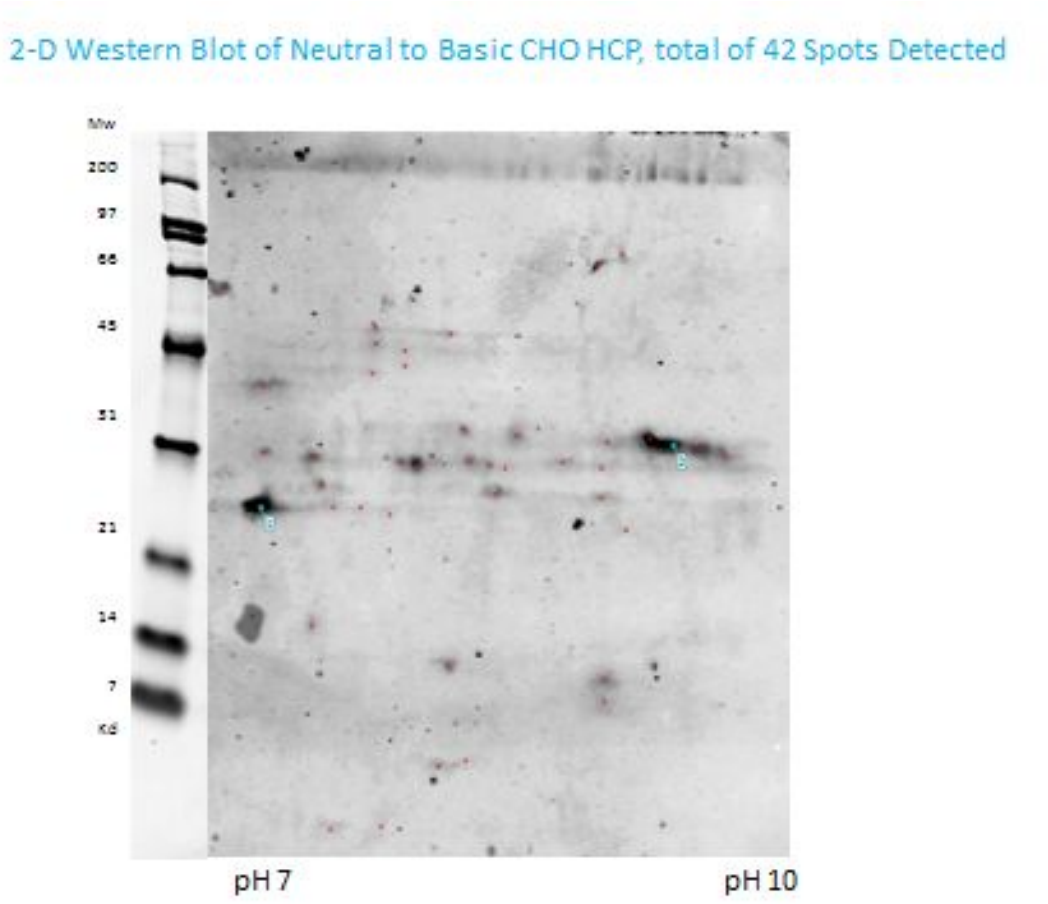
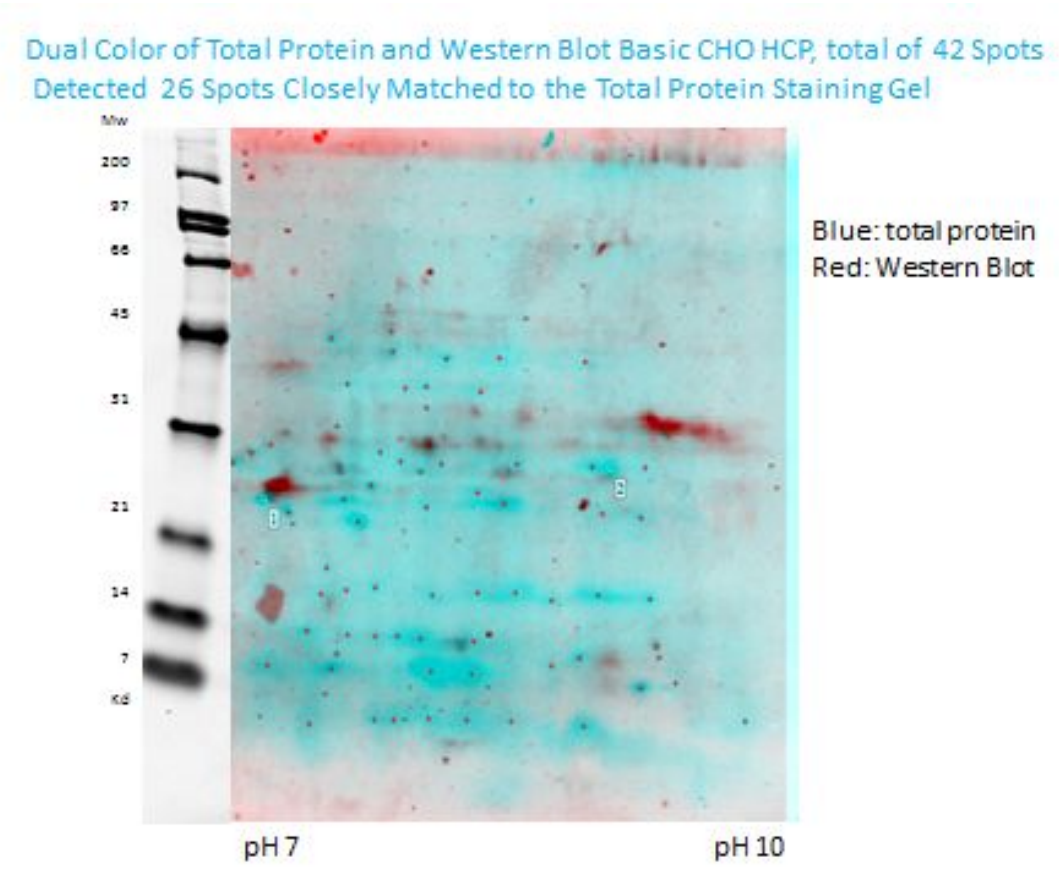


Figure 7. Total protein and Western Blot overlay for antibody coverage analysis



## Calculation of Anti-CHO HCP Antibody Coverage

1. Based on the Bio-Rad Published Method: (Total spots detected in Western Blot) /Total Spots detected in protein staining + additional spots detected in Western Blot)

Total protein spots detected: 197+ 67 = 264
Total Western Blot spots detected: 157 + 42 = 199
Total matched spots: 126 + 26 = 152
Total additional (unmatched Western Blot) spots: 31 + 16 = 47

Total Coverage (Bio-Rad method): 199/(264 + 47) = 199/311 = 64%.

2. Based on Celltrion Suggested Method: (Total spots matched in Western Blot/Total spots detected in protein staining)

Total Coverage: 152/264 = 58%.

Summary of the 2-D total protein staining and 2-D Western Blot analysis for anti-CHO HCP coverage: based on the analysis methods recommended by Bio-Rad, the anti-CHO HCP antibody coverage toward CHO HCPs are 64% (FDA required more than 50% coverage therefore this HCP antibody production meets the FDA and EMA requirement).

Figure 8. 2-D Western Blot Analysis of Anti-CHO HCP Antibody Coverage---Short Exposure

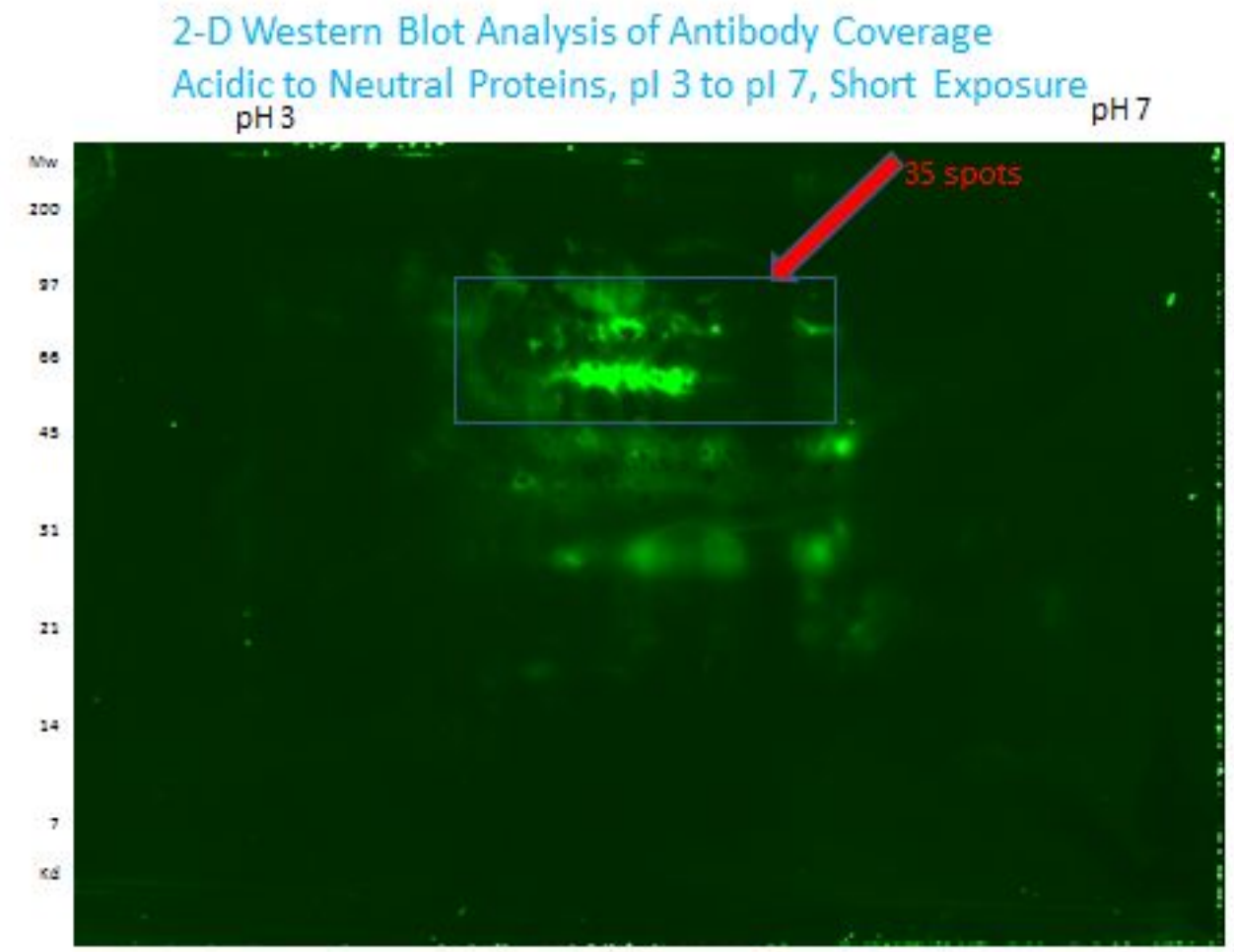

2-D Western Blot Analysis of Antibody Coverage
Acidic to Neutral Proteins, pI 3 to pI 7, Short Exposure
pH 3
pH 7
Mw
200
97
66
45
31
21
14
7
Kd
35 spots

Figure 9. 2-D Western Blot Analysis of Anti-CHO HCP Antibody Coverage---Medium Sensitivity

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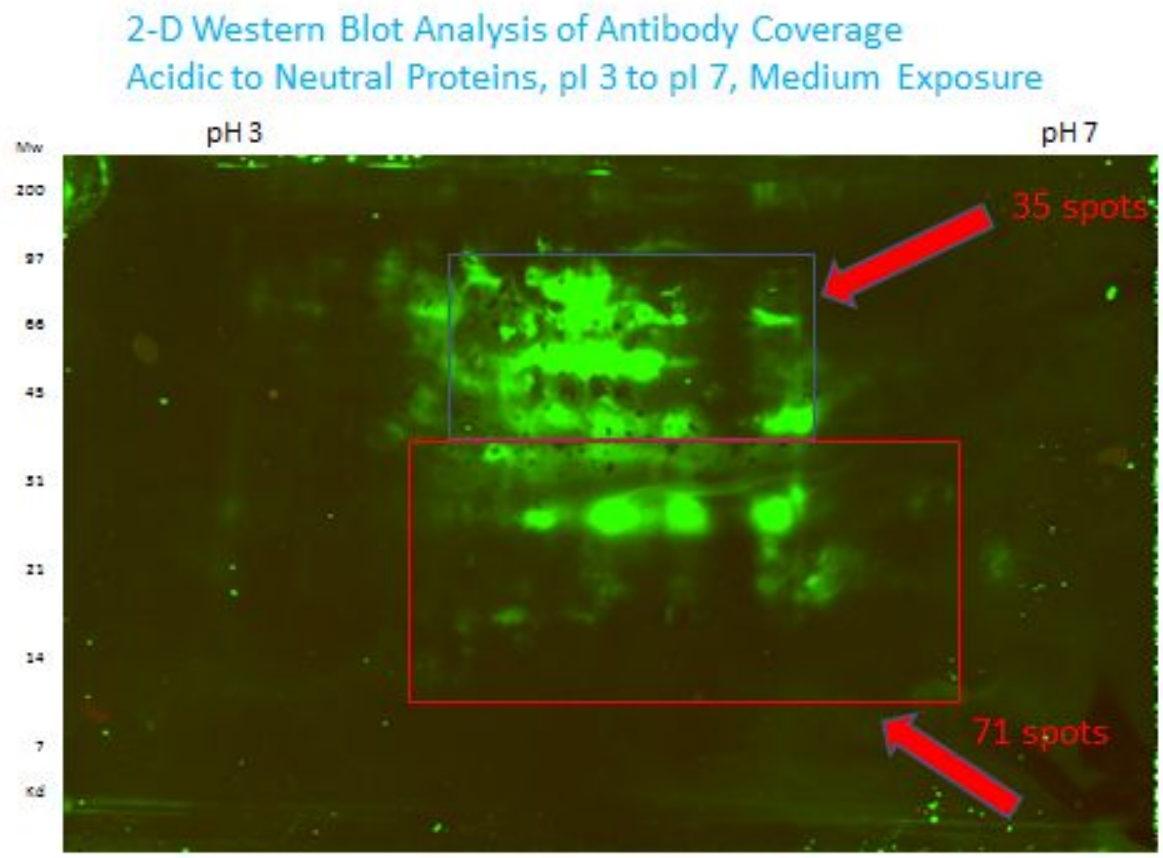
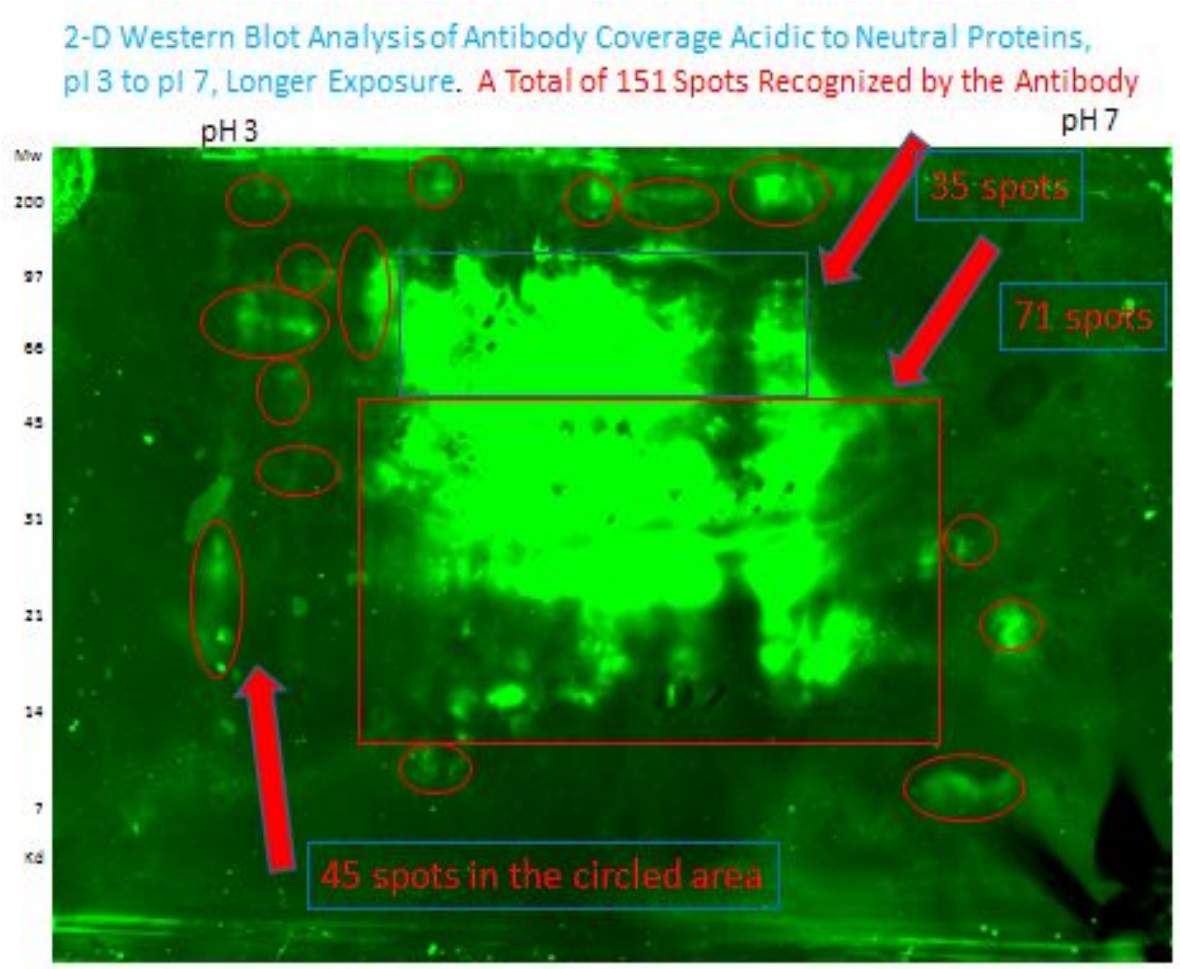


Figure 10. 2-D Western Blot Analysis of Anti-CHO HCP Antibody Coverage---Long Exposure




Based on the total protein spots detected by Melenie, a total of 264 spots were detected. In the sequencial exposure Western Blot analysis (fig. 13, 14 and 15 respectively), a total of 194 spots were detected (151 spots in acidic to neutral pH range and 43 spots in neutral to basic pH range). The anti-CHO HCP antibody coverage is calculated as 194/264 = 73%.

## CONCLUSIONS

2-D Western Blot analysis showed that the anti-CHO HCP antibodies generated provides good coverage toward the CHO HCP preparations with over 50% coverage. These results suggested that the CHO HCP antibody preparation could be used in both HCP quantitation using the sandwich ELISA and sensitive HCP detection in Western Blot analysis.