KRIBIOLISA™ CHO HCP ELISA

Validation Guide

Catalog No.: KBBP122

Kit Format	Sandwich ELISA
Detection Range	1 – 128 ng/ml
Limit of Detection	0.19 ng/ml
HCP Coverage (LC-MS)	86.8% (unique peptide ≥ 2)
HCP Coverage (2D)	71.0 – 82.6%
Manufacturing	ISO 13485
Regulatory Compliance	USP <1132>, EP <2.6.34>, ICH Q2(R2), ICH M10

1. Introduction

The KRIBIOLISA™ CHO HCP ELISA Kit (One-Step ELISA) is a high-sensitivity immunoassay for quantitative detection of Host Cell Proteins (HCPs) derived from Chinese Hamster Ovary (CHO) cell lines. HCPs are process-related impurities that may co-purify with biopharmaceutical drug substances and pose potential immunogenicity or safety risks. Their accurate measurement is a regulatory requirement across biopharmaceutical manufacturing.

This Validation Guide presents performance data generated in compliance with:

- USP <1132> – Residual Host Cell Protein Measurement in Biopharmaceuticals
- EP <2.6.34> – Host-Cell Protein Assays
- ICH Q2(R2) – Validation of Analytical Procedures
- ICH M10 – Bioanalytical Method Validation
- ChP <9012> – Guidance for Method Validation of Quantitative Analysis of Biological Samples
- YY/T1183-2010 – Chinese Pharmaceutical Industry Standard (ELISA Reagent/Kit)

2. Kit Overview & Intended Use

Parameter	Details
Kit Name	KRIBIOLISA™ CHO HCP ELISA Kit (One-Step ELISA)
Catalog Number	KBBP122
Assay Format	Double Antibody, Sandwich ELISA
Host Cell Line	Chinese Hamster Ovary (CHO)
Detection Range	1 – 128 ng/ml
Limit of Detection (LOD)	0.19 ng/ml
LLOQ	1 ng/ml
ULOQ	128 ng/ml
Intended Use	Quantitative detection of CHO HCPs in biopharmaceutical process samples
Regulatory Compliance	USP <1132>, EP <2.6.34>, ICH Q2(R2), ICH M10
Manufacturing Standard	ISO 13485

3. Linearity and Range

Linearity was assessed across the full working range of 1 to 128 ng/ml in three independent test runs. R^2 and percent CV/relative bias were evaluated at each calibration point.

3.1 Acceptance Criteria

- $R^2 \geq 0.990$ for all test runs
- CV ≤ 25% and relative bias within ±25% at LLOQ and ULOQ
- CV ≤ 20% and relative bias within ±20% at all intermediate concentrations

3.2 Standard Curve (Observed vs. Theoretical)

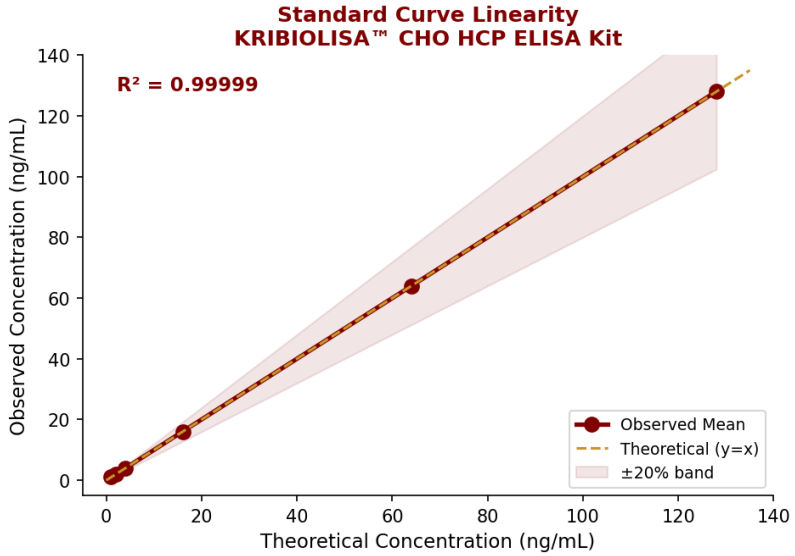


Figure 1. Standard curve linearity across the 1–128 ng/ml range. All three runs achieved $R^2 \geq 0.999$. Gold dashed line = theoretical y=x; shaded band = ±20%.

3.3 Linearity Data Table

Conc. (ng/ml)	Run 1 Mean	CV (%)	Bias (%)	Run 2 Mean	CV (%)	Bias (%)	Run 3 Mean	CV (%)	Bias (%)
1	1.08	11.8	+7.8	1.06	5.8	+6.2	0.99	5.5	-0.8
2	1.95	6.2	-2.7	2.03	8.2	+1.4	1.94	5.7	-3.1
4	3.94	3.2	-1.6	3.84	2.4	-4.0	4.10	3.5	+2.5
16	16.06	4.5	+0.4	16.10	1.7	+0.6	15.96	4.1	-0.3
64	63.97	1.8	0.0	63.95	1.7	-0.1	64.07	6.6	+0.1
128	128.02	0.6	0.0	128.08	4.6	+0.1	128.04	3.8	0.0
R^2	**1.0**			**0.99**			**1.0**		

4. Quantitation and Detection Limits

4.1 LLOQ and ULOQ

Concentration (ng/ml)	Mean Value (ng/ml)	CV (%)	Relative Bias (%)
1 (LLOQ, n=10)	0.94	8.8	-6.3
128 (ULOQ, n=10)	125.55	2.7	-1.9

Both LLOQ (1 ng/ml) and ULOQ (128 ng/ml) met acceptance criteria of CV ≤ 25% and relative bias within ±25%.

4.2 Limit of Detection (LOD)

The LOD was determined as blank signal mean + 2SD. The LOD of the KRIBIOLISA™ CHO HCP ELISA Kit is 0.19 ng/ml.

5. Accuracy

Accuracy was evaluated using QC samples at five concentration levels. Acceptance criteria: 75–125% recovery for LLOQ/ULOQ; 80–120% for all other QC levels.

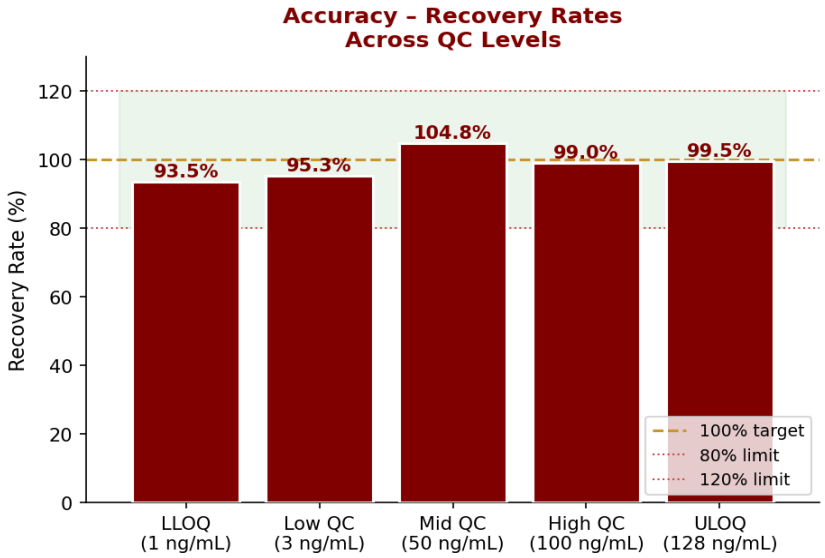


Figure 2. Recovery rates across all five QC concentration levels. All values fall well within acceptance limits (gold dashed = 100% target; red dotted = 80/120% limits).

QC Level	Theoretical Conc. (ng/ml)	Mean Value (ng/ml)	Recovery Rate (%)
ULOQ (n=3)	128	127.42	99.5%
High QC (n=3)	100	99.04	99.0%
Mid QC (n=3)	50	52.38	104.8%
Low QC (n=3)	3	2.86	95.3%
LLOQ (n=3)	1	0.93	93.5%

6. Precision

6.1 Repeatability (Within-Run)

Three QC levels tested in 10 replicates per run. All CV values ≤ 20%.

QC Level	Theoretical Conc. (ng/ml)	CV (%)
High QC	100	3.5%
Mid QC	50	3.6%
Low QC	3	5.6%

6.2 Intermediate Precision (Between-Run / Between-Analyst)

Five levels tested across 3 independent runs by 2 technicians (n=30/level). Acceptance: CV ≤ 25% for LLOQ/ULOQ; CV ≤ 20% for all others.

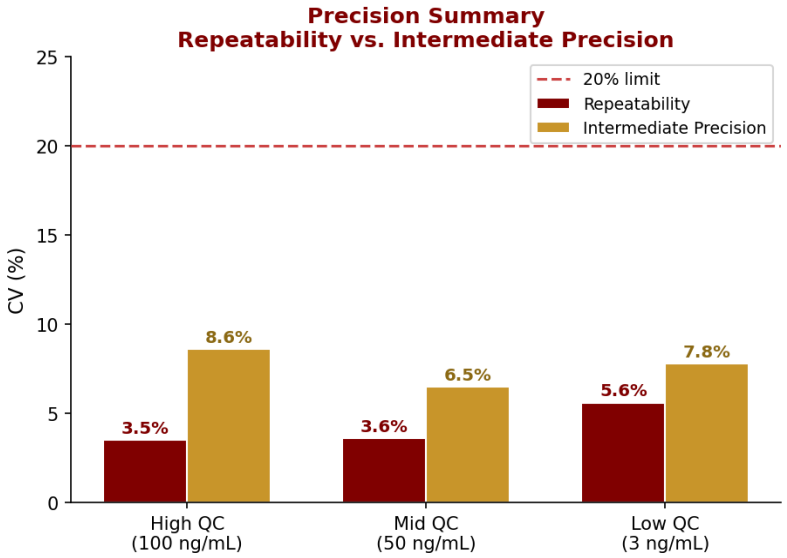


Figure 3. Precision summary – repeatability (maroon) vs. intermediate precision (gold). All values well within the 20% acceptance threshold (red dashed line).

ULOQ (128 ng/ml)	High QC (100 ng/ml)	Mid QC (50 ng/ml)	Low QC (3 ng/ml)	LLOQ (1 ng/ml)
113.04 ng/ml	91.49 ng/ml	48.52 ng/ml	2.91 ng/ml	1.00 ng/ml
7.5%	**8.6%**	**6.5%**	**7.8%**	**14.6%**

Row 1: Mean observed value (ng/ml) | Row 2: CV (%). All values within acceptance criteria.

7. Specificity

7.1 Cross-Reactivity with Non-CHO HCPs

HCPs from 7 non-CHO host cell lines were prepared at 1280 ng/ml and tested for cross-reactivity. All background signals were expected below the LLOQ; spiked CHO HCP recovery expected within 75–125%.

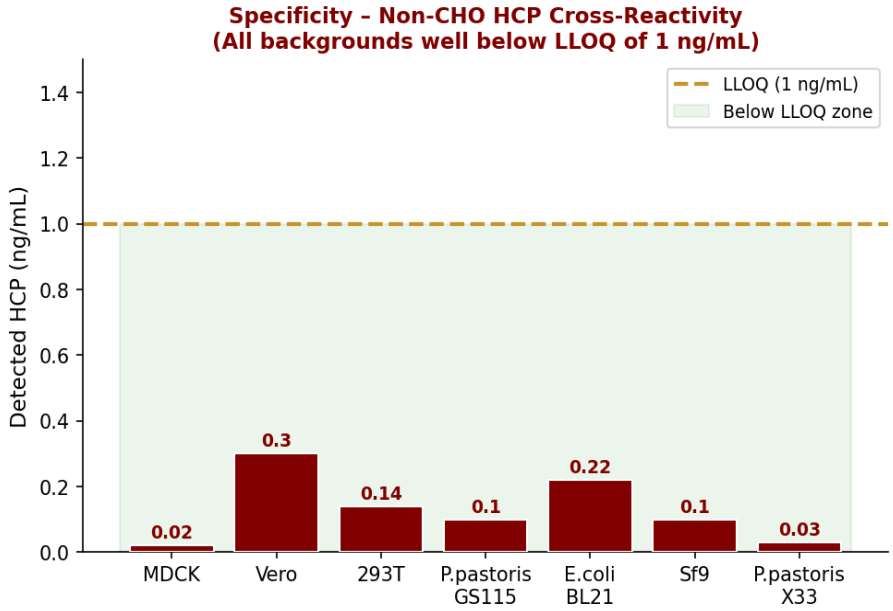


Figure 4. Cross-reactivity background signals for non-CHO host cell line HCPs (1280 ng/ml each). All backgrounds are well below the LLOQ of 1 ng/ml (gold dashed line).

Host Cell Line	Background (ng/ml)	Spiked Conc. (ng/ml)	Recovery @ 1 ng/ml	Recovery @ 128 ng/ml
MDCK	0.02	1 / 128	79.8%	88.9%
Vero	0.30	1 / 128	89.8%	83.6%
293T	0.14	1 / 128	83.9%	87.6%
P. pastoris GS115	0.10	1 / 128	90.7%	93.8%
E. coli BL21	0.22	1 / 128	76.2%	95.3%
Sf9	0.10	1 / 128	98.9%	99.9%
P. pastoris X33	0.03	1 / 128	106.0%	112.7%

7.2 Matrix Effect / Selectivity (pH Range)

Sample Matrix	Spiked Conc. (ng/ml)	Recovery Rate (%)
20 mM Phosphate Buffer pH 6.5	1	102.4%
20 mM Phosphate Buffer pH 7.0	1	102.2%
20 mM Phosphate Buffer pH 8.5	1	79.4%

8. HCP Antibody Coverage

HCP antibody coverage reflects the breadth of the polyclonal antibody system's ability to recognize the full CHO HCP repertoire. Two orthogonal methods were applied as recommended by USP <1132> and EP <2.6.34>.

8.1 Coverage Methodology – Venn Diagram

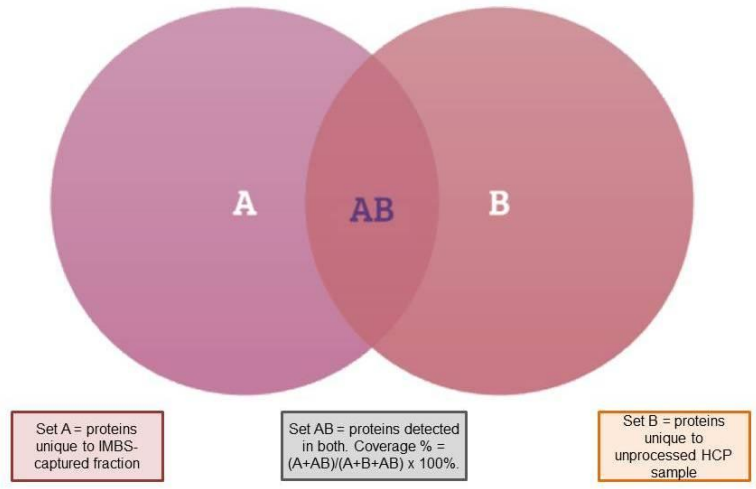


Figure 5. Antibody coverage analysis schematic. A = proteins unique to IMBS-captured fraction; B = proteins unique to unprocessed HCP sample; C = proteins detected in both. Coverage% = (A+C)/(A+B+C) × 100%.

8.2 IMBS-LC-MS Method

Anti-CHO HCP antibodies were covalently immobilized on magnetic beads (IMBS) to capture CHO HCPs. Captured and uncaptured fractions were processed by denaturation, reduction, alkylation, and tryptic digestion, then analyzed by UPLC-ESI-Orbitrap MS. Data processed using Proteome Discoverer 2.5 against the Cricetulus griseus UniProt database.

QC Indicator 1 – Enzyme Digestion Efficiency:

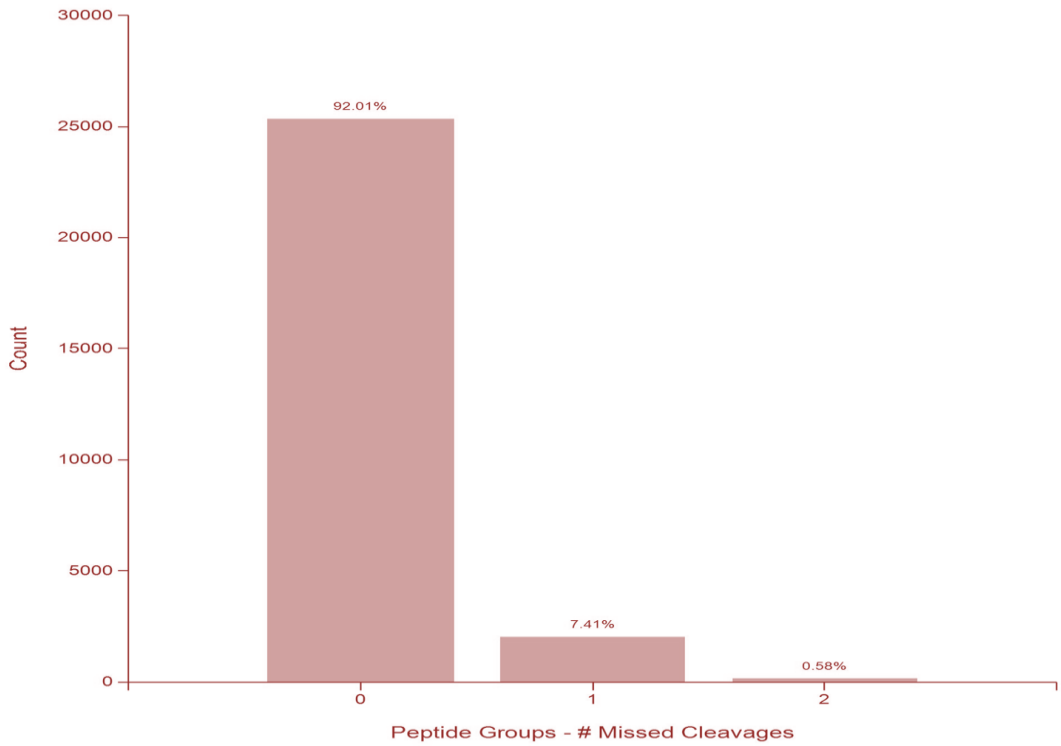


Figure 6. Missed cleavages analysis: >92% of peptides had zero missed cleavage sites, confirming highly efficient tryptic digestion.

QC Indicator 2 – Mass Spectrometry Accuracy:

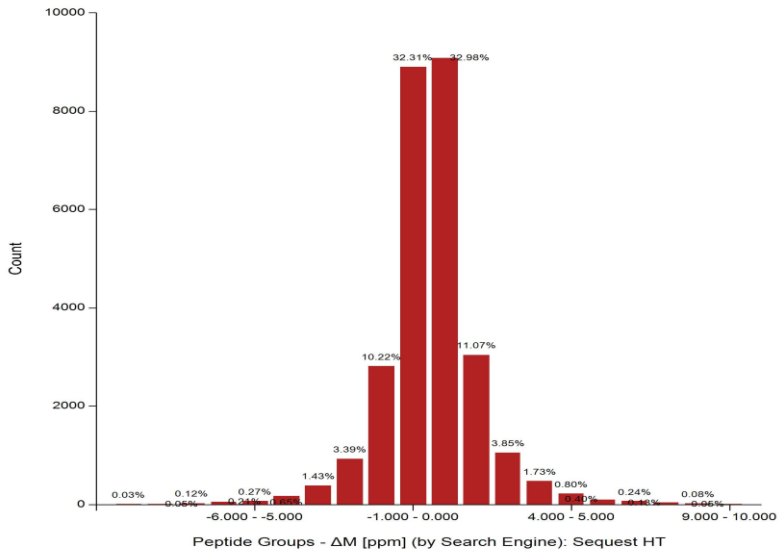
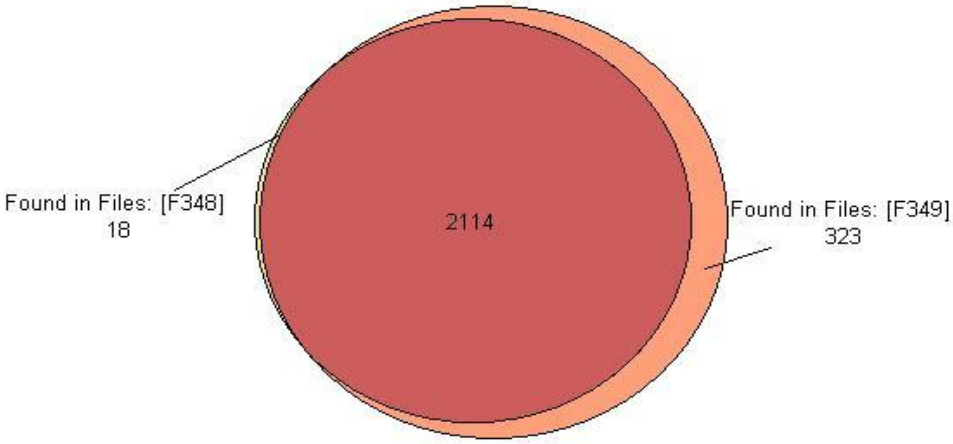


Figure 7. Peptide mass tolerance analysis (ΔM, ppm). The deviation between identified and expected masses is within ±10 ppm, confirming high instrument accuracy and reliability.

Coverage Result – Protein Identification Overlap:



	Exclusive	Total	Label
A	18	2132	Found in Files: [F348]
B	323	2437	Found in Files: [F349]
A | B	2114	2114	Found in Files: [F348] | Found in Files: [F349]
Sum	2455		

Figure 8. Protein identification Venn diagram: CHO HCP sample (F349) = 2,437 proteins; IMBS-captured sample (F348) = 2,132 proteins; overlap = 2,114. IMBS-LC-MS coverage = 86.8%.

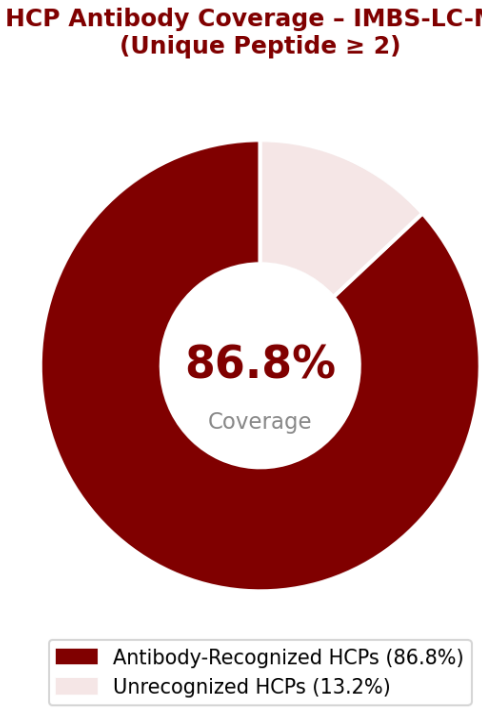


Figure 9. Antibody coverage donut chart – 86.8% of identified CHO HCPs are recognized by the KRIBIOLISA™ polyclonal antibody system (IMBS-LC-MS method, unique peptide ≥ 2).

Parameter	Result
Total proteins in CHO HCP sample (F349)	2,437
Total proteins in IMBS sample (F348)	2,132
Proteins detected in both (C)	2,114
Proteins unique to IMBS fraction (A)	18
Proteins unique to unprocessed HCP (B)	323
HCP Antibody Coverage	86.8%

8.3 IMBS-2D Electrophoresis Method

Two-dimensional silver-stain SDS-PAGE (18 cm, pH 3–10 NL strips, 150 µg protein load) was used as an orthogonal confirmatory method. Spot matching was performed with PDQuest Basic v8.0.1 (Bio-Rad).

First-Dimension Isoelectric Focusing Quality Control:

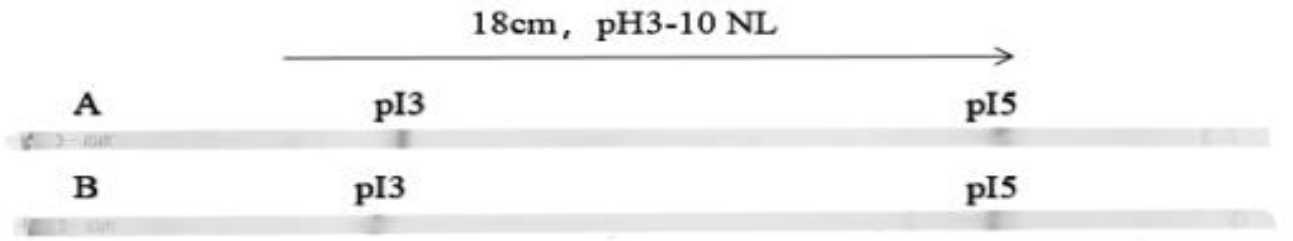


Figure 10. First-dimension IEF strip scans. pI3 and pI5 quality control markers are clearly resolved in both untreated HCPs (A) and IMBS-treated HCPs (B), confirming valid IEF separation.

2D SDS-PAGE Silver Stain – CHO HCPs (Untreated):

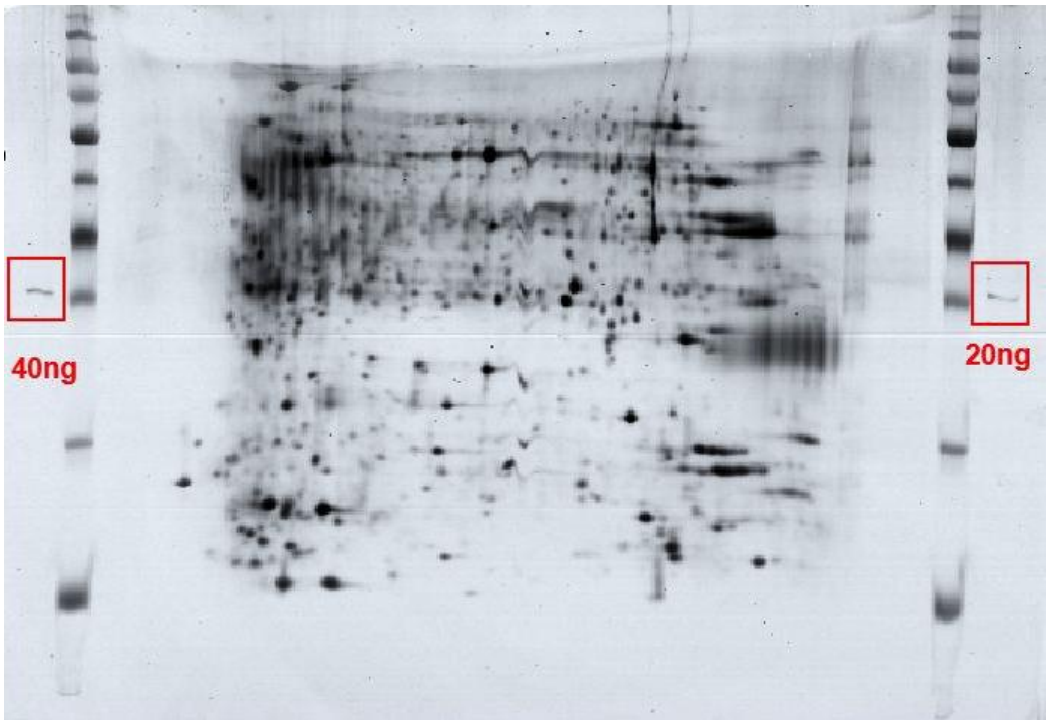


Figure 11. 2D electrophoresis silver stain of CHO HCPs (18 cm, pH 3–10 NL, 150 µg). Quality control bands (40 ng and 20 ng) confirm high silver stain sensitivity. 988 protein spots were identified.

2D SDS-PAGE Silver Stain – CHO HCPs After IMBS Treatment:

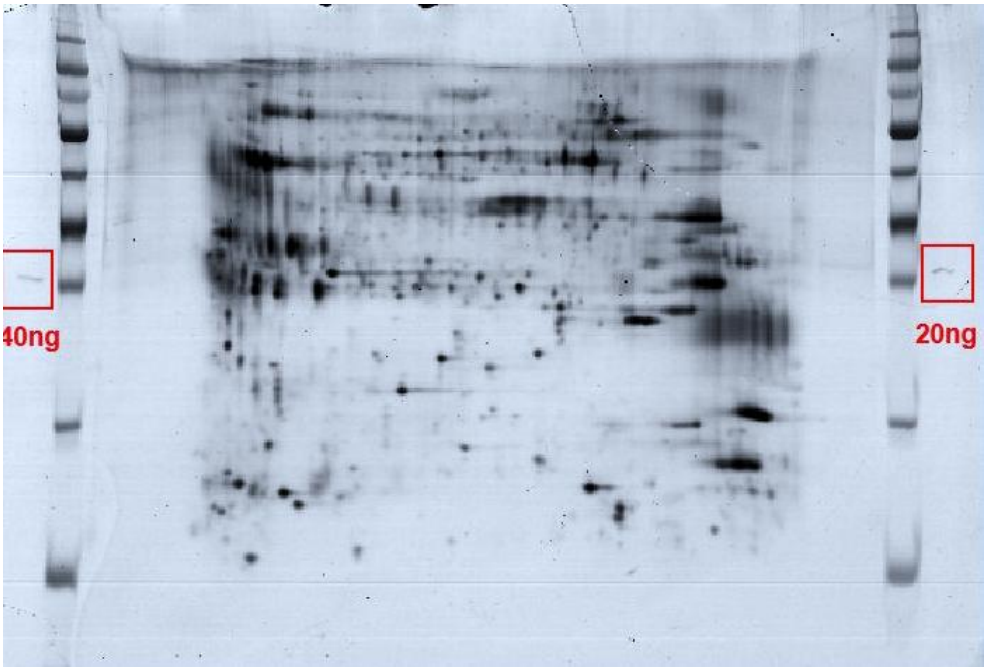


Figure 12. 2D electrophoresis silver stain of IMBS-treated CHO HCPs. The reduced but well-distributed spot pattern reflects proteins captured by the polyclonal antibody. 816 spots were identified.

PDQuest Spot Matching Analysis:

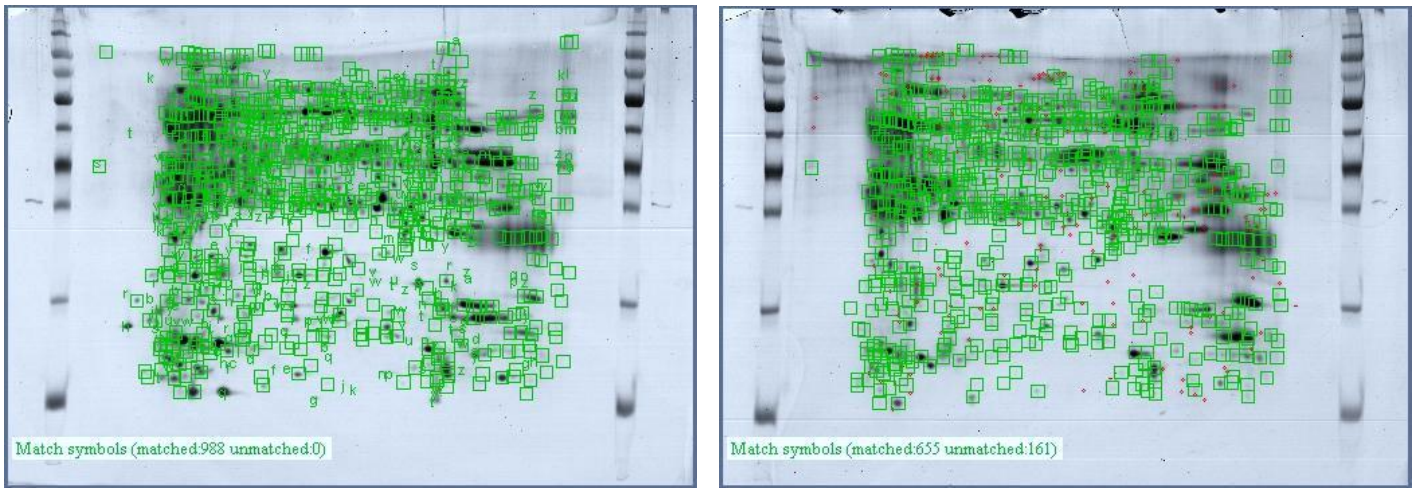


Figure 13. PDQuest spot mapping: CHO HCPs (left, 988 matched spots) and IMBS-treated HCPs (right, 655 matched of 816 total). Green boxes = matched spots; red crosses = unmatched.

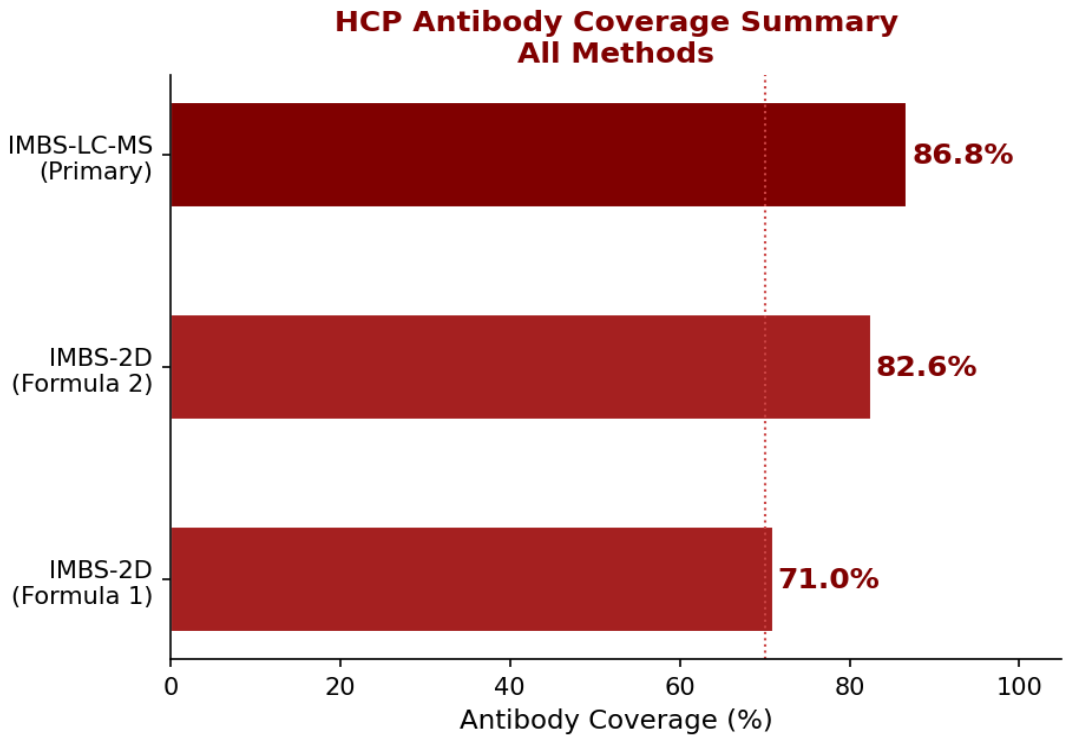


Figure 14. HCP antibody coverage by all methods. IMBS-LC-MS provides the highest resolution result (86.8%); IMBS-2D confirms coverage in the 71.0–82.6% range using two calculation formulas.

Method	Coverage Result	Reference
IMBS-LC-MS (primary)	86.8%	USP PF49(3) <1132.1>
IMBS-2D Formula 1	71.0%	USP40 <1132>, EP <2.6.34>
IMBS-2D Formula 2	82.6%	USP40 <1132>, EP <2.6.34>

9. Robustness

Robustness was assessed at the boundary limits of the recommended incubation temperature (25°C ± 3°C) — at 22°C and 28°C. Acceptance: CV ≤ 25% and relative bias within ±25% for LLOQ/ULOQ; CV ≤ 20% and bias within ±20% for internal QC.

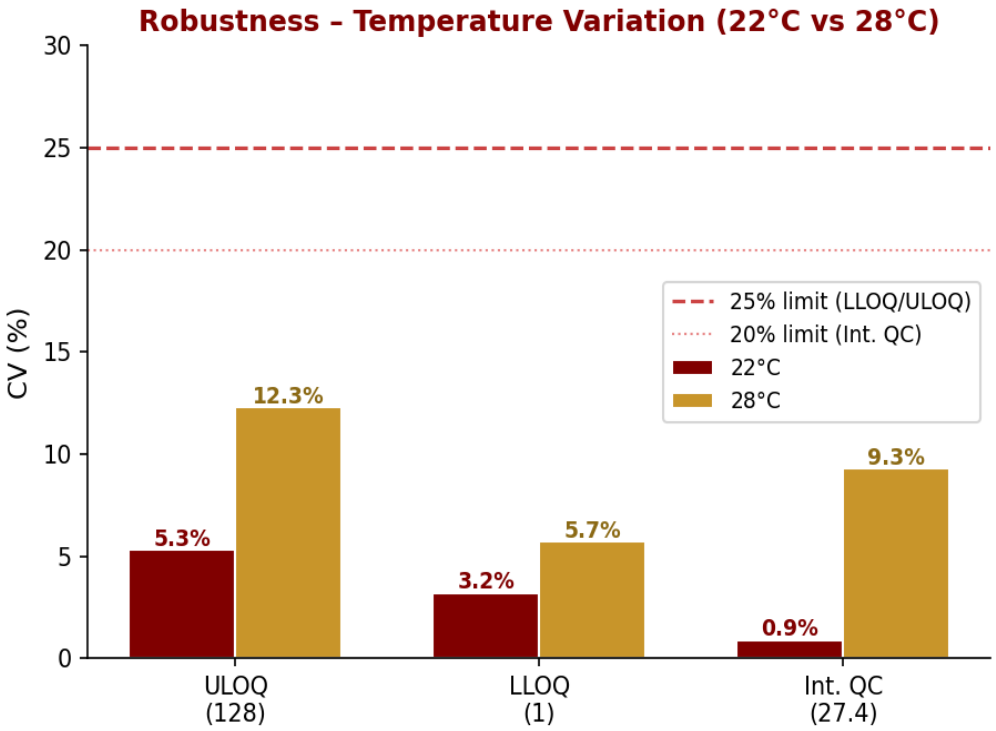


Figure 15. Robustness – CV (%) at 22°C (maroon) and 28°C (gold) across all QC levels. All values remain within the 20–25% acceptance thresholds (red lines), confirming robust performance across the ±3°C working temperature range.

QC Level	ULOQ 22°C	LLOQ 22°C	Int. QC 22°C	ULOQ 28°C	LLOQ 28°C	Int. QC 28°C
Theor. Conc. (ng/ml)	128	1	27.4	128	1	27.4
Mean Value (ng/ml)	126.73	1.04	27.60	113.14	1.22	31.25
CV (%)	5.3%	3.2%	0.9%	12.3%	5.7%	9.3%
Relative Bias (%)	−1.0%	+4.3%	+0.7%	−11.6%	+22.2%	+14.0%

10. Validation Summary

Parameter	Result	Status
Linearity (R^2)	≥ 0.999 across all 3 runs	✓ Pass
LLOQ	1 ng/ml | CV 8.8% | Bias −6.3%	✓ Pass
ULOQ	128 ng/ml | CV 2.7% | Bias −1.9%	✓ Pass
LOD	0.19 ng/ml	✓ Pass
Accuracy	Recovery 93.5% – 104.8% across all QC levels	✓ Pass
Repeatability (CV)	3.5% – 5.6% (all < 20%)	✓ Pass
Intermediate Precision	6.5% – 14.6% (all within limits)	✓ Pass
Specificity	All non-CHO backgrounds < LLOQ	✓ Pass
Selectivity (pH 6.5–8.5)	Recovery 79.4% – 102.4%	✓ Pass
HCP Coverage (LC-MS)	86.8% (unique peptide ≥ 2)	✓ Pass
HCP Coverage (2D)	71.0% – 82.6%	✓ Pass
Robustness (22–28°C)	All CV and bias within limits	✓ Pass

All validation parameters meet predefined acceptance criteria per USP <1132>, EP <2.6.34>, ICH Q2(R2), and ICH M10. The KRIBIOLISA™ CHO HCP ELISA Kit is validated for reliable, sensitive, and specific quantification of CHO-derived HCPs across 1–128 ng/ml.

11. References

1. USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals
2. USP PF49(3) <1132.1> Residual Host Cell Protein Measurement in Biopharmaceuticals
3. EP <2.6.34> Host-Cell Protein Assays
4. ICH Q2(R2) Validation of Analytical Procedures
5. ICH M10 Bioanalytical Method Validation
6. ChP <9012> Guidance for Method Validation of Quantitative Analysis of Biological Samples

12. Citations

Long term culturing of CHO cells: phenotypic drift and quality attributes of the expressed monoclonal antibody

R Kaur, R Jain, N Budholiya, AS Rathore - Biotechnology Letters, 2023 - Springer

… at the time of subculturing using CHO HCP ELISA kit from Krishgen Biosystems as per manufacturer'… The platform CHOP ELISA is a sandwich ELISA with the detection antibody directly …

Polymer-coated fiber optic sensor as a process analytical tool for biopharmaceutical impurity detection

AM Nair, A Dhawangale, S Chandra… - IEEE Transactions …, 2020 - ieeexplore.ieee.org

… applied to histone ELISA plates in the same buffer as per the protocol. The ELISA-based histone … The anti-CHO HCP proteins showed binding response with the IgG impurities as well as … Save Cite Cited by 9 Related articles All 3 versions