Amersham™ HCP CHO solution (supernatant)

IMAGING SYSTEMS, SOFTWARE, AND ACCESSORIES

Amersham™ HCP CHO is part of our complete solution that supports host cell protein (HCP) risk mitigation. From process development to batch release, our products provide robust tools for enzyme-linked immunosorbent assays (ELISAs), 2D electrophoresis, image acquisition, and image analysis.

This Amersham™ HCP CHO (supernatant) solution includes an HCP ELISA and antibody reagents designed to sensitively and robustly quantitate HCP contamination within therapeutics manufactured in Chinese hamster ovary (CHO) cell lines. The purified antibodies react strongly with supernatant proteins derived from CHO-K1 and CHO-S backgrounds. The complete HCP solution facilitates method optimization and data submission throughout process and product development.

Our solution includes:

- Amersham™ HCPQuant™ CHO (supernatant) Kit: This kit is a 96-well microtiter strip format sandwich ELISA. It uses pre-immobilized anti-CHO HCP antibodies, horseradish peroxidase (HRP)-conjugated detection and 3,3',5,5'-tetramethylbenzidine (TMB) substrate to measure residual HCP in downstream purification.
- Amersham™ Anti-CHO HCP (supernatant) Antibody: A flexible antibody reagent for orthogonal HCP quantification methods. This antibody also supports coverage assays, including fluorescent-based differential in-blot electrophoresis (DIBE™) and affinity-based chromatography methods.

Key benefits

- **Sensitive antibody with strong HCP coverage** minimizes the risk of undetected HCP.
- **Wide dynamic range enables excellent dilution linearity,** increasing confidence across the entire purification process.
- **Consistently low inter- and intra-plate variation** ensures reproducible data.

Rockland Immunochemicals, Inc., in collaboration with Cytiva, developed and extensively validated these assays.

An ELISA with broad sample compatibility and high sensitivity

The Amersham™ HCPQuant™ CHO (supernatant) ELISA is designed to deliver reliable results across diverse sample types and purification steps. Its broad compatibility minimizes matrix effects, while high sensitivity detects low-level HCPs. This helps researchers make confident decisions from development to release.

Excellent compatibility with a wide range of samples from different processes

To demonstrate compatibility with biologic therapeutics, we tested commercial drug substances and biosimilars for residual HCP using the Amersham™ HCPQuant™ CHO Kit and Cygnus 3G kit (F550). We tested each substance in duplicate across several dilutions, and performed each assay in triplicate.

We interpolated the measured absorbance values for each sample to concentration (in ng/mL) using a four-parameter logistic (4-PL) fit of the standard curve included with each kit. We converted the average concentrations to parts per million (ppm) of drug substance.

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We used a *t*-test to calculate any statistically significant differences between the measured concentrations of samples from each kit. For each assay, we excluded absorbance values from samples which were outside their corresponding standard curves from analysis. The results indicate that Amersham™ HCPQuant™ CHO (supernatant) detects more HCP in five out of nine substances tested versus the competitor kit (Fig 1).

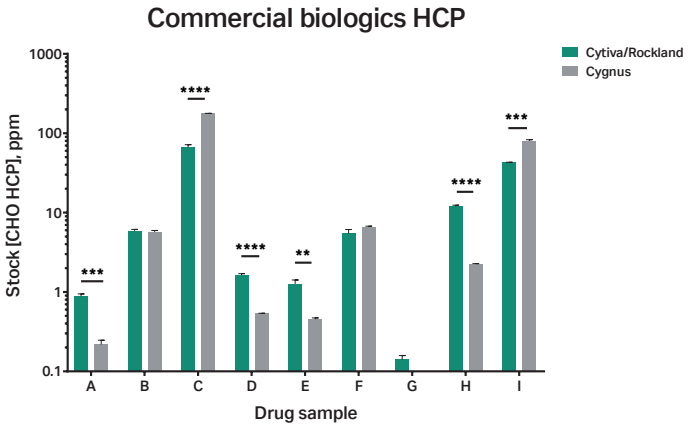

Fig 1. Residual HCP in commercial drug substances expressed as ppm (ng/mg). Asterisks denote statistical significance (*t*-test). Error bars represent standard deviation.

** = $P < 0.01$
*** = $P < 0.001$
**** = $P < 0.0001$

Importance of linearity

Dilution linearity across many in-process steps helps scientists demonstrate good sample compatibility and maintain confidence in data. You should be able to calculate the same stock concentration of HCP across several dilutions of the same sample — this parameter is also known as parallelism. Parallelism in downstream steps demonstrates a robust assay and enables accurate HCP level tracing — helping you develop a good purification strategy while saving time and resources.

Here, we demonstrate sample compatibility and parallelism across several downstream purification steps for two cell lines; CHO-K1 and CHO-S.

We used a standard downstream process to purify supernatant from CHO-K1 and CHO-S cell lines expressing monoclonal antibody (1). To demonstrate dilution linearity, we tested samples from each purification step for HCP in triplicate assays across several dilutions. We performed the tests using the Amersham™ HCPQuant™ CHO (supernatant) Kit and Cygnus 3G kit (F550).

We interpolated the measured absorbance values for each sample to concentration (in ng/mL) using a 4-PL fit of the standard curve included with each kit, and converted the average concentrations to ppm.

We used a *t*-test to calculate any statistically significant differences between the measured concentrations from each kit for all samples. For each assay, we excluded absorbance values from samples which were outside their corresponding standard curves from analysis. The results indicate that Amersham™ HCPQuant™ CHO (supernatant) detected more HCP than the competitor kit at each purification step for CHO-K1 (Fig 2, Fig 3) and CHO-S (Fig 4, Fig 5). Amersham™ HCPQuant™ CHO also demonstrated excellent parallelism for both CHO-K1 and CHO-S across the entire purification process, from harvest to final purified product (Table 1).

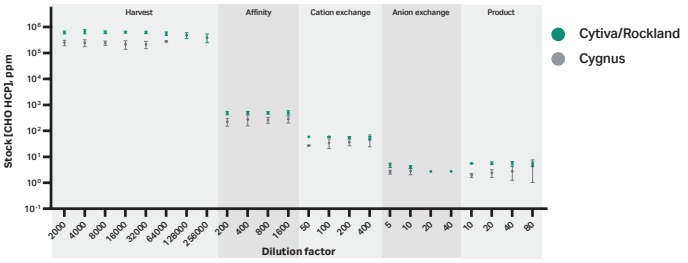

Fig 2. Parallelism across in-process purification steps for a monoclonal antibody produced in a CHO-K1 cell line. Error bars represent standard deviation.

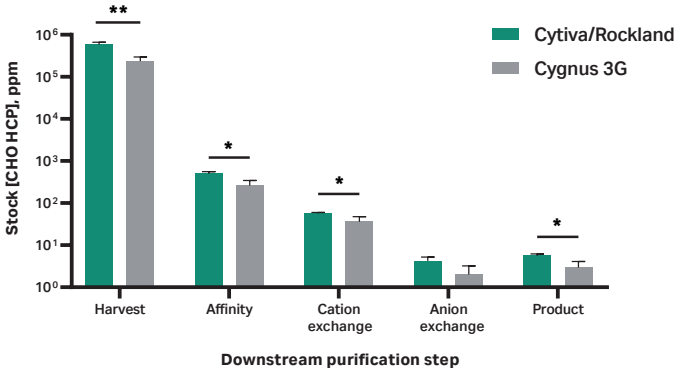

Fig 3. HCP concentration across in-process purification steps for a monoclonal antibody produced in a CHO-K1 cell line, expressed as ppm. Asterisks denote statistical significance (*t*-test). Error bars represent standard deviation.

* = $P < 0.05$
** = $P < 0.01$

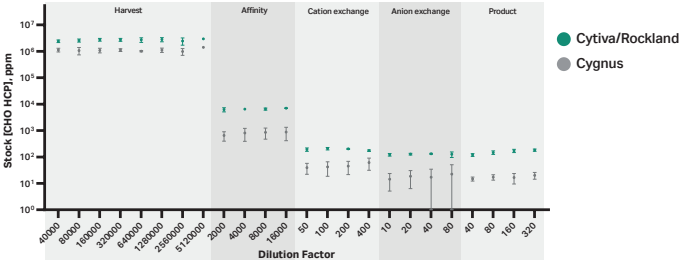

Fig 4. Parallelism across in-process purification steps for a monoclonal antibody produced in a CHO-S cell line. Error bars represent standard deviation.

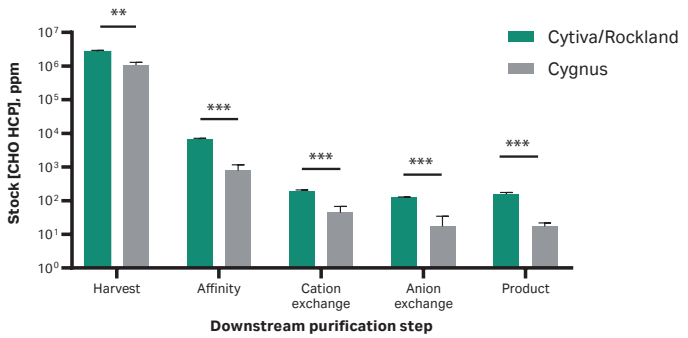

Fig 5. HCP concentration across in-process purification steps for a monoclonal antibody produced in a CHO-S cell line expressed as ppm. Asterisks denote statistical significance (*t*-test). Error bars represent standard deviation.

** = $P < 0.01$
*** = $P < 0.001$

Table 1. Parallelism (expressed as the coefficient of variation, or CV%) of in-process purification steps for a monoclonal antibody produced in CHO-K1 and CHO-S cell lines. CV < 20% indicates good sample compatibility.

	CHO-K1		CHO-S	
Purification step	**HCPQuant™**	**Cygnus 3G**	**HCPQuant™**	**Cygnus 3G**
Harvest	18.10	10.78	10.49	13.48
Affinity	8.16	14.87	7.06	14.48
Cation exchange	6.85	21.27	9.60	15.51
Anion exchange	13.74	10.00	12.59	34.95
Product	7.15	20.64	17.90	17.01

High sensitivity

The lower limit of detection (LLD) is the lowest concentration that can be distinguished from the background. The lower limit of quantitation (LLQ) is the lowest concentration that can be measured reliably and reproducibly.

To calculate the LLD and LLQ, we spiked known concentrations of Amersham™ HCPQuant™ protein standard into sample buffer and measured absorbance in the ELISA Kit. We calculated LLD as the concentration for which the signal was greater than three standard deviations (σ) from the mean of the zero standard [0.106 + (3 × 0.003) = 0.115] (Table 2). We calculated LLQ as the lowest concentration for which the coefficient of variation (CV) was less than 20% and recovery was between 80% and 120% (Table 3). We determined LLD and LLQ from eight replicates across two plates.

Table 2. LLD

HCP concentration (ng/mL)	**6.0**	**5.0**	**4.0**	**3.0**	**2.0**	**1.0**	**0.5**	**0**
Mean A_{450}	0.184	0.168	0.156	0.149	0.132	0.118	0.112	0.106
Standard deviation (σ)	0.005	0.007	0.005	0.006	0.004	0.003	0.005	0.003
Blank + 3σ								0.115
Mean abs > blank + 3σ	Yes	Yes	Yes	Yes	Yes	Yes	No	–

Table 3. LLQ

HCP concentration (ng/mL)	**6.0**	**5.0**	**4.0**	**3.0**	**2.0**	**1.0**	**0.5**
Mean interpolated concentration (ng/mL)	5.74	4.55	3.68	3.13	1.95	0.88	0.48
Recovery (%)	95.60	90.97	92.02	104.18	97.64	87.91	95.67
CV (%)	4.90	4.09	4.23	10.03	19.91	21.03	69.90

We used spike assays to confirm the LLD and LLQ, testing a total of 72 replicates at 1 and 2 ng/mL across three plates.

For LLD, the average absorbance from 72 replicates of the blank plus 3σ was 0.0683, and the average absorbance from 72 replicates of the 1 ng/mL standard was 0.0723. This indicates an LLD around 1 ng/mL.

For LLQ, the CV for 72 replicates of the 2 ng/mL protein standard was 18.3% with a recovery of 91.9%, indicating an LLQ around 2 ng/mL.

Broad dynamic range

Our Amersham™ HCPQuant™ CHO (supernatant) Kit has a broad dynamic range that reduces the time and number of plates needed for linear experiments.

We used the protein standard from the HCPQuant™ CHO (supernatant) ELISA Kit to prepare a standard calibration curve from 200 to 1.389 ng/mL (Fig 6, Table 4). To perform the assay in triplicate, we measured in duplicate across three assays (six replicates total). The standard curve shows a broad range (1.39 to 200 ng/mL) and a strong goodness of fit (R^2 > 0.999).

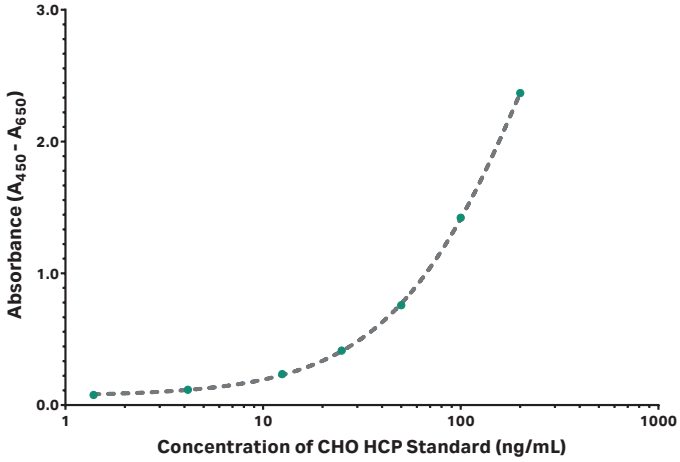

Fig 6. Standard curve (4-PL fit) of the Amersham™ HCPQuant™ CHO (supernatant) Kit.

Table 4. Mean absorbance and inter-plate CV of the standard curve

Concentration (ng/mL)	**Mean absorbance (A_{450}–A_{650})**	**Inter-plate variation (CV%)**
200	2.369	2.315
100	1.422	2.787
50	0.758	3.073
25	0.414	3.504
12.5	0.236	2.809
4.167	0.116	5.702
1.389	0.077	7.656

To avoid assay repetition due to out-of-range detection, scientists need to determine the optimal dilution factors for each assay sample. For the best performance, we recommend performing two dilution series in parallel; a two-fold eight-step serial dilution, and a five-fold eight-step serial dilution. This combination enables you to identify a dilution where the sample generates a reading within the detection range.

A robust assay with reproducible results

Assay precision is the coefficient of variation (CV) within a single experiment (intra-assay) and across multiple experiments (inter-assay). To perform precision analyses, we spiked the assay with three sample concentrations (160, 60, and 8 ng/mL). We calculated intra-assay precision using 10 replicates for each concentration (Table 5), and calculated inter-assay precision from the averaged means of three replicate assays (three plates) (Table 6).

Table 5. Intra-assay precision

Spiked concentration of HCP (ng/mL)	Calculated concentration (ng/mL)	Recovery (%)	Recovery CV%
160	165.12	103.20	3.40
60	61.77	102.95	4.10
8	9.28	115.95	4.70

Table 6. Inter-assay precision

Spiked concentration of HCP (ng/mL)	Average calculated concentration (ng/mL)	Average recovery (%)	Recovery CV%
160	154.69	96.68	4.70
60	56.86	94.77	6.72
8	7.87	98.34	5.82

Flexible and compatible with commonly used buffers

To test for possible matrix effects, we performed spike recovery assays with buffers commonly used in monoclonal antibody purification. We spiked the assay with 160, 60, and 8 ng/mL CHO supernatant HCP standard and then compared the calculated concentration of HCP with the known spiked value to test for recovery. Additionally, we spiked the CHO standard into a monoclonal antibody (manufactured in a murine cell line to negate the effect of HCP from the matrix) to test for possible effects of a high concentration of drug substance. As shown in Table 7, the recovery rates demonstrate broad buffer compatibility with 100% recovery (± 20%) at 1:10 dilution.

For optimal performance, we recommend diluting assay samples in a 1:1 ratio into the provided sample buffer, and then performing a series of dilutions into the sample buffer. Scientists should determine the optimal dilution factors for each assay sample.

We also recommend routinely measuring the recovery of a spiked sample to detect any process-specific matrix effects. If you discover any precipitates or aggregates in your test samples, centrifuge them to remove insoluble proteins and avoid complications. If you do not use the sample buffer from the kit, (e.g., in cases when the sample's HCP concentration is very low), you should test for potential matrix effects. To do this, spike a known concentration of control protein into a control sample matrix.

Table 7. Matrix recovery rates (%) demonstrate broad buffer compatibility

Matrix		Sample buffer	50 mM Na acetate pH 3.5		50mM Na acetate pH 5.5, 100 mM NaCl		25 mM Phosphate pH 7.5		Monoclonal antibody (5 mg/mL)		
Dilution		0	1:1	1:10	1:1	1:10	1:1	1:10	0	1:1	1:10
Spiked HCP	160 (ng/mL)	97	61	107	111	95	112	105	101	101	94
	60 (ng/mL)	95	56	96	107	95	105	100	99	93	84
	8 (ng/mL)	98	67	120	113	106	110	113	105	104	105

Automated plate washing accelerates your workflow

Manual washing procedures present a bottleneck that hinders efficient workflows and requires more tedious hands-on effort compared to automated methods. To increase throughput and reduce hands on time, we tested automated wash methods using an AquaMax 4000 (Molecular Devices) Microplate Washer. We used representative in-process samples from a standard downstream purification of a therapeutic monoclonal antibody and measured residual HCP at each process step, across triplicate assays. We tested the same samples using the manual washing method to act as a control. To compare the mean HCP concentrations between the washing methods, we performed a *t*-test on the calculated ppm from each in-process step.

The results indicate that there was no significant difference ($p < 0.05$) in the detected level of residual CHO HCP with either wash method (Fig 7). This supports the use of automated plate washing using the AquaMax 4000 washer with the Amersham™ HCPQuant™ CHO (Supernatant) ELISA.

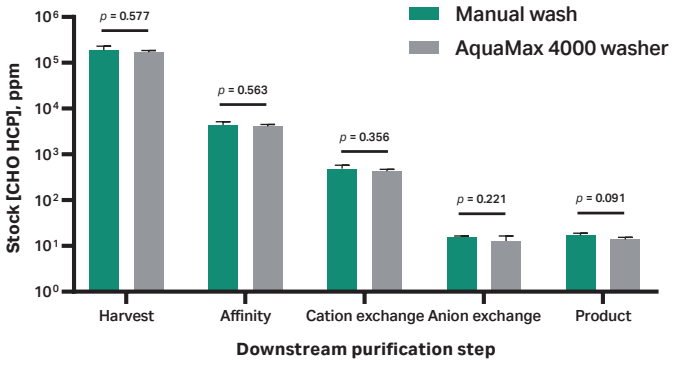

Fig 7. HCP concentration across in-process purification steps for a monoclonal antibody produced in a CHO-K1 cell line expressed as ppm. The results from each statistical comparison (*t*-test) are given above each group. Error bars represent standard deviation.

Reagent stability

We used a real-time stability study to determine shelf life. We stored kit compontents at 4°C and tested assay performance at regular intervals over 24 months using the criteria in Table 8.

All components passed the acceptance criteria at day 720. This indicates that the predicted shelf life of the kit is 24 months from the manufacuture date when stored between 2°C to 8°C.

Table 8. Test criteria and results of the accelarated stability study

Parameter	Pass criteria	Result (day 720 at 4°C)
Absorbance of 100 ng/mL standard	> 1.0	Pass
Absorbance of sample buffer	< 0.25	Pass
Intra-assay CV	< 20%	Pass
LLD (ng/mL)	< 1.39	Pass
Recovery of each protein standard	80%–120%	Pass

Reliable coverage solution for accurate ELISA validation

The Amersham™ Anti-CHO HCP (supernatant) Antibody contains the same polyclonal primary antibodies as our ELISA Kit, enabling you to accurately manage the risk of HCP impurities in biologics produced from the supernatant of CHO cells. You can use this antibody with fluorescent-based DIBE™ technology to simplify ELISA validation and increase the accuracy of HCP coverage analysis.

HCP coverage analysis with DIBE™ technology

The conventional enhanced chemiluminescence (ECL™) method for HCP coverage assessment requires two separate analyses for each process-specific sample using two independent gels run under identical conditions (Fig 8). After separation by isoelectric point and molecular weight, scientists stain one gel with a total protein stain to detect all possible HCPs. They transfer the second gel to a membrane, probe it with anti-HCP antibodies, and conduct detection with ECL™ to identify HCPs recognized by the anti-HCP antibody. Then the scientists compare the signals from the gel and the probed membrane, and calculate coverage as the percentage of the total protein spots in the gel that could be identified on the antibody probed membrane.

When using this conventional method to assess HCP coverage with two gels, scientists need to align two independent 2D patterns — which can be challenging and time-consuming. Additionally, the patterns might not match due to typical gel-to-gel variation which can increase the complexity of analysis. Overlaying two different signal types (total protein stain and ECL™) can also require multiple exposures for the best results. These experimal considerations mean that this coverage method can become fragmented, increasing the risk for errors that lead to an inaccurate assessment of antibody coverage.

DIBE™ HCP coverage analysis (Fig 8) uses CyDye™ labeled HCPs and bound antibodies visualized through a CyDye™ secondary detection reagent. This method detects the HCPs and bound antibodies in distinct channels from the same membrane. Using a single membrane improves accuracy compared to the ECL™ method, as it eliminates the risk of errors arising from gel-to-gel variation and misalignment.

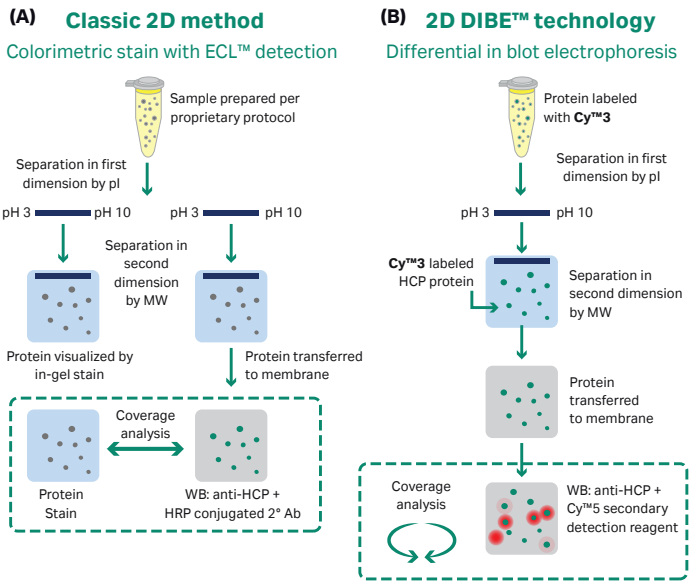

Fig 8. Coverage analysis using (A) conventional ECL detection and (B) 2D DIBE™ technology. WB: Western blot.

The DIBE™ workflow gives flexibility to meet different user needs

2D DIBE™ technology is compatible with a wide range of gel sizes and imagers, providing the flexibility to meet different user needs.

For accurate data submission to regulatory authorities, scientists need to combine high image quality with advanced analysis software. We recommend using an **Amersham™ Typhoon™ 5** or **RGB** imager, along with large format (24 cm) isoelectric focusing (IEF) Immobline™ DryStrips and DIGE™ gels. This combination enhances spot detection and the resulting robustness of your assay.

For optimization and development, we recommend using the **Amersham™ ImageQuant™ 800 Fluor** system and small format (7 cm) gels, which support quicker analysis and savings on reagents. Both options deliver high image quality and resolution, enabling precise spot definition with the 3D view in Melanie 9.2 coverage software (Fig 9).

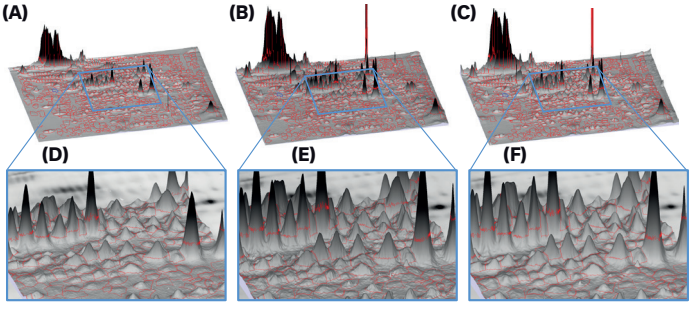

Fig 9. Images of 3D view from Amersham™ Typhoon™ (A and D), ImageQuant™ 800 (B and E), and ImageQuant™ 800 SNOW (C and F). A, B, and C show the entire 2D electrophoresis area. D, E, and F show close-ups of the areas framed in blue.

Antibody coverage is an important variable in HCP risk mitigation

Different antibodies have different reactivities to process-specific samples. These variations are an important factor when choosing an antibody or generic ELISA for HCP analysis. Scientists should perform a coverage analysis with the antibodies using their process-specific samples as part of ELISA validation.

To demonstrate this antibody-dependent variability, we performed coverage analysis on supernatant derived from two CHO mock cell lines using the Amersham™ Anti-CHO HCP (supernatant) Antibody. We labeled purified supernatant proteins from the mock cell lines with CyDye™ reagents DIGE™ assay Cy™3 dye. We then separated the labeled samples with IEF gel electrophoresis using an IPGphor™ 3 IEF system and 7 cm pH 3–11 NL Immobiline DryStrips strips.

We separated the proteins in the second dimension by molecular weight using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Next, we transferred the gels to a polyvinylidene difluoride membrane and blotted them with the anti-HCP primary antibodies. To perform detection, we incubated with Cy5-labeled secondary antibodies raised against the host species of the primary antibody (rabbit). We scanned the membranes on an Amersham™ Typhoon™ biomolecular imager, and calculated coverage using the DIBE™ coverage module in Melanie 9 software.

As shown in Fig 10 and Table 9, our data demonstrates some variability in the total antigen coverage of the anti-HCP antibodies. These differences can be due to cell culture conditions, sample preparation, and antibody specificity to subpopulations of HCP. The variability also underscores the importance of measuring coverage as part of an HCP risk mitigation strategy.

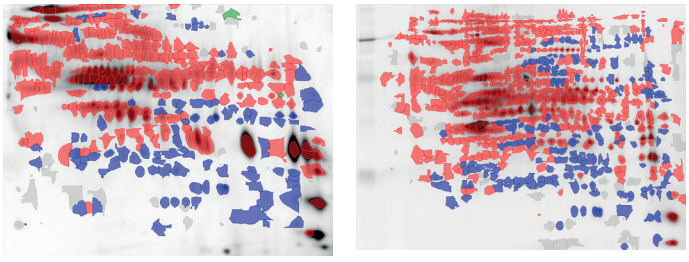

Fig 10. 2D DIBE analysis of CHO supernatnant samples using Amersham™ Anti-CHO HCP (supernatant) Antibody. Blue spots: Only present in protein channel. Green spots: Only present in antibody channel. Red spots: present in both protein and antibody channels.

Table 9. Average coverage percentage of two CHO supernatant samples

CHO supernatant sample	Percent coverage
CHO (A)	73%
CHO (B)	67%

Increased confidence in HCP antibody suitability using mass spectrometry

2D coverage methods like DIBE are reliable applications to determine antibody coverage and are recommended in regulatory guidelines. However, 2D Western blotting cannot be used to determine if an ELISA antibody detects specific proteins of interest, which can useful if faced with troublesome or problematic HCPs during process development. ELISA-based immunocapture combined with mass spectrometry offers additional insight into antibody coverage so researchers make more informed decisions about HCP assay suitability.

Compatibility with problematic HCPs

Several CHO HCPs have potential to cause issues in biologics. They can trigger immune responses or have other unwanted biological effects in patients. These proteins can also affect drug quality by causing problems like aggregation, fragmentation, and degradation of the drug or its excipients. Two examples of these problematic HCPs are clusterin and lipoprotein lipase (2). Clusterin can bind to the Fc and Fab regions of antibodies and cause them to aggregate. Lipoprotein lipase can break down polysorbate, potentially affecting drug stability and risking immune responses in patients. It's important to check if an immunoassay can detect problematic HCPs to prevent these issues.

To identify problematic HCPs detectable with Amersham™ HCPQuant™ CHO ELISA kit, we collaborated with Alphalyse A/S to screen our anti-CHO HCP antibodies for activity against the CHO proteome using immunocapture LC-MS/MS. Briefly, CHO HCPs from two samples were captured using the CHO-HCP antibody-coated 96-well strip plate from HCPQuant CHO ELISA kit. The captured proteins underwent partial trypsin digestion, reduction and alkylation, desalting, and concentration before LC-MS/MS analysis on a Sciex TripleFOT 6600 mass spectrometer (3). Information dependent acquisition data was searched against a CHO proteome database using Sciex Protein Pilot software. 624 unique proteins were identified above the limit of quantification across the two samples. From this protein list, 16 were identified as HCPs of concern, including clusterin and lipoprotein lipase (4, Table 10).

Table 10. A list of commonly problematic HCPs detected by the HCPQuant CHO ELISA kit, confirmed by immunocapture LC-MS/MS.

Uniprot ID	Protein name
G3I8R9	78 kDa glucose regulated protein
G3I5A4	Annexin A5
A0A061IFE2	Carboxyesterase
G3H0L9	Cathepsin B
G3INC5	Cathepsin L
Q9EPP7	Cathepsin Z
G3I4W7	Cathepsin D
G3HNJ3	Clusterin
G3H6V7	Lipoprotein lipase
G3HQY6	Lysosomal acid lipase
G3HRK9	Matrix metalloproteinase-19
G3GTT2	Monocyte chemoattractant Protein-1
P14851	Peptidyl-prolyl cis-trans isomerase A
Q9JKY1	Peroxiredoxin
G3I6T1	Phospholipase B-like 2
G3HC31	Protein S100

Specifications

Specification	Parameter
LLD	1 ng/mL
LLQ	2 ng/mL
Range	1.39 to 200 ng/mL
Precision	< 20% intra- and inter-assay variability
Total coverage of ELISA protein standard*	> 70%

* Coverage is sample-dependant and will vary with process-specific samples.

Kit contents

Components for HCPQuant™ CHO (supernatant) ELISA Kit
CHO-HCP (supernatant) detection antibody, 100 µL/vial
CHO-HCP (supernatant) antibody-coated 96-well strip plate, 1 plate
CHO-HCP (supernatant) protein standard, 1 µg/vial
HCP Kit assay buffer, 50 mL/bottle
HCP Kit wash buffer (10×), 60 mL/bottle
HCP Kit TMB buffer (HRP substrate), 20 mL/bottle
HCP Kit stop buffer, 20 mL/bottle
Plate sealer, 3 sheets

Ordering information

HCP ELISA Kit

Generic ELISA	Product code
Amersham™ HCPQuant™ CHO Kit (supernatant), 1 unit, 96-well plate	29496737

DIBE workflow

Instrument and software	Product code
Amersham™ Typhoon™ 5	29187191
Amersham™ Typhoon™ RGB	29187193
Amersham™ ImageQuant™ 800 Fluor	29399484
Melanie 9 Coverage software 1 license floating	29705442
Melanie 9 Coverage software 1 license node locked	29705440
Melanie 9 DIGE™ 1 license floating	29705336
Melanie 9 DIGE™ 1 license node locked	29705338
Melanie 9 Package 1 license floating	29705331
Melanie 9 Package 1 license node locked	29705340
EPS 3501 XL Power Supply Unit	18113005
DIGE Unit LF24	29701935
Transfer Unit LF24	29701936
IPGphor™ 3, IEF	11003364

Consumables	Product code
Amersham™ Anti-CHO HCP (supernatant) Antibody, 100 µg	29496739
Amersham™ Anti-CHO HCP (supernatant) Antibody, 1 mg	29496740
Amersham™ DIBE™ HCP Detection Kit	29613962
DIGE Gels LF24	29706670
DIGE Buffer Kit	28937452
2D Clean-Up Kit	80648451
2D Quant Kit	80648356
Immobiline DryStrip pH 3-11 NL, 7 cm	17600373
Immobiline DryStrip pH 3-11 NL, 24 cm	17600377

DIBE™ training	Product code
Amersham™ HCP DIBE™ Three Day Training	29477924

References

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2. Application note: Host cell protein identification and quantitation by mass spectrometry enabled by USP analytical reference materials. USP Biologics; BIO.1023
3. Pilely K, Nielsen SB, Draborg A, et al. A novel approach to evaluate ELISA antibody coverage of host cell proteins—combining ELISA-based immunocapture and mass spectrometry. Biotechnology Progress. 2020;36(4). doi:10.1002/btpr.2983
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