Amersham HCPQuant CHO

The Amersham™ HCPQuant CHO kit is designed to quantify Chinese hamster ovary (CHO) host cell protein (HCP) contamination in process-derived samples. This assay has been developed by Rockland Immunochemicals, Inc. in collaboration with GE Healthcare Life Sciences, and extensively validated. The Amersham HCPQuant CHO assay delivers enhanced detection and quantification of host cell protein with strong detection of CHO-HCP proteins, especially in the low molecular weight range. The assay is a 96-well microtiter strip format sandwich ELISA, utilizing HRP-conjugated antibodies and TMB as the substrate.

The purified antibodies used are generated against Chinese hamster ovary K1 cell lysates that were derived from cells grown in chemically-defined media. These antibodies react strongly with CHO-K1 cell lysates, showing extremely broad antigen coverage.

Fig 1. The Amersham HCPQuant CHO kit.

Key features and benefits

- Broad protein coverage and high sensitivity promote better understanding of HCP contamination and exceed the requirements of current regulatory guidelines.
- Robust data with low inter-plate and intra-plate variation for reliable, reproduceable results supports regulatory data submission to demonstrate risk management throughout the development process.
- High sample recovery and broad buffer compatibility to fit your existing workflows.
- Broad dynamic range for easy linear assay set-up saves time and uses fewer plates.
- Standard and rapid protocols are included to offer maximum flexibility.

Enhanced detection of low molecular weight (LMW) proteins

Amersham HCPQuant CHO enables the detection of HCP in the LMW range of both acidic and alkaline proteins where most difficult-to-detect HCPs are found.

Two types of assay were performed utilizing different coverage techniques to demonstrate the percentage of coverage. The first assay used fluorescence, and the second used enhanced chemiluminescence (ECL).

The first experiments were based on 2D DIBE™ technology with fluorescence. The CHO HCP standard (included in kit) was prelabelled with CyDye™ DIGE Cy™3 and then separated by 2D electrophoresis using 7 cm IPG strips. The proteins were transferred to PVDF membrane, then probed using a biotinylated anti-CHO HCP antibody. Detection was performed with a streptavidin Cy5 complex. The membranes were scanned on an Amersham™ Typhoon and coverage was calculated using Melanie™ 9.

The coverage was 95%, with a range between 89% and 93% for the LMW proteins. (Fig 2).

For appropriate coverage across a 2D blot, we recommend using the criteria in Table 1.

Table 1. Quadrant set-up for coverage assays.

Product	Description
Quadrant 1 (Q1)	HMW proteins above 50 kDa in low pI region above pH 6.5
Quadrant 2 (Q2)	HMW proteins above 50 kDa in high pI region above pH 6.5
Quadrant 3 (Q3)	LMW proteins below 50 kDa in the low pI region
Quadrant 4 (Q4)	LMW proteins below 50 kDa in the low pI region

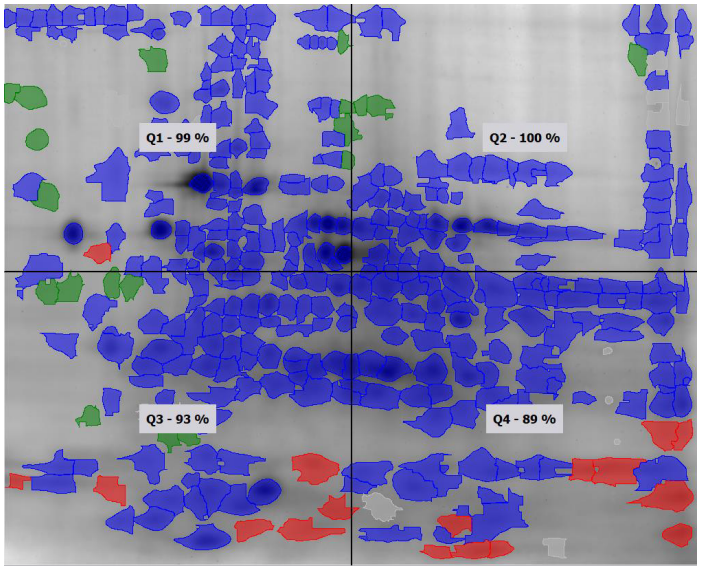

Fig 2. Quadrant image for assay performed with 2D-DIBE technology on Amersham Typhoon.

For accurate data submission to regulatory authorities, it's important to combine high image quality with advanced analysis software. The image quality and high resolution of Amersham Typhoon allow precise spot definition with the 3D view in Melanie 9 Coverage software (Fig 3).

Table 2. Quadrant analysis performed with 2D DIBE technology on Amersham Typhoon at 25 µm and analyzed with Melanie 9 Coverage.

Area	Coverage	Spots	Primary	Secondary	Common
Total	95%	365	17	19	329
Q1 HMW Low pI	99%	122	1	9	112
Q2 HMW High pI	100%	66	0	3	63
Q3 LMW Low pI	93%	85	6	7	72
Q4 LMW High pI	89%	92	10	0	82

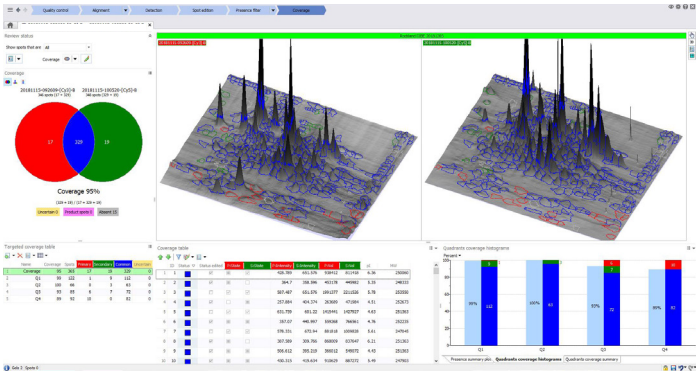

Fig 3. Coverage analysis using Melanie 9.2 Coverage software.

The second set of experiments used the ECL method. CHO HCP standard was separated by 2D electrophoresis using 7 cm strips in duplicate. The antigens were stained in one gel with Oriole and imaged with a CCD imager (Fig 4). The second gel was then transferred to PVDF membrane and blotted with an anti-CHO HCP antibody. Chemiluminescent detection was performed by incubating with an HRP-conjugated anti-rabbit antibody, and subsequent reaction with ECL reagents (Fig 5). Images were captured using a CCD camera and coverage was calculated using Melanie 9 software.

The total coverage was 89%, with a range between 76% and 92% for LMW proteins (Fig 6 and Table 3).

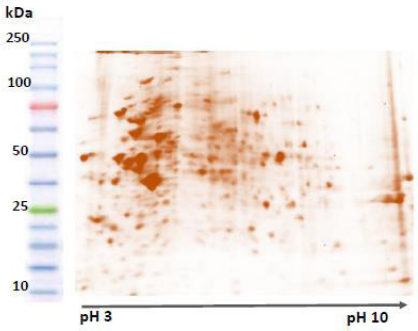

Fig 4. Total CHO HCP, 50 µg stain with Oriole.

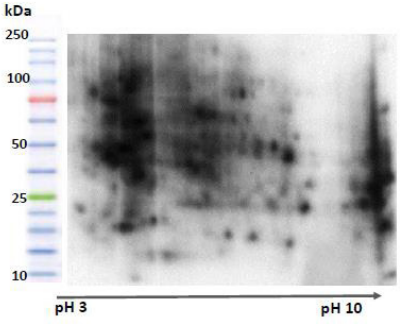

Fig 5. Total CHO HCP, 50 µg on PVDF detected with chemiluminescence.

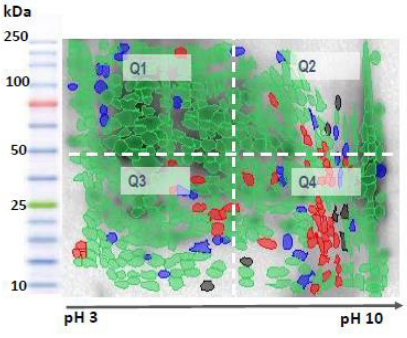

Fig 6. Quadrant analysis analyzed with Melanie 9 Coverage, Red (Spot in stain only), Green (spot in stain + antibody), Blue (spot only in antibody).

Table 3. Coverage percentage with the classical method.

Total Coverage	Q1	Q2	Q3	Q4
89%	99%	91%	92%	76%

With both methods, the anti-HCP antibody in the Amersham HCPQuant CHO kit showed exceptional coverage, with high coverage in the difficult-to-detect LMW range of HCPs.

High sensitivity

The lower limit of detection (LLD) for an assay is usually calculated as the concentration where the signal is greater than 3 standard deviations (SD) from the mean of the zero standard as recommended by regulatory authorities.

The LLD of Amersham HCPQuant was determined by using a signal greater than 8 SD above the mean of the zero standard [0.172 + (8 x 0.004) = 0.204] (Table 4) to give a more robust calculation. The lower limit of quantitation (LLQ) is defined as the lowest concentration for which CV is <20% (Table 5). The LLD and LLQ were determined from 8 replicates.

Table 4. Lower limit of detection (LLD).

ng/mL	128	64	32	16	8	4	2	1	0.5	0.25	0.125	0
Mean OD 450	1.779	0.982	0.592	0.389	0.279	0.231	0.206	0.190	0.178	0.175	0.172	0.172
SD	0.075	0.051	0.027	0.011	0.007	0.007	0.008	0.023	0.008	0.007	0.003	0.004

Table 5. Lower limit of quantification (LLQ).

ng/mL	128	64	32	16	8	4	2
% CV	4.66	6.4	6.48	5.33	6.67	13.56	29.69

Broad dynamic range

Amersham HCPQuant has broad dynamic range that reduces the time and the number of plates required to set up a linear experiment.

CHO K1 cell lysate grown in suspension with chemically defined media and harvested at 60-85% viability was used to perform a linear assay. The standard curve shows a broad range (2-200ng/mL) and strong linearity, R^2=0,998 (Fig 7).

Optimal dilution factors must be determined for each assay sample. We recommend performing two dilution series in parallel: a two-fold, eight-step serial dilution, and an additional five-fold, eight-step serial dilution. This will avoid assay repetition due to out-of-range detection. This dilution combination allows the user to assess the appropriate dilution needed for the sample to generate a reading within the detection range.

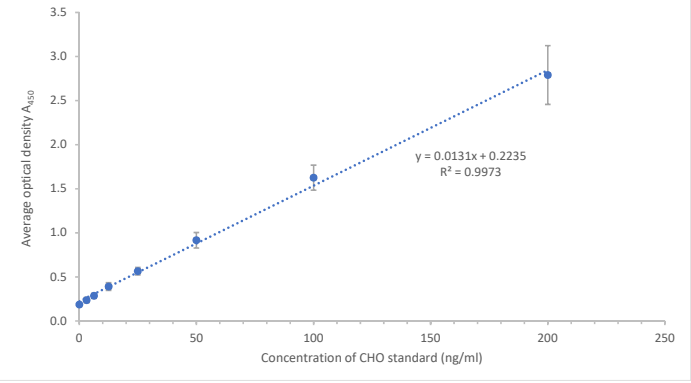

Fig 7. Standard curve, Amersham HCPQuant CHO with CHO K1 cell lysate.

A robust assay for reproducible results

Precision is commonly reported as the coefficient of variation (CV) within a single experiment (intra-assay) and across multiple experiments (inter-assay).

Precision assays were performed by spiking the assay with three sample concentrations (low, medium, high). Intra-assay precision was calculated from 25 replicates for each concentration (Table 6). Inter-assay precision was calculated from the averaged means of five replicates (five plates) (Table 7).

Table 6. Intra-assay precision.

Spike ng/mL	Mean conc. Ng/mL	CV%	Passing criteria
200	207.73	1.39	<20%
100	103.29	0.92	<20%
20	20.17	6.63	<20%

Table 7. Inter-assay precision.

Spike ng/mL	Mean conc. Ng/mL	CV%	Passing criteria
250	296.85	13.22	<20%
100	95.4	18.28	<20%
20	8.23	11.83	<20%

HCPQuant CHO is compatible with many commonly used buffers

To test commonly used matrices, buffers were spiked with 50 ng/mL CHO HCP standard and tested for recovery at various dilutions. The recovery rates, as shown in Table 8, demonstrate broad buffer compatibility with 100% recovery (+/-20%) at 1:10 dilution.

We recommend that end users dilute their assay samples at a 1:1 ratio into the provided sample buffer first. Then, perform a series of dilutions into the sample buffer. Optimal dilution factors for each assay sample must be determined by the user. If a test sample is not diluted into the provided sample buffer, specific matrix effects must be validated by the user by measuring the recovery of a spiked sample. If precipitates or aggregates are discovered in the test samples, centrifuge them to remove insoluble proteins to avoid any unexpected complications.

Table 8. Matrix recovery rates demonstrate broad buffer compatibility.

Matrix	Recovery % at 1:1	Recovery % at 1:10
0.1M Citric Acid pH 4.5	101	98
0.1M Citric Acid pH 3.5	10	109
9.9 mg/20 mL Histidine HCL; 6.4 mg/20 mL L-Histidine; 400 mg/20 mL Trehalose; 1.8 mg/20 mL PS20; pH 6	126	101
47mM Histidine; 3.0 mM Glycine; 5.6 % Mannitol	101	95
0.1M Glycine pH 2.5	6	101

Specifications

Specificity (ECL detection method)	Total Coverage of CHO-K1	89.5 % in 2D gel assays
	HMW protein, coverage	95% average
	LMW protein coverage	84% average
Precision	Intra-assay precision (high, medium, low)	<20%
	Inter-assay precision (high, medium, low)	<20%
Sensitivity	LLD	2 ng/mL
	LLQ	4 ng/mL
	Assay range	2–200 ng/mL
Accuracy	Recovery/matrix effect	100% ± 20% (at ≤ 1:10 dilution)
Linearity	R^2	0.998

Kit contents

Component	Size
CHO-HCP Detection Antibody	35 µL/vial
CHO-HCP Antibody-coated 96-well strip plate	1 plate
CHO-HCP Protein Standard	1 µg/vial
HCP kit sample buffer	50 mL/bottle
HCP kit wash buffer (10X)	60 mL/bottle
HCP kit TMB buffer (HRP Substrate)	15 mL/bottle
HCP kit stop buffer	15 mL/bottle
Plate sealer	1 sheet

GE Healthcare provides a full line of products and technical support to help biopharma labs with HCP workflows.

Ordering information

Generic ELISA Kit

Product	Description	Product code
Amersham HCPQuant CHO kit	1 unit 96-well plate	29340032

Coverage instruments and software

Product	Description	Product code
Amersham Typhoon 5	1 unit	29187191
Amersham Typhoon RGB	1 unit	29187193
Melanie 9 Coverage software	1 license, floating	29270737
Melanie 9 Coverage software	1 license, node locked	29270543
Melanie 9 DIGE	1 license, floating	29270537
Melanie 9 DIGE	1 license, node locked	29270536

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