Amersham HCP **DIBE** CHO kit

ANTIBODIES AND ASSAYS

The Amersham™ HCP DIBE™ CHO kit is designed to measure the antibody coverage of Amersham HCPQuant CHO ELISA on host cell proteins (HCP) from process-derived samples (Fig 1). The kit uses the same polyclonal primary antibodies as the ELISA kit, enabling accurate risk management of HCP impurities in biologics produced in Chinese hamster ovary (CHO) cells from a K1 background. This assay uses fluorescent-based differential in blot electrophoresis (DIBE technology) that simplifies the ELISA assay validation and increases accuracy of HCP coverage analysis. The Amersham HCP DIBE CHO kit was developed by Rockland Immunochemicals, Inc. in collaboration with Cytiva and has been extensively validated. When combined with the Amersham HCPQuant CHO ELISA, Amersham Typhoon™ biomolecular imager, Melanie™ 9 coverage software, and the 2D electrophoresis range, this kit completes Cytiva's HCP workflow. Together, these products allow for in-house ELISA validation and full control of HCP risk mitigation.

Key features and benefits:

- Accurate HCP risk management by using the same antibodies as Amersham HCPQuant CHO ELISA kit
- Robust coverage data with single-gel DIBE technology eliminating risk of error from gel-to-gel variation
- High sensitivity and signal stability using CyDye™ differential gel electrophoresis (DIGE)
- Simplified 2D analysis without the need for alignment of two independent patterns

HCP coverage analysis with DIBE technology

The conventional enhanced chemiluminescence (ECL) method for HCP coverage assessment (Fig 2A) requires two separate analyses of samples using two independent gels. After separation by isoelectric point (pI) and molecular weight (MW), one gel is stained with a total protein stain and the second is transferred to a membrane, probed with the anti-HCP antibodies, and detected by ECL. The process of assessing HCP coverage using two gels requires the alignment of two independent 2D patterns which is time consuming and challenging. Additionally, due to gel-to-gel variation, the gel separation patterns are unlikely to be identical.

Fig 1. Amersham HCP DIBE CHO kit for measurement of HCP coverage and ELISA assay validation.

The overlay of two different types of signals (total protein stain and ECL) requires multiple exposures for the best results. This fragmented detection method increases the risk of errors that can lead to false interpretation of the antibody coverage and thus incorrect assessment of ELISA performance.

DIBE HCP coverage analysis (Fig 2B) uses CyDye labeled HCPs and bound antibodies visualized through a CyDye secondary detection reagent. The HCPs and bound antibodies are detected in distinct channels on the same membrane. The use of a single membrane reduces risk of misinterpretation and improves accuracy versus the ECL method as there is no longer a need to factor in potential error from gel-to-gel variation and the possibility of misalignment.

2D DIBE technology increases HCP coverage analysis accuracy

2D DIBE technology reduces the risk of errors that are common in conventional ECL detection. These two assay types were compared to demonstrate the increased robustness and simplicity of 2D DIBE technology versus ECL.

2D DIBE technology yields better data quality

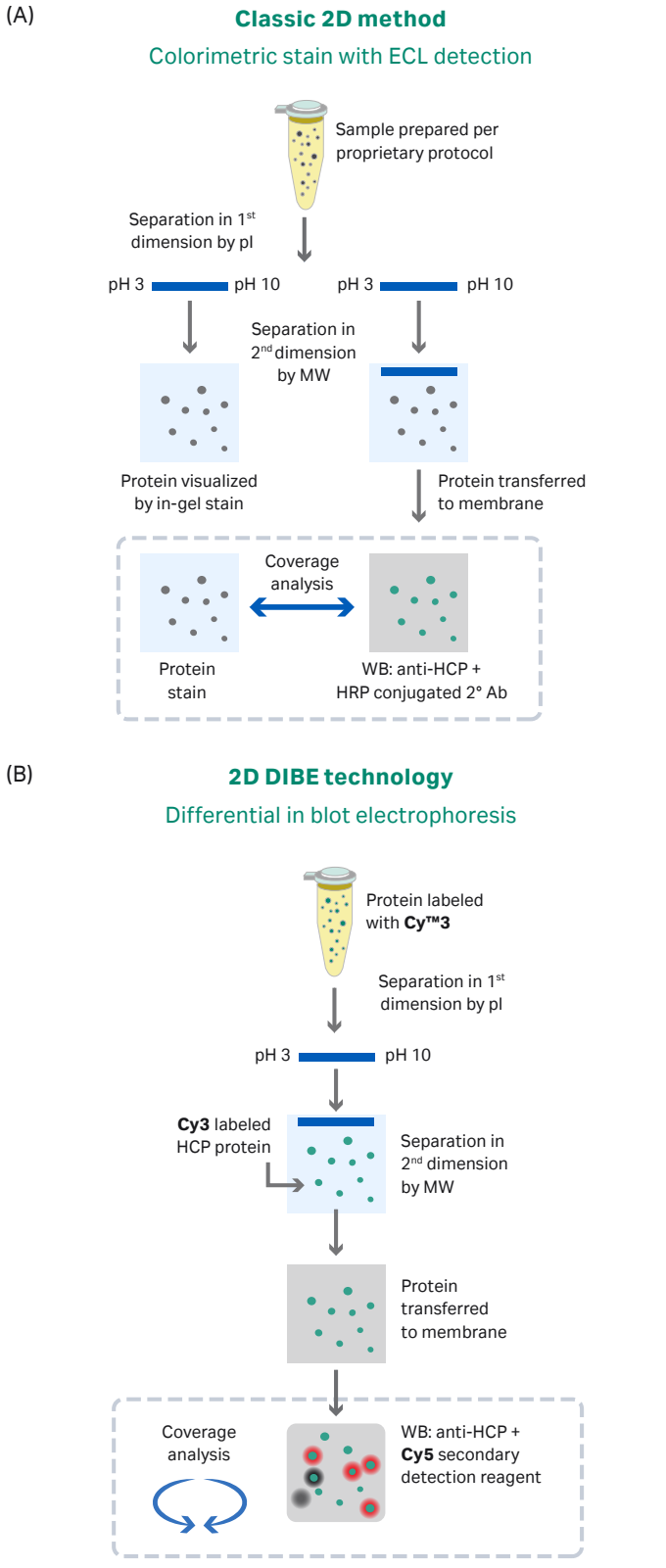

Fig 2. Coverage analysis using (A) conventional ECL detection and (B) 2D DIBE technology; WB = Western blot.

Box 1. Coverage analysis by quadrant increases understanding of HCP risk. Coverage is calculated as the percentage of antibody detected protein spots relative to the total number of protein spots in the sample (Fig 3). When measuring coverage, it can be easy to overlook troublesome sub-populations of proteins if the overall coverage percentage is high. Sub-dividing the analysis into quadrants based on MW and pI (Table 1) gives a more complete understanding of HCP coverage, allowing the user to better mitigate risk.

Fig 3. Quadrant definitions and coverage calculation for HCP coverage analysis.

Table 1. Quadrant definitions for coverage assay; HMW = high molecular weight, LMW = low molecular weight

Product	Definition
Quadrant 1 (Q1)	HMW proteins above 50 kDa in pH region below 6.5
Quadrant 2 (Q2)	HMW proteins above 50 kDa in pH region above 6.5
Quadrant 3 (Q3)	LMW proteins below 50 kDa in pH region below 6.5
Quadrant 4 (Q4)	LMW proteins below 50 kDa in pH region above 6.5

For analysis using ECL detection (Fig 4), CHO HCP standard was separated by pI on two 7 cm pH 3-10 NL immobilized pH gradient (IPG) strips for isoelectric focusing (IEF). The proteins were then separated in the second dimension by MW using SDS-PAGE. The antigens were stained in one gel with Oriole fluorescent gel stain and imaged on a charge-coupled device (CCD) imager. The second gel was then transferred to a polyvinylidene fluoride (PVDF) membrane and blotted with an anti-CHO HCP antibody. Chemiluminescent detection was performed by incubation with a horseradish peroxidase (HRP)-conjugated anti-rabbit antibody and a subsequent reaction with ECL reagents. ECL images were captured using a CCD camera and coverage was calculated using the coverage module of Melanie 9 software and the quadrant criteria defined in Box 1. Using ECL, the mean total coverage from triplicate experiments was 89% with a range of 76% to 92% for low molecular weight (LMW) proteins (Table 2).

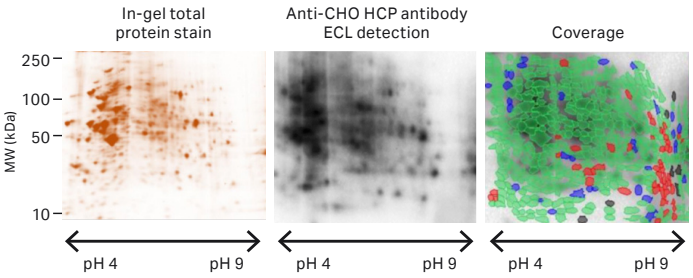

Fig 4. Conventional ECL coverage analysis using CHO HCP standard. The pI range shown is not the full range of the strip. Images have been cropped to remove artifacts and are representative of the pI range noted on the axis.

For analysis using DIBE technology (Fig 5), the pre-labeled DIBE CHO K1 Control Protein Cy3 reagent was separated by pI using 7 cm pH 3-10 NL IPG strips for IEF. The proteins were then separated in the second dimension by MW using SDS-PAGE. Next, the gel was transferred to a PVDF membrane and blotted with the DIBE CHO K1 HCP Antibody. Detection was performed by incubating with DIBE Cy5 detection reagent. The membrane was scanned on an Amersham Typhoon gel and blot imaging system, and coverage was calculated using the coverage module of Melanie 9 software with the quadrant criteria defined in Box 1. The mean total coverage from triplicate experiments using DIBE technology was 96% with a range of 89% to 93% for LMW proteins (Table 2).

For accurate data submission to regulatory authorities, it is 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 9.2 coverage software.

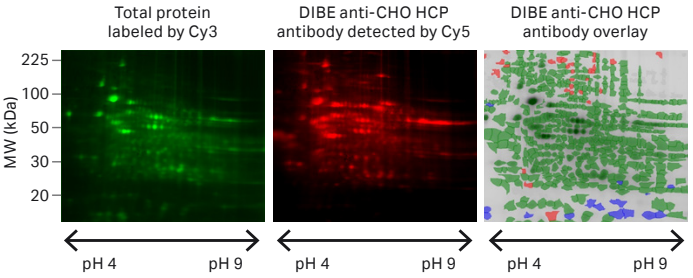

Fig 5. DIBE technology coverage analysis using CHO HCP standard. The pI range shown is not the full range of the strip. Images have been cropped to remove artifacts and are representative of the pI range noted on the axis.

Table 2. Coverage analysis by quadrant for the conventional ECL method and 2D DIBE technology[1]

	Total	Q1	Q2	Q3	Q4
Total stain and ECL	89%	99%	91%	92%	76%
2D DIBE technology	96%	100%	100%	93%	89%

[1] All data reflect mean values, n = 3. The coverage percentage can vary due to user specific equipment, handling, and interpretation in the analysis software.

The increased resolution of large format 2D DIBE technology yields more robust HCP coverage data versus small format

2D DIBE technology enables the robust detection of HCPs with enhanced detection in the LMW range. A 2D DIBE technology coverage analysis can be further improved with the use of large format (24 cm) IEF strips and gels which provide higher spot resolution and increased sample load. To compare coverage based on format size, large (Fig 6) and small (Fig 7) format analyses were compared. The analysis was performed for 2D DIBE technology as previously described using 7 cm and 24 cm pH 3-11 NL IEF strips and DIGE gels for the 24 cm strips.

The mean total coverage from triplicate experiments was 95% for large gels and 92% for small gels (Table 3).

The use of large gels for coverage analysis resulted in the detection of more spots compared to small format DIBE technology (Table 4). The increased dimensions of large gels enable greater resolving power of HCPs, separating proteins of similar pI and MW that could potentially overlap on smaller gels. Additionally, proteins of lower abundance may be resolved from highly abundant neighboring spots by this increased separation, allowing coverage to be measured for these proteins that would otherwise be missed. Another benefit of using large gels is the increased protein loading capacity which might enable the detection of less abundant proteins by bringing them above the limit of detection. Visualization of these masked proteins enables a more accurate measurement of coverage resulting in a more robust analysis.

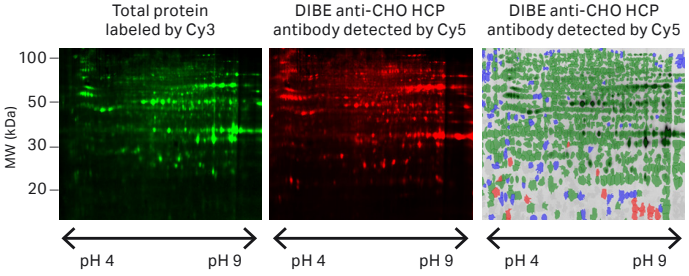

Fig 6. 2D DIBE technology with large DIGE gels, 24 cm. The pI range shown is not the full range of the strip. Images have been cropped to remove artifacts and are representative of the pI range noted on the axis.

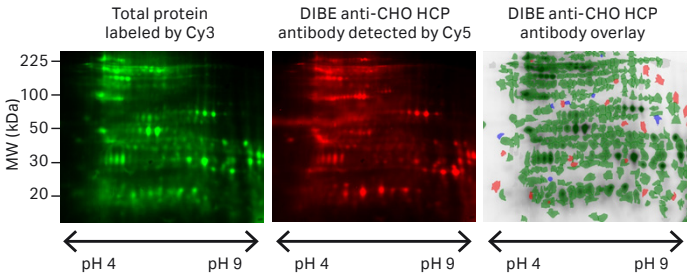

Fig 7. 2D DIBE technology with small, 7 cm gels. The pI range shown is not the full range of the strip. Images have been cropped to remove artifacts and are representative of the pI range noted on the axis.

Table 3. Coverage analysis by quadrant for large and small gels[1]

	Total	Q1	Q2	Q3	Q4
Small gel (7 cm)	92%	89%	78%	92%	95%
Large gel (24 cm)	96%	99%	99%	92%	90%

[1] All data reflect mean values, n = 3. The coverage percentage can vary due to user specific equipment, handling, and interpretation in the analysis software.

Table 4. Spot count by quadrant analysis for large and small gels[1]

	Total	Q1	Q2	Q3	Q4
Small gel (7 cm)	478	133	36	184	126
Large gel (24 cm)	1120	450	192	333	144

[1] All data reflect mean values, n = 3. The coverage number of spots detected can vary due to user specific equipment, handling, and interpretation in the analysis software.

Antibodies matter for HCP analysis

When choosing an antibody or generic ELISA for HCP analysis, it is important to consider that different antibodies have different reactivities to process-specific samples. A coverage analysis should be performed on the antibodies to validate the results of the ELISA. To demonstrate this antibody-dependent variability, coverage analysis was performed on DIBE CHO K1 Control Protein Cy3 using the DIBE CHO K1 HCP antibody and the anti-CHO HCP 3G antibody from Cygnus Technologies. The analysis was performed for 2D DIBE technology as previously described using 7 cm pH 3-10 NL IEF strips. In addition, the control protein from Amersham HCPQuant CHO was tested at a known concentration using the ELISA kit from Cygnus.

Coverage analysis of Amersham DIBE CHO K1 Control Protein Cy3 using the Amersham DIBE CHO K1 HCP Antibody (Fig 8) showed a higher coverage than the antibodies from Cygnus Technologies (Fig 9). The reactivity of the Amersham DIBE CHO K1 HCP antibody with this sample and sub-optimal compatibility of the Cygnus antibodies (Table 5) highlight the importance of performing coverage analysis and selecting the most suitable antibody for HCP quantitation. The use of sub-optimal detection systems might lead to inaccuracies in HCP quantitation and an underestimation of true HCP levels.

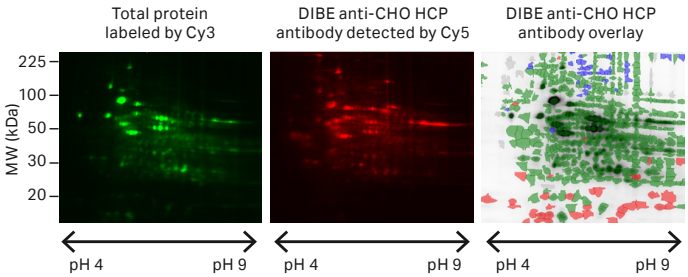

Fig 8. DIBE technology coverage analysis of Amersham DIBE CHO K1 Control Protein Cy3 using Amersham DIBE CHO K1 HCP Antibody.

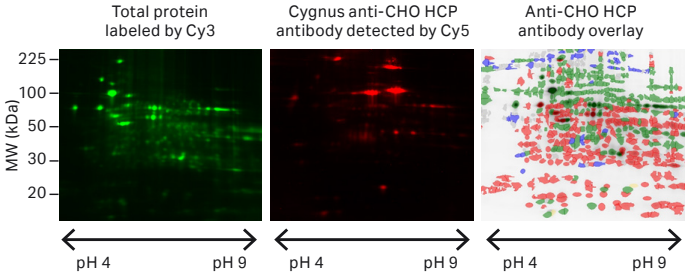

Fig 9. DIBE technology coverage analysis of Amersham DIBE CHO K1 Control Protein Cy3 using Cygnus antibody.

Table 5. Coverage analysis against Amersham DIBE CHO K1 Control Protein Cy3 between Cygnus and Amersham HCP DIBE CHO antibodies1[1]

	Bicinchoninic acid (BCA) assay	DIBE CHO K1 HCP antibody	Anti-CHO HCP antibody (Cygnus Technologies)[2]	*p* value[3]
Amersham DIBE CHO K1 Control Protein Cy3 (ng/mL)	200	197.4[4]	6.47[5]	<0.0001
DIBE coverage (%)	n/a	96	66	< 0.0001
Spot count	n/a	494	480	NS[6]

[1] All data reflect mean values, n = 3. Experiments conducted by Rockland Immunochemicals, Inc. Raw data available upon request.
[2] Cygnus Technologies product catalog number 3G-0016-AF
[3] Unpaired t-test
[4] Measured by ELISA using Amersham HCPQuant CHO
[5] Measured by ELISA using Cygnus Technologies product catalog number F550
[6] Not significant

Specifications

In 7 cm gels with DIBE CHO K1 Control Protein Cy3	
Coverage (%)	≥ 89*
Number of spots	≥ 320*

* The coverage percentage and the number of spots detected can vary due to user specific equipment, handling, and interpretation in the analysis software.

Kit contents

Description	Size	Product code
Amersham HCP DIBE CHO	For 5 mini gels or 1 large gel	29398892
DIBE CHO K1 HCP Antibody	1 vial	
DIBE Detection Reagent Cy5	100 µg	
DIBE CHO K1 Control Protein Cy3	250 µg	
DIBE Blocking Buffer (10 ×)	50 mL	
DIBE Wash Buffer (10 ×)	2 × 60 mL	
CyDye DIGE Cy3	5 nmol	25801083

Ordering information

Description	Size	Product code
Amersham HCP DIBE CHO kit	1 unit	29402111

Coverage instruments and software

Description	Size	Product code
Amersham Typhoon 5	1 unit	29187191
Amersham Typhoon RGB	1 unit	29187193
Amersham AI680 RGB	1 unit	29270772
DIGE gels	3 gels	28937451
Melanie 9 package (coverage, DIGE and Classic)	1 license node locked	29321923
Melanie 9 package (coverage, DIGE and Classic)	1 license, floating	29321924
Melanie 9 package (coverage, DIGE and Classic)	3 months lease	29321928
Melanie 9 coverage software	1 license, floating	29321922
Melanie 9 coverage software	1 license, node locked	29321665
Melanie 9 coverage software	3 months lease	29321927
Melanie 9 DIGE	1 license, floating	29270537
Melanie 9 DIGE	1 license, node locked	29270536

cytiva.com/HCPDIBECHO

CY14630-09Sep20-DF

cytiva