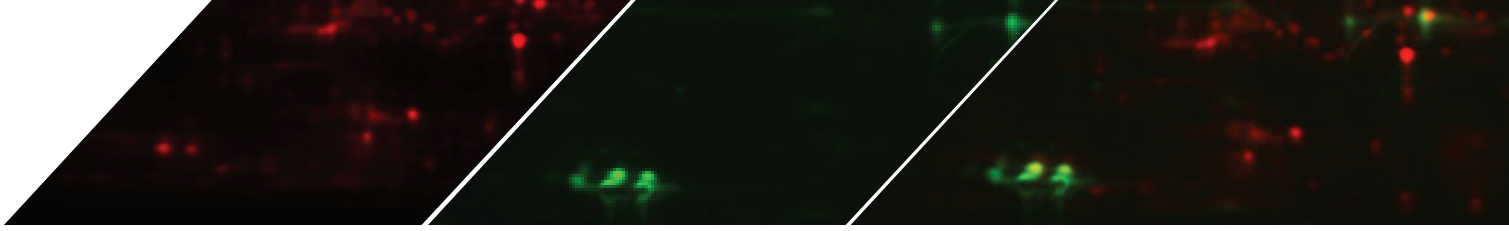

Introducing the latest DIBE technology for reliable HCP detection in biologics production

Efficient coverage analysis to validate your HCP ELISA assay

Host cell protein (HCP) is a primary impurity and a critical quality attribute (CQA) for biopharmaceuticals (biologics). HCP affects product quality, safety and efficacy.

HCP ELISA is the gold standard of HCP detection and measurement, which requires polyclonal Antibodies (Ab) with broad reactivity against a wide range of potential HCPs (Fig 1).

US and EU Pharmacopeia requires the characterization of the **ELISA Abs** used in the HCP ELISA assay. 2-D gel electrophoresis followed by Western blotting is the recommended approach to characterize HCP ELISA antibodies and their **coverages.** (USP 1132 & Ph. Eur. 2.06.34).

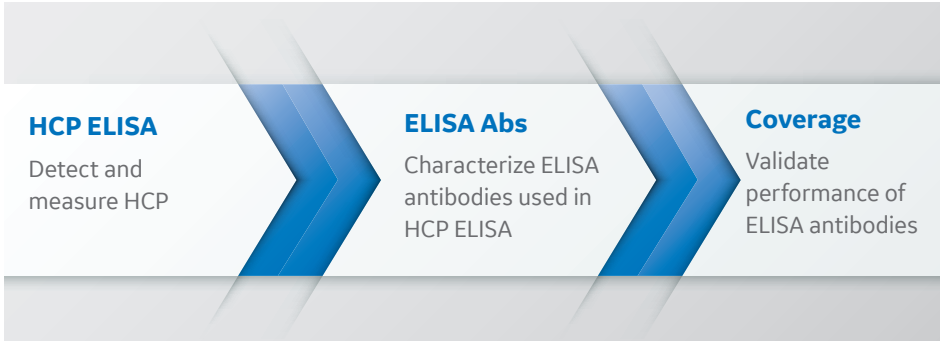

Fig 1. HCP analysis methods consists of 3 key components, HCP ELISA, ELISA Abs and Coverage assay.

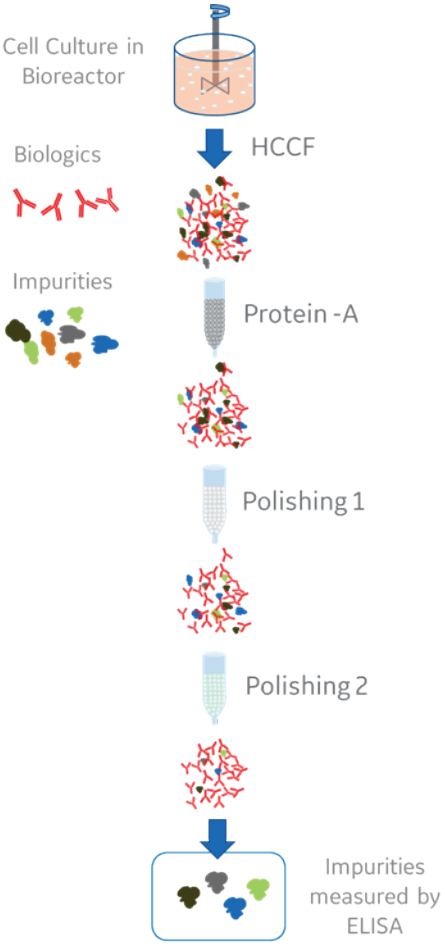

Fig 2. HCP purification steps

HCP removal is achieved by several steps of downstream chromatography purification (Fig 2). Continuous monitoring of HCP levels throughout the process and in the final product is required.

With growing attention to the safety of biologics and biosimilar drugs, HCP analysis is getting more attention from regulating authorities.

Shahrokh, Z. *et al* (2016). Science, Risks, and Regulations: Current Perspectives on Host Cell Protein Analysis and Control. *BioProcess Int.* **14**(8).

Challenges of current coverage assays

- Sensitivity to maximize HCP detection
- Variations caused by gel electrophoresis and membrane transfer
- Reliability between total HCP and immune anti-HCP antibody detection
- Time-consuming experimental preparation and data analysis for coverage

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Streamlined step-by-step workflow

2D differential in blot electrophoresis (2D-DIBE) combined with Western blotting is a powerful technology for separation and visualization of complex protein mixtures such as HCPs. To simplify the process and to improve quantitation, GE Healthcare has all the key components across the entire workflow, from sample prep to data analysis, for successful results.

High sensitivity

Fluorescent multiplexed methodology based on CyDye™ pre-labeled Western blotting, and image acquisition with Amersham™ Typhoon™ laser scanner deliver high sensitivity for HCP detection.

Minimal variation

Labeled proteins can be directly compared to the proteins detected by CyDye pre-labeled antibodies on the same membrane.

No mismatches

Multiplex fluorescence image acquisition with the Amersham Typhoon simultaneously captures both HCP antigen and anti-HCP antibody images from a single membrane.

Fast evaluation

Melanie™ Coverage software helps investigators evaluate the data in less time, with greater confidence.

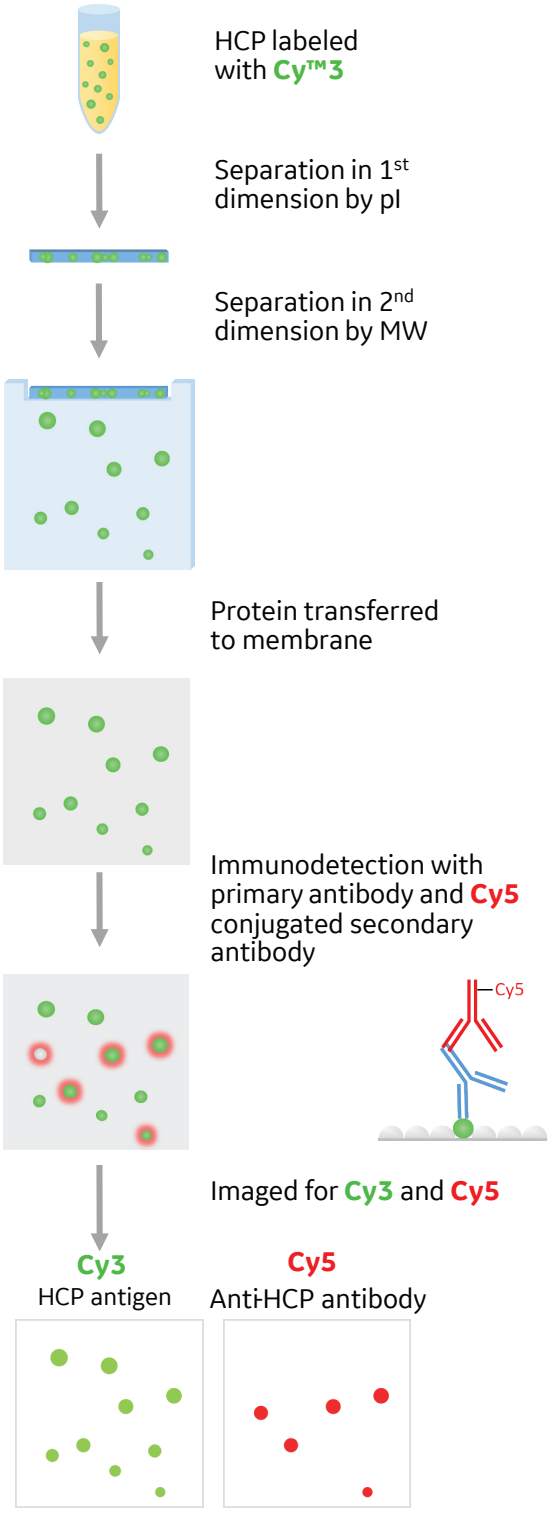

Fig 3. 2D-DIBE workflow

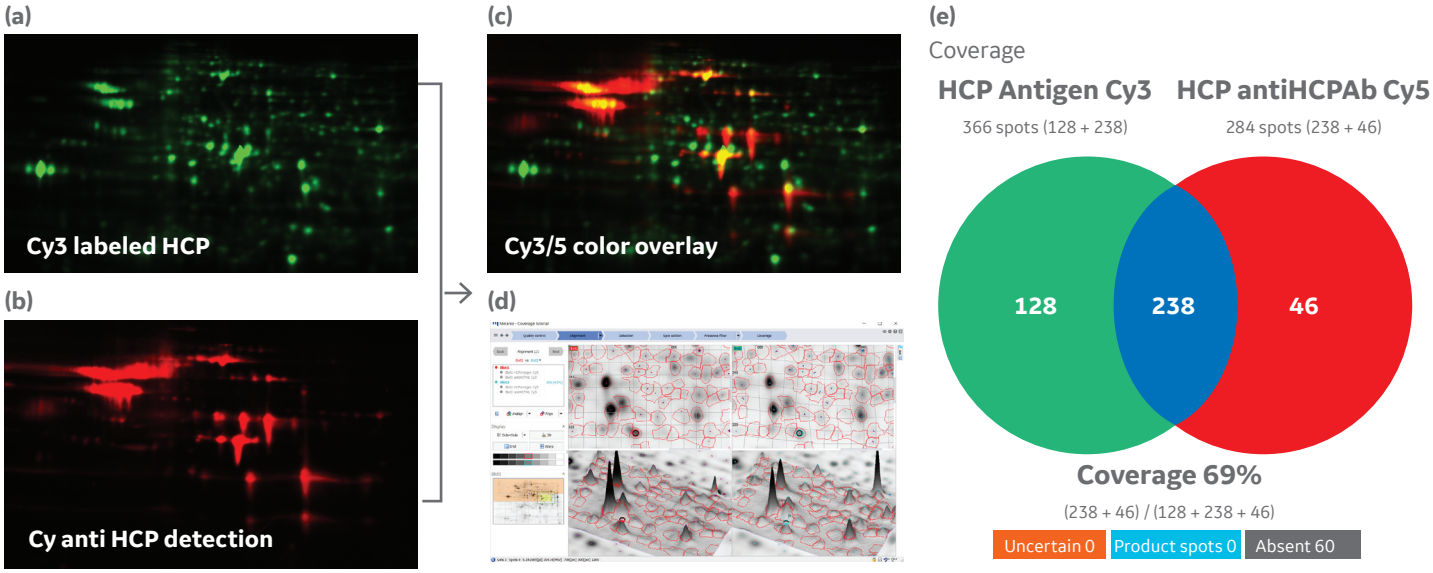

Fig 4. 2D-DIBE (a) Cy3 and, (b) Cy5 color images, (c) Cy3 & Cy5 color overlay, (d) Melanie 2/3D views and (e) Coverage % value report out calculate by Melanie software.

2D-DIBE for HCP coverage assay

Figure 4 shows an example of DIBE analysis. Total HCPs are labeled with CyDye DIGE Cy3 minimal dye. After 2D electrophoresis, protein spots are transferred onto a PVDF membrane (A). The HCP antibody is applied to the membrane and visualized by Western blot with Cy5 fluorescence (B). Those two images are then overlaid (C). Cy3 labeled total HCP spot and Cy5 immunodetected spot overlay is confirmed by Melanie Coverage analysis software with 3D visualization (D). Finally, Melanie Coverage provides a coverage percentage value for this assay (E).

Synchronized solutions for 2D-DIBE for HCP coverage assay

From sample preparation to image analysis, the success of each step will affect the steps that follow, and ultimately your results. GE Healthcare solutions span the entire process, leading with quality, reliability, efficiency and continuous improvement. Extending our capabilities for 2D-DIBE analysis, Melanie Coverage software allows you to characterize your anti-HCP ELISA antibodies with confidence, and supports your requirements for documentation, record-keeping and regulatory reporting. With the goal of helping you achieve the best results, we deliver 2D-DIBE products that improve data quality when compared to traditional 2D experiments and Western blotting, and can be integrated into a complete HCP analysis solution.

End-to-end products for HCP coverage assay

Sample preparation	Protein labeling	Protein separation	Protein transfer	Western blotting	Image acquisition	Image analysis
2-D Quant Kit 2-D Clean-Up Kit Illustra™ TriplePrep Kit	CyDye DIGE fluor Cy3/5	IPG buffer, DeStreak & Drystrip IPGphor™ IEF unit DALT gel SE 600 unit Mini VE unit	Amersham Hybond™ PVDF & Protran™ NC Whatman™ blotting paper TE 77 Transfer unit	Blocking buffer ECL Plex™ Cy3/5	Amersham Typhoon RGB/5 IQ/OQ & service contract	Melanie 9 Coverage/DIGE

Ordering information

Sample preparation	Quantity	Code number
2-D Quant Kit	500 assays	80648356
2-D Clean-Up Kit	50 samples	80648451
Illustra TriplePrep Kit	50 preps	28942544

Protein labling	Quantity	Code number
CYDYE DIGE CY3 MINIMAL 25 NMOL	25 nmol	25190028
CYDYE DIGE CY5 MINIMAL 25 NMOL	25 nmol	25190030
CYDYE DIGE CY3 MINIMAL 10 NMOL	10 nmol	25800861
CYDYE DIGE CY5 MINIMAL 10 NMOL	10 nmol	25800862
CYDYE DIGE CY3 MINIMAL 5 NMOL	5 nmol	25801083
CYDYE DIGE CY5 MINIMAL 5 NMOL	5 nmol	25801085

Protein transfer	Quantity	Code number
Amersham Hybond LFP PVDF 0.2 um (100 x 100 mm)	25	10600090
Amersham Hybond LFP PVDF 0.2 um (254 mm x 4 m)	1 roll	10600022
Amersham Protran Premium NC 0.45 um (100 x 100 mm)	25	10600078
Amersham Protran Premium NC 0.45 um (300 mm x 4 m)	1 roll	10600003
GB003 (10 x 10 cm)	50	10426880
GB003 (30 x 60 cm) , 0.8 mm thick	25	10426890
GB005 (58 x 58 cm), 1.2 mm thick	25	10426994
TE 77 Semi-dry transfer unit (21x26cm)	1	11001342

Western blotting	Quantity	Code number
ECL™ Prime Blocking Reagent	40 g	25190028
ECL PLEX Goat-Anti-Mouse IGG, Cy3	150 µg	25190030
ECL PLEX Goat-Anti-Mouse IGG, Cy5	150 µg	25800861
ECL PLEX Goat-Anti-Rabbit IGG Cy3	150 µg	25800862
ECL PLEX Goat-Anti-Rabbit IGG Cy5	150 µg	25801085

Protein separation	Quantity	Code number
IPG BUFFER, PH 3-11 NL	1 ml	17600440
DeStreak rehydration solution	5 x 3 ml	17600319
IMM. DRYSTRIP PH 3-11 NL, 7 CM	12	17600373
IMM.DRYSTRIP PH 3-11 NL, 13CM	12	17600375
IMM.DRYSTRIP PH 3-11 NL, 13CM	12	17600375
IMM. DRYSTRIP PH 3-11 NL, 24CM	12	17600377
ETTAN™ IPGPHOR 3	1	11003364
SE 600 RUBY, COMPLETE	1	80647957
MINIVE COMPLETE UNIT	1	80641877
DIGE gels	3	28937451

Image acquisition	Quantity	Code number
Amersham Typhoon 5	1	29187191
Amersham Typhoon RGB	1	29187193
Amersham Imager 680 RGB	1	29270772
IQOQ Amersham Typhoon 5	1	29145023
IQOQ Amersham Typhoon RGB	1	29245024
IQ/OQ documentation AI680	1	29098345

Image analysis	Quantity	Code number
Melanie 9 Coverage Perpetual Node-locked license	1	29270543
Melanie 9 Coverage Perpetual Floating license	1	29270737
Melanie 9 DIGE Perpetual Node-locked license	1	29270536
Melanie 9 DIGE Perpetual Floating license	1	29270537

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This version of Melanie has been developed by the SIB Swiss Institute of Bioinformatics in collaboration with GeneBio and GE Healthcare.
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