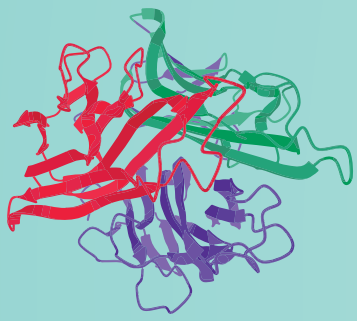
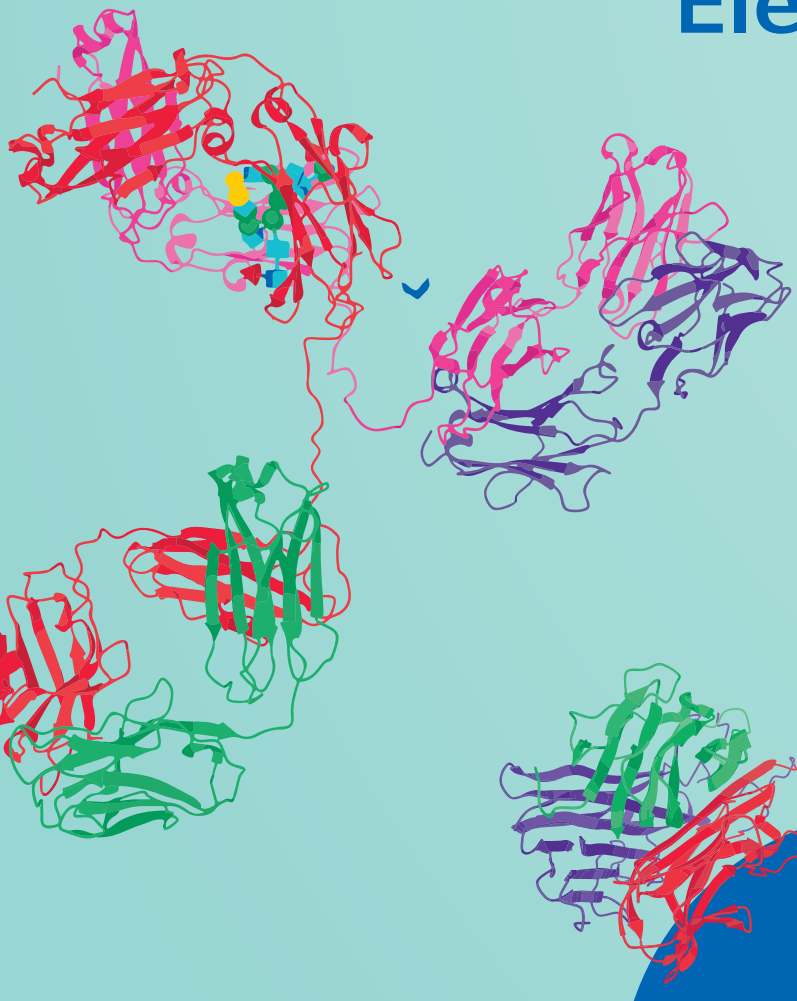
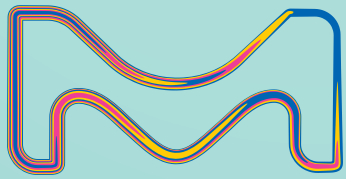
MERCK



Rapid, regulatory-compliant HCP assay development and validation with

Auto2D® 2-D Electrophoresis Device





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Auto2D Plus
Recipe Name : pH3-10 M
MAIN
SAMP. LOAD
IEF
SDS-PAGE
LOG
Ready
Touch START button
Operation Schedule
ON Gel Swelling
ON Sample Loading
ON IEF
ON SDS Equilibration
ON SDS-PAGE
START
STOP
OPEN
CLOSE
SETTING
MENU
Touch START button after checking the setting.
13:43
Auto2D

Residual HCP measurement in biopharmaceuticals

Residual host-cell protein (HCP) impurities introduced during biologics manufacturing processes can trigger immunogenic responses in patients and reduce drug efficacy. Regulatory compliance calls for the monitoring and removal of HCPs to acceptably low levels to ensure safety. Pharmacopoeias in Japan, Europe, and the United States have published guidelines for the development and validation of HCP assays for biopharmaceutical product manufacturing.

To meet compliance, HCP assays must be methodically developed with rigorous qualification of all assay components. For antibody validation, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is required to ensure comprehensive HCP coverage. Gel electrophoresis methods including 2D-PAGE are also advised for the characterization of antigens used as calibration standards or immunogens for polyclonal antibody production.

Though a powerful technique for analysis, 2D electrophoresis has been regarded as a high skilled, tedious technique that requires multiple manual procedures for sample loading, gel equilibration, and transfer. We introduce a fully automated 2D gel electrophoresis system for HCP assay development and validation. The Auto2D® 2-D Electrophoresis Device eliminates the need for advanced user training, facilitating implementation into bioprocess workflows to meet compliance with industry standards.

Auto2D® systems for fully automated 2D gel electrophoresis

The Auto2D® system fully automates two-dimensional gel electrophoresis. The applied sample enters the first-dimension gel passively, separating proteins by their isoelectric points. After equilibration, the first-dimension gel is set onto a horizontal SDS-PAGE gel, which resolves proteins by molecular weight. Auto2D® 2-D Electrophoresis Device is user-friendly and enable two-dimensional electrophoresis in 1-2 hours for faster, more reproducible results.

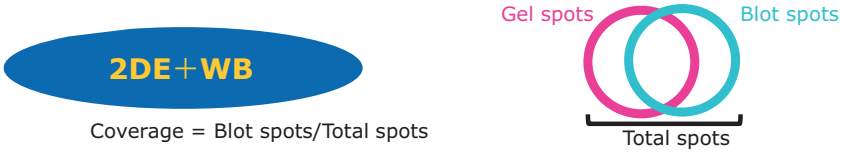
- Fully automated to eliminate tedious handling steps
- Easy to use, with no advanced training required
- Quick workflow implementation for reduced lab downtime
- Fast results in 1-2 hours
- Better reproducibility
- Decreased inter-operator variability
- IQ/OQ support for GMP compliance

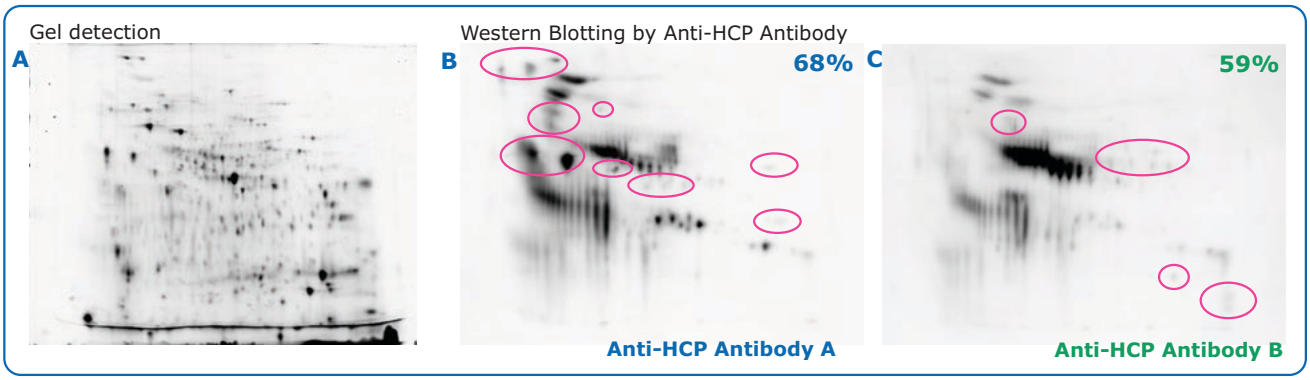
Anti-HCP antibody validation for ELISA and other immunoassays

ELISAs are the most common assay format for HCP analysis. This immunoassay method relies on polyclonal antibodies that recognize residual HCPs with broad range coverage.

Japanese and European Pharmacopoeia specify the use of 2D gel electrophoresis followed by Western blot analysis (2D Western blotting) to evaluate HCP antibody coverage using. U.S. Pharmacopoeia specifies the use of 2D Western blotting or immunoaffinity purification followed by gel electrophoresis to evaluate antibody coverage.

HCP coverage using the Auto2D® system

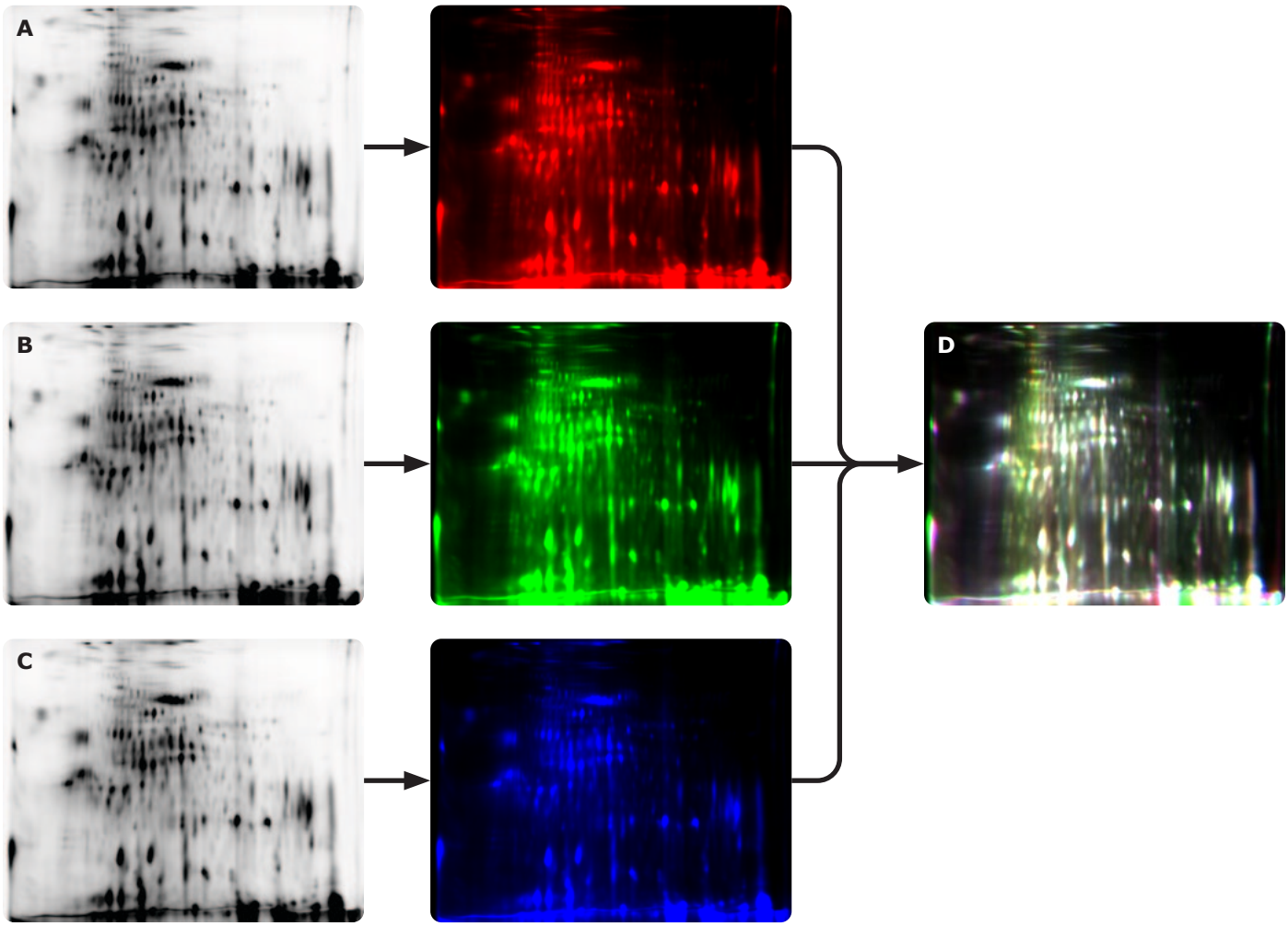




CHO cell culture supernatant was desalted with Protein-Concentrate Kit (Cat. No. 2100/2102) and the pellet was resuspended in rehydration solution. A portion of sample was labeled with Cy5 NHS ester. Approximately 2 µg of the Cy5 labeled CHO HCP antigens and 8 µg of non-labeled CHO HCP antigens were separated on the Auto2D® system using IEF Chip pH3-10 and 10.0% PAGE Chip (A). Proteins were subsequently transferred to Immobilon®-FL using the fast transfer system. Two different anti-HCP antibodies were used for the Western blot immunodetection (B, C). HCP coverage was calculated for the gel image and Western blot image using the SpotMap software. This data suggests that the coverage of Anti-HCP Antibody A is higher than Anti-HCP Antibody B. The spots in the red circles are unique spots detected by each antibody and a Western blot using a mixture of both antibodies will increase total coverage.

The Auto2D® system helps you meet compliance with regulatory methods for anti-HCP antibody validation, providing a simpler workflow with results in a significantly shorter timeframe compared to manual 2D gel electrophoresis techniques.

High reproducibility of Auto2D® System



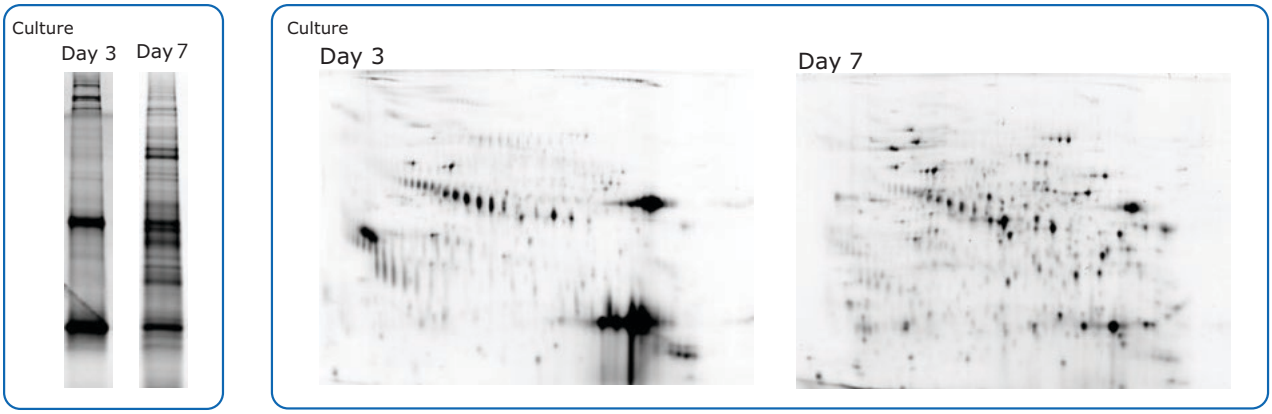
0.75 µg of Cy5-labeled CHO HCP antigen from a commercial source was separated by 2D gel electrophoresis and analyzed on the Auto2D® system using a IEF Chip pH 3-10NL and PAGE Chip 12.5%. The same sample was measured a total of three times (A-C). White spots (D) show overlap of results. The data suggest high reproducibility of two-dimensional gel electrophoresis using the Auto2D® system.

Consistent, reproducible processes are essential in biologics manufacturing and quality control. In addition to faster results and higher throughput, the fully automated Auto2D® gel electrophoresis system eliminates day-to-day and inter-operator variability in 2D-PAGE and 2D-DIGE methods, offering significantly better reproducibility for more reliable results.

Antigen profiling

Immunization with HCP antigen is required to obtain polyclonal antibody pools for ELISA and other immunoassays. HCP antigen is also used as a calibration standard for detection and quantitation. Process-specific HCP antigens are generated using non-transfected null cell cultures. These antigens must be characterized to show the pattern of HCPs, confirm the presence of a broad spectrum of proteins, and demonstrate that HCP antigens from null cultures are representative of those in production cultures. Japanese, European, and U.S. Pharmacopoeia advise the use of SDS-PAGE or 2D gel electrophoresis for antigen characterization and profiling. U.S. Pharmacopoeia also suggests the use of 2D gel electrophoresis as a complementary method for residual HCP monitoring.

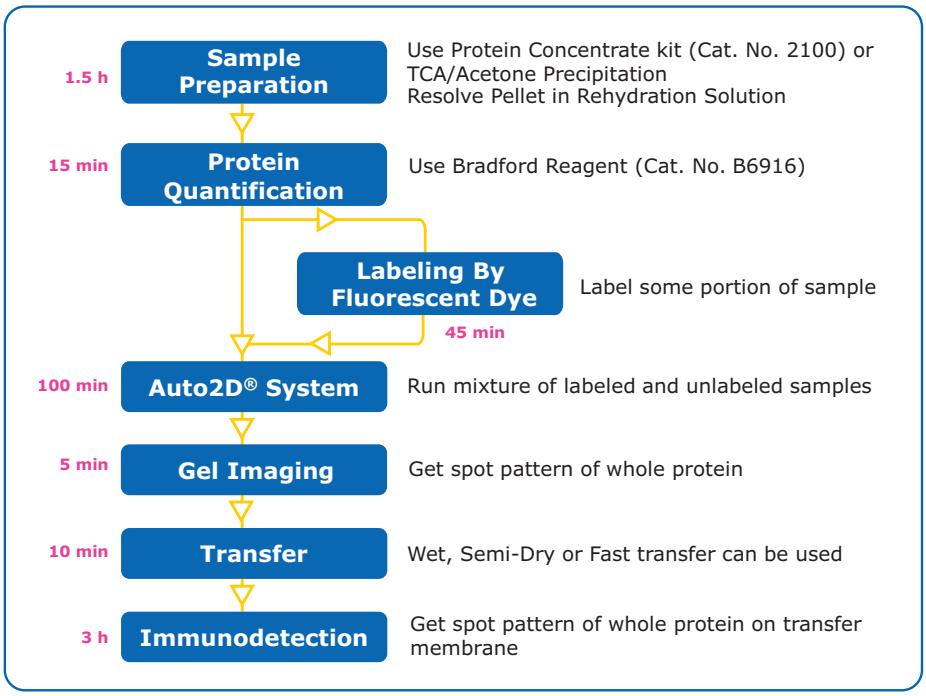
HCP antigen profiling using the Auto2D® automated system



Culture supernatants of CHL-YN cell cultured for 3 and 7 days were desalted with Protein-Concentration Kit (Cat. No. 2100/2102) and the pellets were resuspended in rehydration solution. Samples were labeled with Cy3 or Cy5 NHS ester. Approximately 3 µg each of Cy3 labeled CHL-YN HCP Day 3 and Cy5 labeled CHL HCP Day 7 were separated by SDS-PAGE using 10% Tris-Glycine gel and Auto2D® system using IEF Chip pH3-10NL and 10.0% PAGE Chip. HCP profile can change depending on cell culture conditions. HCP Profiling can be done by SDS-PAGE or Mass Spec as well as 2DE. By using 2DE, comparison of the HCP profiles is easier than SDS-PAGE, and the profiling result can be compared with the coverage test result which is done by 2DE. Samples were provided from Noriko Yamano-Adachi Ph.D., Osaka University

The Auto2D® system ensures compliance with regulatory methods for HCP antigen profiling and characterization, offering higher throughput and easy implementation for reduced downtime between productions.

Recommended Workflow for HCP Coverage Test with Tips & Tricks



Tips & Tricks

One run generates both whole protein spot pattern and Immunodetection spot pattern.

Easy to check transfer and match spots.

Auto2D® system for regulatory compliance

The Auto2D® system helps you meet compliance with regulatory requirements for HCP analysis in biopharmaceutical manufacturing. This fully automated system eliminates day-to-day and inter-operator variability for reliable results in less than 2 hours. Easy to implement into bioprocess workflows, the Auto2D® system reduces lab downtime associated with production changes. There's no need to outsource –protect your intellectual property using in-house resources for HCP analysis. Talk to a specialist to learn more.

Ordering information

Product name	Quantity	Cat. No.
Auto2D® Base Unit		
Auto2D® 2-D Electrophoresis Device	1 unit	**BM-100**
Auto2D® Special Consumables		
Electrode Chip	1 chip	**BM-1E**
Solution Chip	10 chips	**BM-1S**
Auto2D® Plus Special Consumables		
Electrode Chip Plus	1 chip	**BM-1EP**
Solution Chip Plus	10 chips	**BM-1SP**
Auto2D®/Auto2D® Plus Common Consumables		
IEF Chip pH 3-10	10 chips	**BM-113010**
IEF Chip pH 3-10NL	10 chips	**BM-113010NL**
IEF Chip pH 4-7	10 chips	**BM-114070**
IEF Chip pH 4-5.5	10 chips	**BM-114055**

Product name	Quantity	Cat. No.
IEF Chip pH 5-6.5	10 chips	**BM-115065**
IEF Chip pH 6-10	10 chips	**BM-116010**
IEF Chip pH 7-10	10 chips	**BM-117010**
PAGE Chip 6.5%	10 chips	**BM-12065**
PAGE Chip 7.5%	10 chips	**BM-12075**
PAGE Chip 10.0%	10 chips	**BM-12100**
PAGE Chip 12.5%	10 chips	**BM-12125**
Tris-Glycine Reagent Kit	1 kit	**BM-1RYSJ1**
Tris-Tricine Reagent Kit	1 kit	**BM-1RYTJ1**
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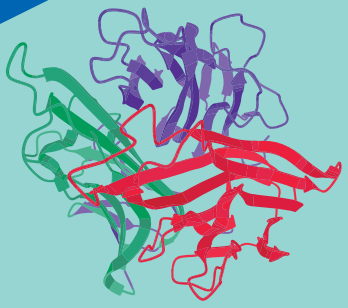

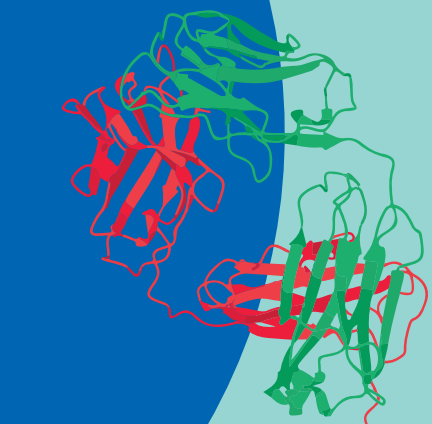

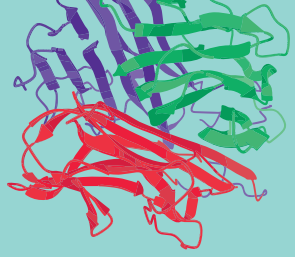

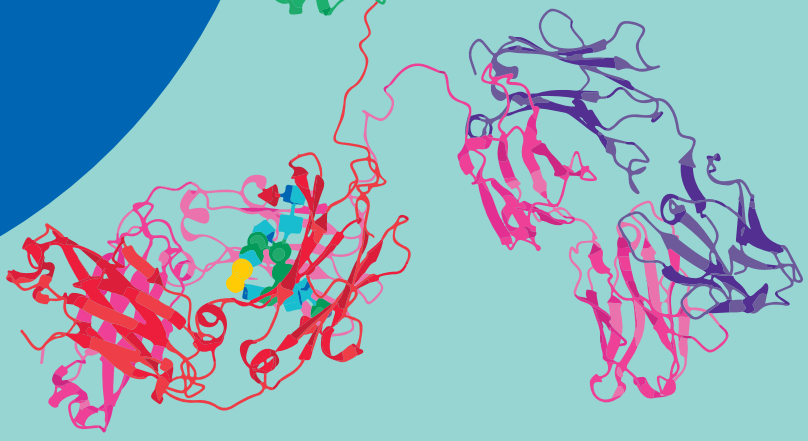

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