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Application Note

Auto2D® 2-D Gel Electrophoresis for Food Allergy Applications

Rapid and automated 2-D gel electrophoresis capabilities to separate and identify allergenic proteins from diverse protein sources.

Given the large and growing number of people who suffer from allergies worldwide, it is increasingly important to detect and identify allergens, particularly in food sources. Two-dimensional gel electrophoresis (2D-E) is traditionally used for high-resolution protein separation necessary for the immunodetection of allergenic proteins. However, this traditional separation process is often time-consuming and technically challenging. Our Auto2D® system enables fully automated 2-D electrophoresis in 1-2 hours with quick and reliable results. Here we show that our Auto2D® system is suitable for identifying allergenic proteins from multiple different sources.

Introduction

Allergies are a common worldwide health issue, with the three major sources of allergens including food (estimated to affect 4% of the world population), pharmaceutical drugs (estimated 10% of the population), and plant pollen (estimated 10-30% of the population and rising)[1-3]. Allergic reactions occur through an IgE-mediated immunological response and have traditionally been tested through a skin prick test; however, allergen testing, and diagnostics can also be achieved through molecular approaches[1]. The radioallergosorbent test (RAST) and the enzyme-linked immunosorbent assay (ELISA) are two methods used to detect and quantify IgE against specific allergens. Since RAST and ELISA do not include a protein separation step, cross-reactivity between antibodies and food matrix components can cause false-positive results in some instances[1].

Gel electrophoresis is a common and widely used protein separation method. While standard one-dimensional gel electrophoresis separates proteins based on their molecular weight, two-dimensional gel electrophoresis provides higher resolution by separating proteins based on isoelectric point (pI). This increased resolution allows researchers to distinguish between proteins that are close in molecular weight, particularly in the case of isoforms that may have differing allergenicity. The 2D gel electrophoresis technique also has low technical variability, making it a good option for research that has human health implications, such as allergen identification[4].

The 2D gel electrophoresis technique and subsequent immunodetection has been used to identify a variety of allergenic proteins from diverse sources. Plant pollen researchers have used this technique to identify novel IgE binding proteins from red oak pollen as well as to confirm clinically relevant allergens in a natural grass extract, including multiple isoforms of one allergenic protein[5,6]. 2D gel electrophoresis was used in a deep dive investigation of the major peanut allergen families, comparing different peanut strains, and has also been used to confirm no differences in allergens between natural and genetically modified soybeans[7,8].

Here, we demonstrate the utility of the Auto2D® gel electrophoresis system in detecting allergens from four food sources: three plants (soybean, walnut, and buckwheat) and one animal source (salmon roe). Additionally, our system was successfully used to identify specific allergens following immunodetection using patient serum. We used the Auto2D® system to resolve single bands from SDS-PAGE into multiple distinct protein spots corresponding with major known allergens in the soybean and salmon roe samples. These results suggest that the Auto2D® gel electrophoresis system can be used for allergen identification, reducing both time and necessary technical expertise while still providing high-quality, reliable results.

Results

Soybean extract was obtained by soaking 2.5 g of commercially available soybeans in 25 mL of distilled water overnight, crushing them with a mill, and squeezing them with gauze. The protein concentration was similar to soy milk (approx. 30 µg/µL). Then, 30 µg of sample was subjected to analysis using the Auto2D® system and 0.5 µL to conventional SDS-PAGE. For 2D-E with the Auto2D® system, IEF Chip pH3-10NL was selected as the 1st-Dimension and PAGE Chip 12.5% as the 2nd-Dimension. The sample was run using the standard program (pH3-10NL M). After electrophoresis, proteins in 1D SDS-PAGE and Auto2D® gels were stained with Coomassie Brilliant Blue Stain (CBB). A separate sample was run on our Auto2D® device and was transferred to an Immobilon®-P blotting membrane via semi-dry blotting. For immunodetection, the blotting membrane was blocked with 5% NFDM in PBS-T. After blocking, soybean allergic patient serum (International Bioscience, Inc.) was diluted 20x in blocking solution, added to the membrane, and incubated with gentle agitation. After washing with PBS-T, the membrane was reacted with HRP-conjugated secondary antibody diluted in blocking solution followed by standard chemiluminescence detection.

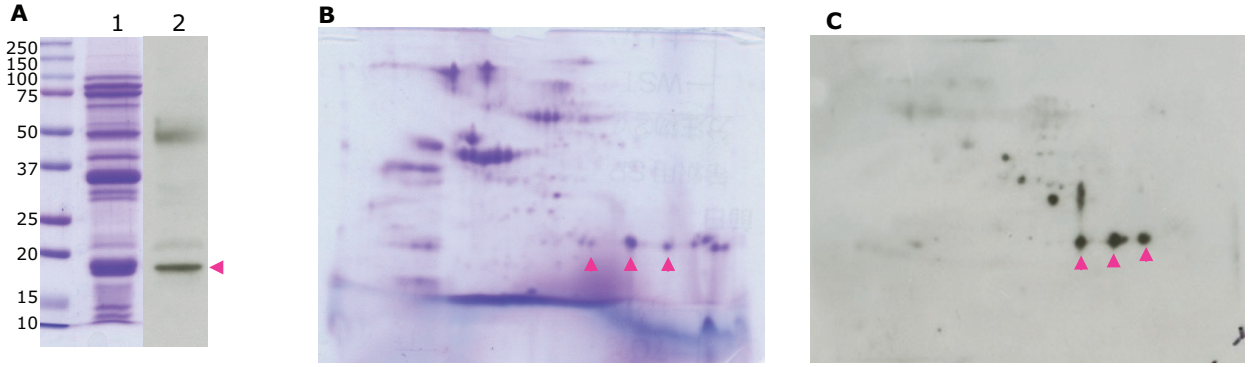

Figure 1. 2D-E separation of soybean extract and immunodetection using patient serum.
Soybean protein extract was separated by SDS-PAGE (A: 15 µg) and the Auto2D® system (B, C: 30 µg) using IEF Chip: pH3-10NL, PAGE Chip: 12.5% and the Tris-Glycine Reagent Kit. Whole protein in the gel was stained with CBB (A-1, B). Immunodetection was performed using soybean-allergic patient serum (A-2, C). Corresponding to the main band in SDS-PAGE, spots considered to be Gly m 6, a major soybean allergen component were detected around 18 kDa (red arrows).

Each additional antigen sample was prepared under the following conditions respectively. Buckwheat flour was defatted with diethyl ether, treated with coca buffer (85 mM NaCl, 32.7 mM $NaHCO_3$, 42.5 mM phenol), and dialyzed with PBS. To remove salts and ionic impurities that can affect the 1st-D IEF process, buckwheat extract was further processed using a protein precipitation kit (ProteoExtract® kits). Walnuts were crushed with a mortar and protein extraction was performed using the Mammalian Cell Lysis Kit (MCL1, Sigma). Salmon roe tissue was homogenized in 1M KCl-PBS. Walnut and salmon roe extracts were desalted using gel-filtration spin columns. Depending on the desalting method, protein samples were dissolved in or exchanged to rehydration solution (8M urea, 2 M thiourea, 4 % CHAPS, 50 mM DTT, 0.02 % ampholyte).

Protein quantitation for each prepared sample was performed using BCA or Bradford protein assay. Protein separation was performed using the pre-installed standard Auto2D® program (“pH3-10NL M” recipe), or by conventional SDS-PAGE. IEF Chip pH3-10NL and PAGE Chip 12.5 % were selected as the 1st-D and 2nd-D gel chip to cover the widest possible separation range. After electrophoresis, separated proteins in Auto2D® gels were stained with Coomassie ReadyBlue™ stain or SYPRO® Ruby Gel stain. Separately prepared Auto2D® gels for immunodetection were removed from the chip and transferred to an Immobilon®-P blotting membrane using a wet transfer device (KS8452, Oriental instrument). PVDF membranes with transferred proteins were blocked with SuperBlock™ Blocking Buffer in PBS for 1hr. Patient sera were diluted 30-fold with PBS containing 0.1 % BRIJ and allowed to react with the membranes while shaking at 4° C overnight. After washing the membrane with the above dilution buffer, alkaline phosphatase (AP) conjugated Anti-human IgE Ab (SeraCare) diluted 2,000-fold was applied as a secondary Ab and incubated at room temperature for 3 hrs. After washing, the membrane was equilibrated in AP reaction buffer (100 mM Tris-HCl buffer pH 9.5 containing 100 mM NaCl and 5mM $MgCl_2$) and reacted with 1-component type of BCIP/NBT substrate (SeraCare) for chromogenic detection of the target proteins.

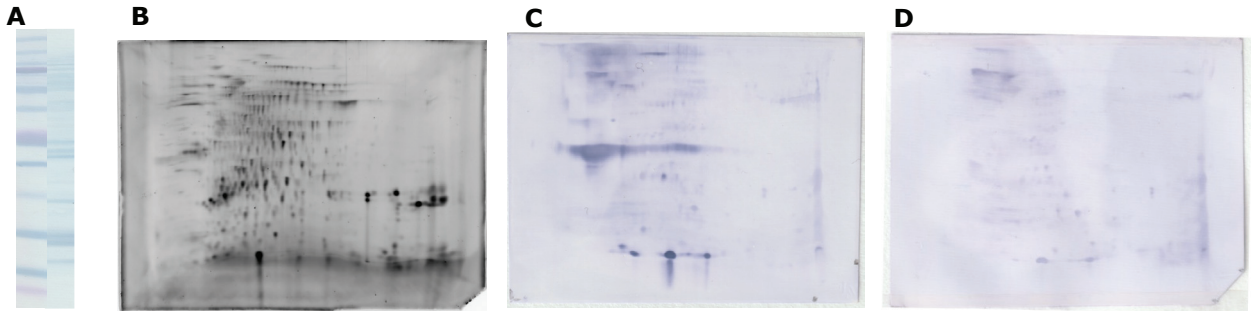

Figure 2. 2D-E separation of walnut protein extract and immunodetection with allergic patient serum.
Walnut protein extract was desalted in a gel-filtration spin column, equilibrated with rehydration solution, and separated by SDS-PAGE (A) and the Auto2D® system (B, C, D: 10 µg) using IEF Chip: pH3-10NL, PAGE Chip: 12.5% and Tris-Glycine Reagent Kit. Whole protein in walnut extract is visualized by amido black on membrane (A), and by SYPRO® Ruby gel stain (B), immunodetection using patient serum (C) and non-allergic serum as a negative control (D).

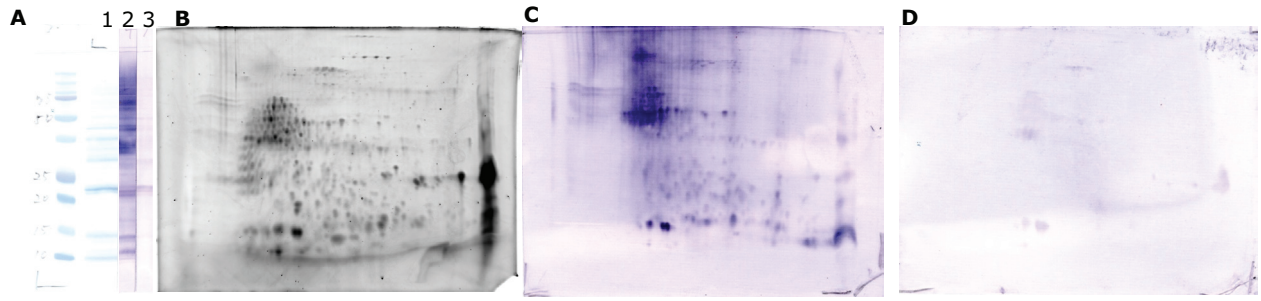

Figure 3. Immunodetection with buckwheat-allergic patient serum.
Buckwheat protein extract treated with the ProteoExtract® precipitation kit was dissolved in rehydration solution and separated by SDS-PAGE (A) and the Auto2D® system (B, C, D: 10 µg) using IEF Chip: pH3-10NL, PAGE Chip: 12.5 % and the Tris-Glycine Reagent Kit. Whole protein in buckwheat extract is visualized by Amido Black on membrane (A-1) or SYPRO® Ruby reagent in gel (B). Immunodetection using patient serum (A-2 and C) and non-allergic serum as a negative control (A-3 and D).

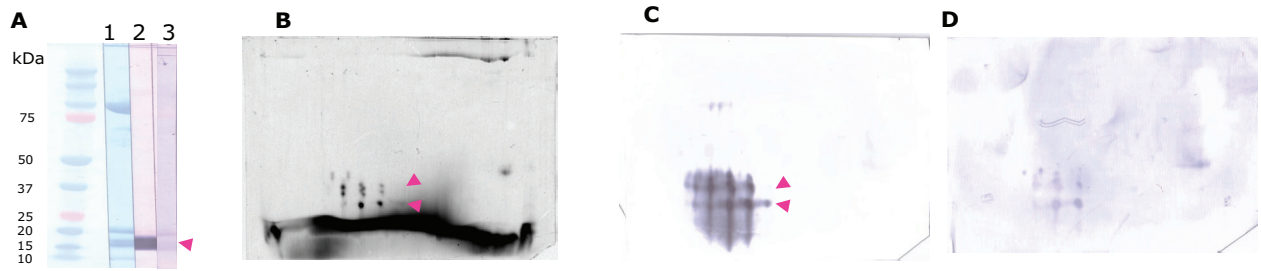

Figure 4. Separation and immunodetection of allergic components by probing with salmon roe-allergic patient serum.
Salmon roe protein extract was desalted in a gel-filtration spin column, equilibrated with rehydration solution and separated by SDS-PAGE (A) and the Auto2D® system (B, C, D) using IEF Chip: pH3-10NL, PAGE Chip: 12.5 %, and the Tris-Glycine Reagent Kit. Whole protein in salmon roe extract is visualized by Amido Black on membrane (A-1) or ReadyBlue™ CBB staining reagent in gel (B, 35 µg). Immunodetection using patient serum (A-2 and C, 10 µg) and non-allergic serum as a negative control (A-3 and D: 10 µg). Spots (red arrows) around 15 to 20 kDa are considered major allergens of salmon roe β'-component, which is a 35 kDa-vitellogenin-fragment consisting of two subunits.

The Western blot images were compared with total protein detection to identify the protein spots that reacted with IgE in allergic sera. To identify the allergenic component, overlapped spots are usually excised from the gel, enzymatically in-gel digested into peptide fragments, and the fragments are analyzed using mass spectral analysis. 2D-E was able to demonstrate superior resolution, showing the presence of multiple proteins in what appeared to be a single band in 1D-E. These data suggest that IgE reacting proteins can be separated and identified in fewer steps by 2D-immunodetection using allergic sera, more easily enabling the identification of causative molecules by mass spectral analysis.

Discussion

Two-dimensional gel electrophoresis is a common protein separation technique used in the detection and identification of allergenic proteins. The Auto2D® system is designed to reduce the time and technical expertise necessary to obtain high-quality results from this technique. Here, we confirm the utility of the Auto2D® system for allergy research by detecting allergens in protein samples from four food sources. Additionally, we were able to resolve a known, major allergen in each of the soybean and salmon roe samples from a single band in one-dimensional SDS-PAGE into multiple, distinct spots using the Auto2D® system. In the soybean sample, we resolved three spots from the 18 kDa band that corresponds to the basic subunit of Gly m 6 (glycinin), a major, known allergenic protein[9]. In the salmon roe sample, we resolved multiple spots from the 16-18 kDa band that corresponds with the two subunits of β'-component (vitellogenin), also a major, known allergenic protein[10]. Overall, these results suggest that the Auto2D® system can be used effectively as a fast, reliable alternative to traditional 2D gel electrophoresis for allergen immunodetection.

For additional information and resources, please explore our Auto2D® Automated 2-D Gel Electrophoresis technical resource page: **SigmaAldrich.com/auto2d**

Acknowledgements

We gratefully acknowledge Prof. Tatsuya Moriyama (Kindai University, Faculty of Agriculture) for providing soybean data and Prof. Yasuto Kondo (Fujita Health University Bantane Hospital) for providing walnut, buckwheat, and salmon roe data.

References

1. De Angelis, Maria, Raffaella Di Cagno, Fabio Minervini, Carlo Giuseppe Rizzello, and Marco Gobbetti. "Two-Dimensional Electrophoresis and IgE-Mediated Food Allergy." Electrophoresis 31, no. 13 (July 2010): 2126–36. **https://doi.org/10.1002/elps.201000101**.
2. "WAO White Book on Allergy | World Allergy Organization." Accessed July 24, 2023. **https://www.worldallergy.org/wao-white-book-on-allergy**.
3. Schmidt, Charles W. "Pollen Overload: Seasonal Allergies in a Changing Climate." Environmental Health Perspectives 124, no. 4 (April 2016): A70–75. **https://doi.org/10.1289/ehp.124-A70**.
4. Pallen, Catherine, Claire Friry-Santini, Corinne Herouet-Guicheney, and Annabelle Capt. "Technical Variability of 2D Gel Electrophoresis – Application to Soybean Allergens." Toxicology Reports 1 (September 16, 2014): 734–42. **https://doi.org/10.1016/j.toxrep.2014.09.002**.
5. Huerta-Ocampo, José Ángel, Alejandra Valenzuela-Corral, María Del Refugio Robles-Burgueño, Ana María Guzmán-Partida, Miguel Ángel Hernández-Oñate, Luz Vázquez-Moreno, Gandhi F. Pavón-Romero, and Luis M. Terán. "Proteomic Identification of Allergenic Proteins in Red Oak (Quercus Rubra) Pollen." The World Allergy Organization Journal 13, no. 3 (March 2020): 100111. **https://doi.org/10.1016/j.waojou.2020.100111**.
6. Corti, Valeria, Angela Cattaneo, Angela Bachi, Renato E. Rossi, Giorgio Monasterolo, Clara Paolucci, Samuele E. Burastero, and Massimo Alessio. "Identification of Grass Pollen Allergens by Two-Dimensional Gel Electrophoresis and Serological Screening." Proteomics 5, no. 3 (February 2005): 729–36. **https://doi.org/10.1002/pmic.200401038**.
7. Mamone, Gianfranco, Luigia Di Stasio, Salvatore De Caro, Gianluca Picariello, Maria Adalgisa Nicolai, and Pasquale Ferranti. "Comprehensive Analysis of the Peanut Allergome Combining 2-DE Gel-Based and Gel-Free Proteomics." Food Research International (Ottawa, Ont.) 116 (February 2019): 1059–65. **https://doi.org/10.1016/j.foodres.2018.09.045**.
8. Batista, Rita, Isabel Martins, Paul Jeno, Cândido Pinto Ricardo, and Maria Margarida Oliveira. "A Proteomic Study to Identify Soya Allergens--the Human Response to Transgenic versus Non-Transgenic Soya Samples." International Archives of Allergy and Immunology 144, no. 1 (2007): 29–38. **https://doi.org/10.1159/000102611**.
9. Hou, Dick Home-jer, and Sam Kow-Ching Chang. "Structural Characteristics of Purified Glycinin from Soybeans Stored under Various Conditions." Journal of Agricultural and Food Chemistry 52, no. 12 (June 1, 2004): 3792–3800. **https://doi.org/10.1021/jf035072z**.
10. Shimizu, Yutaka, Hideki Kishimura, Gaku Kanno, Atsushi Nakamura, Reiko Adachi, Hiroshi Akiyama, Kazuhiko Watanabe, Akihiko Hara, Motohiro Ebisawa, and Hiroki Saeki. "Molecular and Immunological Characterization of β'-Component (Onc k 5), a Major IgE-Binding Protein in Chum Salmon Roe." International Immunology 26, no. 3 (March 2014): 139–47. **https://doi.org/10.1093/intimm/dxt051**.

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