Use of the PROTEAN Plus™ Dodeca™ Cell for Second-Dimension SDS-PAGE

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Abstract

I report the use of the Bio-Rad PROTEAN Plus Dodeca cell (Figure 1) for running 2-D SDS-PAGE slab gels with preparative protein loads. The Dodeca cell was used to run up to 11 vertical 9–16% gradient SDS-PAGE gels. Comparison of the gel images revealed a particularly well-resolved and reproducible set of second-dimension gels with very little smearing or streaking associated with any spots.

Fig.1. PROTEAN Plus Dodeca cell, used for second-dimension electrophoresis of up to 12 large-format vertical SDS-PAGE gels in parallel.

Introduction

In proteome analysis it is crucially important to be able to run reproducible 2-D gels. Such is the importance of running high-quality gels for image analysis that many scientists have settled on performing two separate procedures in order to seek and identify protein expression changes. The first procedure involves running a set of gels with a low amount of protein loaded and using these gels for image analysis. The second procedure requires running additional gels with a much larger amount of protein for protein identification purposes (Görg et al. 2000). This practice has two main disadvantages: first, more gels must be run, and second, due to the poorer quality of heavily loaded gels, it is more difficult to be sure of selecting the correct spot for protein identification. At Roche Welwyn, I have taken the approach of working with more heavily loaded gels and using these gels for both image analysis and protein identification.

I regularly use the Bio-Rad PROTEAN® II xi cell for running second-dimension SDS-PAGE. However, this system is designed to run only two gels per unit for 2-D electrophoresis. Difficulties occurred when sandwich gels were used in order to increase the number of gels from two to four per run. The particular problems that were associated with the sandwich gel system were vertical smearing or "cometing" of spots from higher molecular weight proteins, and poor reproducibility of gels. Therefore, I have evaluated the use of the PROTEAN Plus Dodeca cell (Figure 1, catalog #165-4151, 220/240 V; also available in 100/120 V, catalog #165-4150) to run these gels. Using the conditions described, I obtained very high-quality gels that could be used for both image analysis and mass spectrometric identification of proteins.

Methods

Preparation of Isoelectric Focusing Strips

Cells from a human cell line were lysed with 7 M urea, 2 M thiourea, 2% CHAPS, 50 mM Tris, pH 7.5, 0.4% dithioerythritol (DTE). Total cell protein (500 µg) was loaded, using sample cups, onto pH 4–7 IPG strips at 14°C. Voltage was applied initially at 200 V and gradually increased to 3,500 V over 20 hr, then maintained at 3,500 V for an additional 48–60 hr (Langen et al. 2000).

Gel Casting

Second-dimension gels for the Dodeca cell were cast using the PROTEAN Plus hinged spacer plates, the PROTEAN Plus multi-casting chamber, and the Model 395 gradient former. (The Model 495 gradient former, catalog #165-4121, is now available with a larger volume capacity. This would be a more appropriate choice.) Gradients were formed using Laemmli reagents with 4% glycerol added to the higher

percentage acrylamide solution to increase gradient stability. The reagents for casting twelve 1.0 mm, 9–16% gels were as follows: for the 9% solution, 114 ml of 30% acrylamide (37.5:1 acrylamide:bis-acrylamide, Roche Diagnostics), 142.2 ml 1 M Tris, pH 8.8, 120 ml water, 1.91 ml 20% SDS, 126 ml 10% ammonium persulfate, and 252 µl TEMED; for the 16% solution, 197 ml acrylamide, 138 ml Tris, 32 ml 50% glycerol, 1.85 ml SDS, 1.23 ml ammonium persulfate, and 245 µl of TEMED. TEMED was added to the solutions at the last moment and the gradients pumped into the bottom of the casting chamber over about 6 min. Each gel was immediately overlaid with 0.75 ml of water-saturated butanol, and once set, transferred to 4ºC for storage overnight before use.

Equilibration and Loading of IPG Strips

IPG strips were equilibrated in 10 ml of 50 mM Tris-HCl, pH 8.8, 6 M urea, 30% glycerol, 2% SDS, and Bromophenol Blue (trace), first for 15 min with 100 mg dithioerythritol (DTE), and then for 15 min in a second 10 ml of equilibration buffer with 250 mg iodoacetamide. Strips were then placed on the top surface of the SDS-PAGE gels and sealed with agarose in SDS-PAGE running buffer (Berkelman and Stenstedt 1998).

Running Conditions

The loaded gels (11) were placed into the Dodeca chamber with 23 L of running buffer (made by dilution of Bio-Rad Tris/glycine/SDS buffer, catalog #161-0772) precooled to 18°C using a Techne FC-200 flow cooler and circulator. (Another gel was run separately.) Voltage was applied at 50 V for 0.5 hr, then at 200 V until the Bromophenol Blue line reached the bottom of the gels (about 6 hr). The Dodeca cell is straightforward to use when the PowerPac™ power supply is programmed at constant voltage with no current limit. Under constant voltage conditions, every gel experiences the same running conditions regardless of the number of gels in the tank. The temperature did not rise during the run and the buffer recirculation seemed to be particularly efficient in maintaining a uniform temperature throughout the inner buffer chamber.

Staining and Imaging

Post-electrophoresis, the gels were removed from the PROTEAN Plus hinged spacer plates. Care needs to be taken while removing 1.0 mm thick gels from their cassettes, as they are fragile and easily torn. Thicker gels would be easier to handle (Bio-Rad's 1.5 mm cassettes are recommended, catalog #165-4171 for 20 x 20.5 cm and catalog #165-4174 for 25 x 20.5 cm cassette sizes). Ten of the gels were stained with colloidal Coomassie Blue for 48 hr and restained for a further 48 hr with fresh stain; one gel was stained with SYPRO Ruby protein gel stain (image not shown). The gels were destained with and stored in water. Gel images were acquired on an Agfa Arcus II scanner through a single thickness of red document wallet. The images were channel split in Microsoft Paintshop Pro software so that the red channel of the RGB TIFF file was selected.

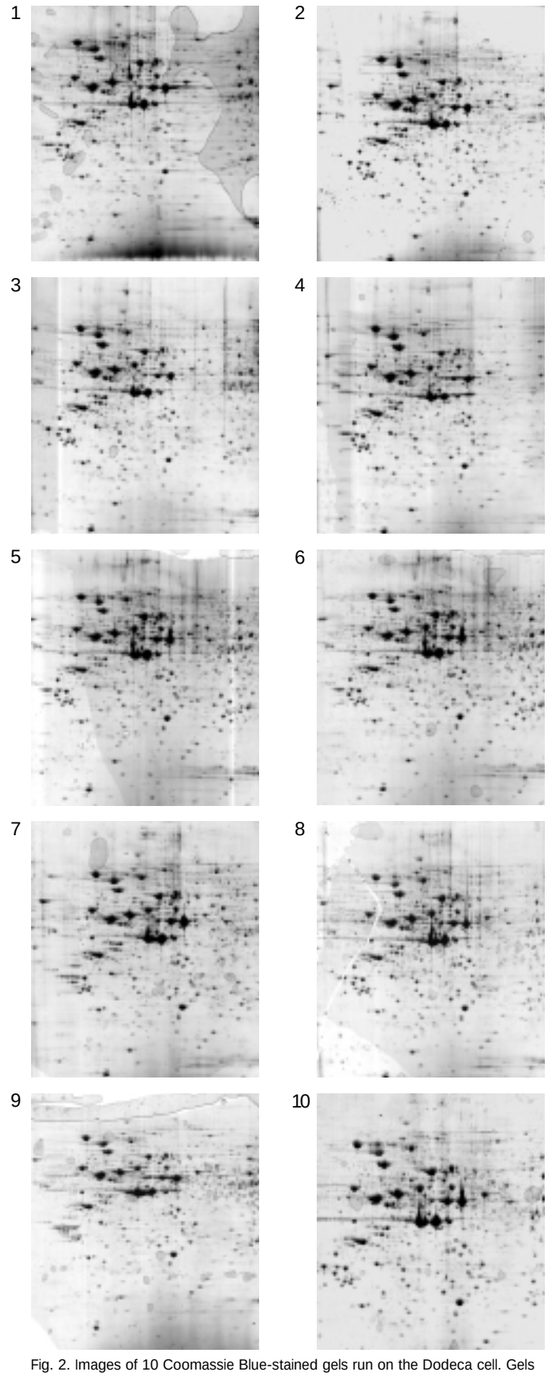

Fig. 2. Images of 10 Coomassie Blue-stained gels run on the Dodeca cell. Gels were 9–16% acrylamide gradients using Laemmli reagents. Proteins (500 µg) were loaded from pH 4–7 IPG strips and gels run for 6.5 hr.

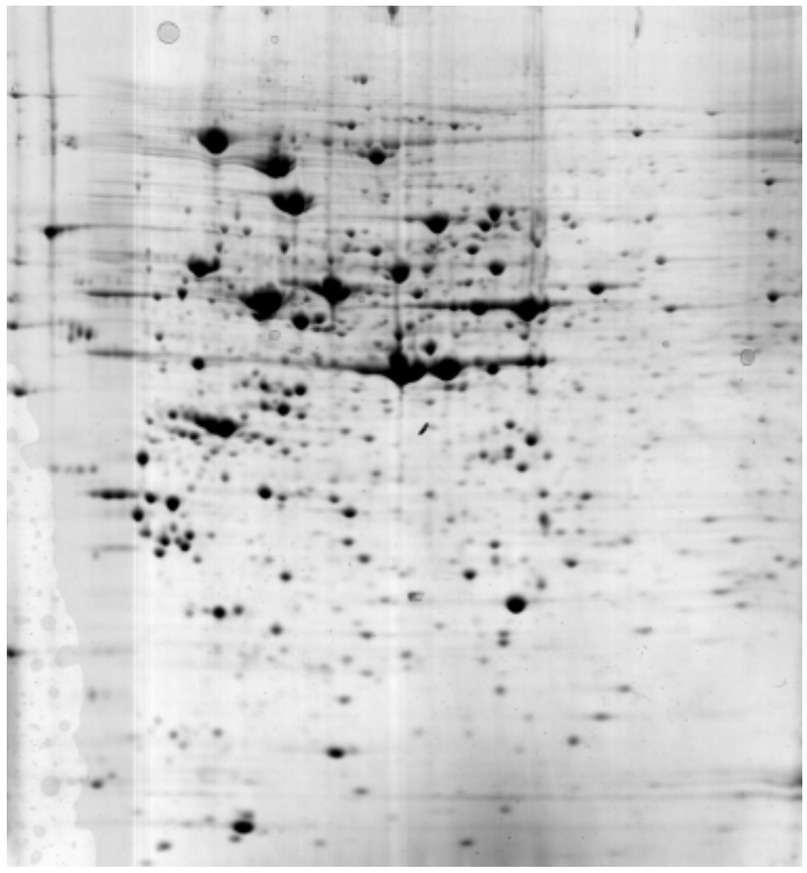

Fig. 3. An enlarged view of gel #4 from Figure 2. All proteins show well-defined spots and the highly expressed proteins show essentially no vertical streaking.

Results and Discussion

Images of the 10 Coomassie Blue-stained gels run on the PROTEAN Plus Dodeca cell are shown in Figure 2. The gels are of excellent quality and are very consistent with the exception of some minor differences due to the isoelectric focusing step. The highly expressed proteins did not suffer from vertical streaking and the other proteins all show well-defined spots (see Figure 3). These high-quality gels are excellent for image analysis comparisons and were sufficiently loaded to allow spot excision for protein identification.

References

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