Mini-PROTEAN® TGX™ Gel: A Versatile and Robust Laemmli-Like Precast Gel for SDS-PAGE

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Introduction

SDS-PAGE is a versatile and widely used method for analyzing protein samples that can provide information on purity, subunit composition, molecular weight, and relative abundance.

When performing SDS-PAGE, samples are treated with a solution containing sodium dodecyl sulfate (SDS) and a thiol reductant (typically β-mercaptoethanol or dithiothreitol [DTT]). SDS is a denaturing detergent that dissociates proteins into their individual subunits and substantially eliminates protein secondary and tertiary structure. The thiol reductant cleaves disulfide bonds within and between proteins, allowing more complete denaturation and dissociation. Heat treatment in the presence of both reagents eliminates the effects of protein conformation and native charge on electrophoretic behavior. SDS-protein complexes are negatively charged and have similar charge-to-mass ratios, allowing electrophoretic migration based on size.

The most commonly used SDS-PAGE method is the Laemmli system, which was first published in 1970 (Laemmli 1970). This system relies on a discontinuous buffer system. Two ions of differing electrophoretic mobility (glycinate and chloride) form a moving boundary when voltage is applied. Proteins have an intermediate mobility, causing them to concentrate or “stack” into a narrow zone at the beginning of electrophoresis. The stacking effect is responsible for the high resolving power of the Laemmli system. Sample is loaded in a relatively broad zone, and the moving boundary concentrates the proteins into sharp bands prior to separation. As the boundary moves through the gel, the sieving effect of the polyacrylamide gel matrix causes different proteins to move at different rates. One advantage of the Laemmli system is that it allows the analysis of relatively dilute samples. The sample concentrates prior to separation so proteins may be loaded in a relatively large volume.

Since its development, the Laemmli system has become the primary tool for analyzing protein mixtures due to its simplicity, versatility, and robustness. It is regarded as the “gold standard” of SDS-PAGE techniques for its ability to cleanly resolve complex samples from a variety of sources in a wide variety of solution backgrounds. It has been particularly valuable as the second dimension of 2-D electrophoresis, which combines isoelectric focusing and Laemmli system SDS-PAGE to resolve hundreds of individual proteins on a single gel.

Most commercially prepared Laemmli system gels typically have a shelf life of only a few months and separation performance degrades steadily over time. Mini-PROTEAN TGX precast gels for SDS-PAGE (8.5 × 10 cm) are based on a modification of the Laemmli system that gives significantly improved stability and performance over time (Berkelman et al. 2009). Mini-PROTEAN TGX gel retains Laemmli-like separation characteristics and uses the same running and sample buffers used for Laemmli system SDS-PAGE. In this study, the performance of Mini-PROTEAN TGX gels is demonstrated with diverse sample types and sample solution compositions, and the suitability of TGX gels is examined for the second dimension of 2-D electrophoresis.

Methods

Sample Preparation

Samples used included rat midbrain extract, salmon muscle extract, soybean extract, rat liver microsomes, bacteriophage T5, spinach leaf soluble extract, and *E. coli* lysate. Rat midbrain extract and salmon muscle extract were prepared for one-dimensional electrophoresis by grinding frozen tissue in 20 volumes of 50 mM Tris-HCl pH 8.0, 50 mM NaCl, 40 mM DTT, 2% (w/v) SDS and removing insoluble material by centrifugation. Soybean extract was prepared by hydrating soybeans overnight in aerated water and grinding in nine volumes of 50 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM PMSF, 40 µM bestatin, 10 µM leupeptin, 10 µM E64 and removing insoluble material by centrifugation. Rat liver microsomes were purchased from Sigma-Aldrich. Bacteriophage T5 was prepared according to Bonhivers et al. 1996, precipitated with acetone and resuspended in 8 M urea. Spinach leaf soluble extract was prepared by grinding fresh spinach leaf in four volumes of 50 mM Tris-HCl pH 8.0,

50 mM NaCl, 1 mM PMSF, 40 µM bestatin, 10 µM leupeptin, 10 µM E64 and removing insoluble material by filtration and centrifugation. The extract was brought to 95% saturation with ammonium sulfate and precipitated protein was collected by centrifugation and resuspended in phosphate-buffered saline. *E. coli* lysate was from Bio-Rad Laboratories, Inc. Rat midbrain extract was prepared for 2-D electrophoresis by grinding in nine volumes of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, and 40 mM Tris base and removing insoluble material by centrifugation.

Lysis, solubilization, and elution buffers tested for their effect on SDS-PAGE included RIPA buffer (25 mM Tris-Cl pH 7.6, 150 mM NaCl, 1% [w/v] NP-40 substitute, 1% [w/v] sodium deoxycholate, 0.1% [w/v] SDS), 2-D sample buffer (7 M urea, 2 M thiourea, 2% [w/v] CHAPS), 1% (w/v) Triton X-100, alkaline lysis buffer (1% [w/v] SDS, 200 mM NaOH), imidazole elution buffer (300 mM imidazole, 300 mM NaCl, 20 mM sodium phosphate buffer pH 7.8), B-Per (a proprietary bacterial cell lysis solution available from Thermo Fisher Scientific), and PBS buffer (10 mM sodium phosphate, 150 mM NaCl pH 7.4).

1-D Electrophoresis

One-dimensional SDS-PAGE was performed on 10-well Mini-PROTEAN TGX 10%, 4–20%, and Any kD™ gels. All samples were prepared by dilution with an equal volume of Laemmli sample buffer (62.5 mM Tris-HCl, 25% [w/v] glycerol, 2% [w/v] SDS, 0.01% [w/v] bromophenol blue, 5% [v/v] β-mercaptoethanol) and heating at 95°C for five minutes. Precision Plus Protein™ unstained standards (Bio-Rad) were used as MW markers. Gels were run in the Mini-PROTEAN Tetra electrophoresis cell at 200 V until the dye front reached the bottom of the gel. The running buffer used was Tris/glycine/SDS (25 mM Tris, 192 mM glycine, 0.1% [w/v] SDS). Gels were stained with Bio-Safe™ Coomassie stain and imaged on a Molecular Imager® GS-800™ calibrated densitometer following standard protocols.

2-D Electrophoresis

Two-dimensional SDS-PAGE was conducted using 7 cm pH 5–8 ReadyStrip™ IPG Strips for the first dimension. Rat midbrain sample prepared for 2-D electrophoresis was diluted into sample solution consisting of 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 0.2% (w/v) Bio-Lyte™ ampholyte pH 3–10, and 0.001% (w/v) bromophenol blue to give approximately 35 µg in a 125 µl sample load. The sample was loaded overnight by passive rehydration. First dimension was run with a current limit of 50 µA per strip and a voltage limit of 4,000 V until 10,000 Vh was reached. Following first dimension IEF, the IPG strips were equilibrated first for 15 min in 6 M urea, 30% (w/v) glycerol, 50 mM Tris-HCl pH 8.8, 2% (w/v) SDS, 1% (w/v) DTT, and 0.001% (w/v) bromophenol blue, then for an additional 15 min in the same equilibration solution containing 2.5% iodoacetamide instead of DTT. Equilibrated IPG strips were applied to Mini-PROTEAN TGX IPG well. The second dimension was run in the Mini-PROTEAN Tetra cell at 50 V for 10 min followed by 200 V until the dye front reached the bottom of the gel. The running buffer was Tris/glycine/SDS. Two-dimensional gels were stained with Flamingo™ fluorescent gel stain (Bio-Rad), imaged with the Molecular Imager® PharosFX™ system (Bio-Rad) and analyzed using PDQuest™ software (Bio-Rad) following standard protocols.

Results

Performance With a Variety of Different Sample Types

Ideally, SDS-PAGE should provide high resolution separations regardless of the origin or complexity of the sample. This capacity was evaluated by running samples from a variety of tissues and organisms with a wide range of relative protein abundance on three different percentages of Mini-PROTEAN TGX gels. Each of the three percentages was found to give high resolution separations characterized by straight, well defined lanes regardless of sample composition or concentration (Figure 1).

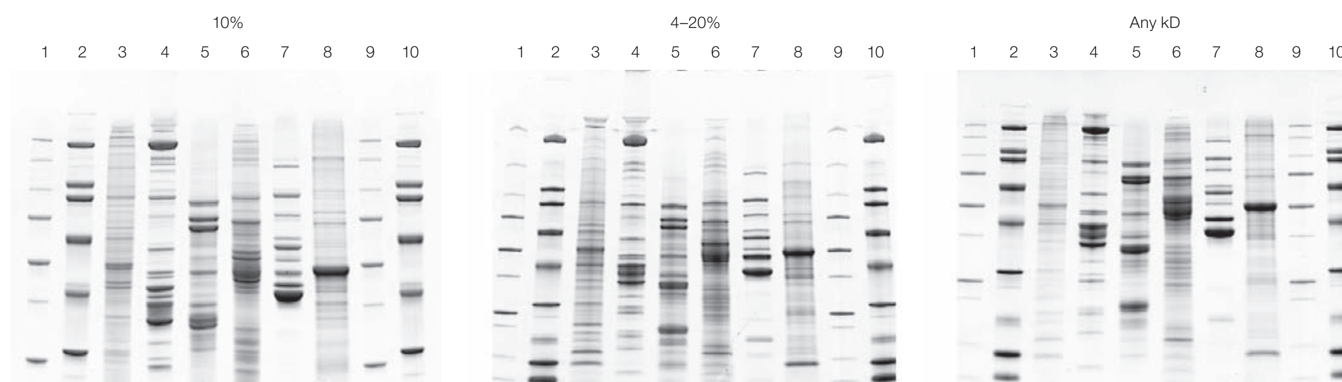

Fig. 1. Various samples run on Mini-PROTEAN TGX gels. Samples were loaded in a volume of 5 µl in the following order: Lanes 1 and 9, Precision Plus Protein unstained standards; 2 and 10, broad range SDS-PAGE standards (Bio-Rad); 3, rat midbrain extract; 4, salmon muscle extract; 5, soybean extract; 6, rat liver microsomes; 7, bacteriophage T5; 8, soluble spinach protein.

Performance With Different Sample Buffer Compositions

Samples to be analyzed by SDS-PAGE are often prepared in solutions containing detergents, salts, and other solutes that can potentially interfere with electrophoretic separation. There may also be a need to separate samples of differing composition on a single gel. Ideally, separation performance should be minimally influenced by these factors. Several commonly used lysis, solubilization, and elution buffers were therefore evaluated for their effect on separation in a Mini-PROTEAN TGX gel (Figure 2). None were found to have any appreciable effect on electrophoretic resolution. Separation behavior was essentially identical regardless of the solution the sample was prepared in.

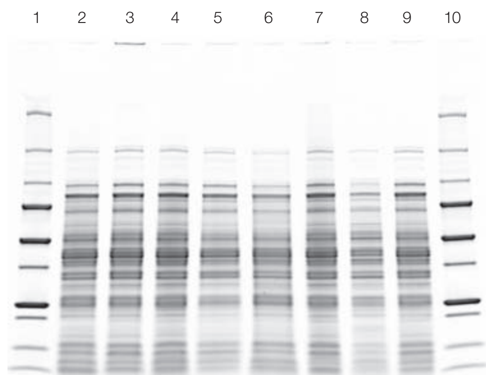

Fig. 2. *E. coli* lysate prepared in various solutions and run on a 4–20% Mini-PROTEAN TGX gel. Lyophilized *E. coli* lysate was resuspended in various lysis, solubilization, and elution buffers to a concentration of 2 mg/ml protein. Each sample was diluted with an equal volume of Laemmli sample buffer and heated at 95ºC for 5 min prior to electrophoresis on a 4–20% Mini-PROTEAN TGX gel. The solutions used are described in the Methods section and their corresponding gel lanes are as follows. Lanes 1 and 10, Precision Plus Protein unstained standards; 2, RIPA buffer; 3, 2-D sample buffer; 4, 1% (w/v) Triton X-100; 5, alkaline lysis buffer; 6, imidazole elution buffer; 7, 20% ethanol; 8, B-PER; 9, PBS buffer.

Performance With 2-D Electrophoresis

2-D electrophoresis is a robust method for analyzing very complex samples. The sample is first separated by isoelectric focusing and the entire separation is applied to SDS-PAGE for a second electrophoretic separation in a perpendicular direction. Ideally, this method should be capable of resolving a complex sample into hundreds of individual protein species. The suitability of Mini-PROTEAN TGX gel for 2-D electrophoresis was evaluated using a sample derived from rat midbrain. Highly resolving separations proved possible as shown in Figure 3. Software analysis showed that the combination of 7 cm IPG strips and Mini-PROTEAN TGX Any kD gel is capable of separating a complex sample into at least 617 individual protein components.

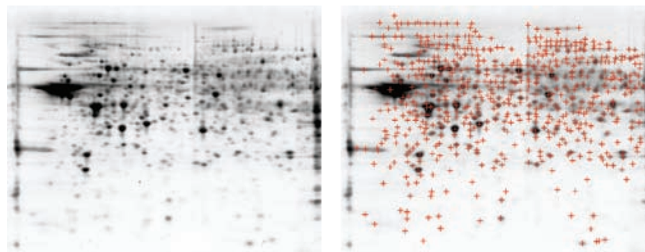

Fig. 3. 2-D analysis of rat midbrain extract using Mini-PROTEAN TGX Any kD gel. Rat midbrain extract was prepared, run, and analyzed as described in the Methods. The left panel shows the high resolution image of the Flamingo fluorescent gel stain. The right panel shows the 617 spots detected and counted using PDQuest software.

Conclusions

SDS-PAGE, particularly the Laemmli system, has been a favored analytical method because of its high tolerance for different sample types and sample solution compositions. Protein samples can have their origin in a wide variety of natural sources. Non-protein substances that can potentially interfere with electrophoresis may be present. Potentially interfering substances may also be present in the reagents used to prepare protein samples. Ideally, an analytical method should be as tolerant as possible of such interferences. In many cases, samples can be prepared for SDS-PAGE simply by dilution with Laemmli sample buffer and heat denaturation. The absence of a need for buffer exchange or any other sample clean-up contributes to the convenience and simplicity of the technique. In this study, we have demonstrated the robustness and versatility of the Mini-PROTEAN TGX gel system. These gels proved capable of handling a wide diversity of sample types and did not exhibit impaired resolution when challenged with a variety of common sample additives. These gels should be suitable for use in many analytical workflows without the need for special sample preparation or clean-up methods.

Mini-PROTEAN TGX Any kD gels are designed to provide optimal resolution in the size range from 10–100 kD. As this is the size range most strongly represented in samples prepared for 2-D electrophoresis, this gel type should be ideally suited for the second dimension of 2-D electrophoresis. This gel is indeed capable of separations in which a complex sample is resolved into several hundred identifiable spots, and the Mini-PROTEAN TGX Any kD gel should prove useful for rapid proteomic analysis with small format gels.

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