

Application Forum

Mini-PROTEAN® TGX™ precast gel for SDS-PAGE with improved stability: comparison with standard Laemmli gels

Tom Berkelman, Shane Petersen, Chengjun Sun, and Sean Cater
Bio-Rad Laboratories, 6000 James Watson Drive, Hercules, CA 94547

Introduction

Bio-Rad's Mini-PROTEAN TGX gels for SDS-PAGE are based on a modification of the Laemmli system that gives significantly improved stability and performance over time. Mini-PROTEAN TGX gels retain separation characteristics similar to Laemmli gels and use standard running and sample buffers. In this study, the performance of TGX gels and standard Laemmli system precast gels are compared following storage at 37°C to mimic accelerated assay conditions, and the separation performance and accuracy of molecular weight estimation of both gel systems are presented.

Methods

Samples

Samples used included broad-range unstained SDS-PAGE standards (Bio-Rad Laboratories, Inc.), Precision Plus Protein™ unstained standards, *Escherichia coli* lysate (Bio-Rad), mouse serum, and soybean extract (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, and 10 μM E64, and removing

insoluble material by centrifugation). Purified proteins used in this study were purchased from Sigma-Aldrich.

Electrophoresis and imaging

Electrophoresis was performed on 7.5%, 10%, 12%, 4–15%, and 4–20% Mini-PROTEAN TGX precast gels and on Ready Gel® Tris-HCl precast gels of the same percentages. Samples were prepared in Laemmli sample buffer containing 5% β-mercaptoethanol. Gels were run using the Mini-PROTEAN Tetra electrophoresis cell at 200 V until the dye front reached the bottom of the gel. Both types of gels were run using Tris/Glycine/SDS running buffer [25 mM Tris, 192 mM glycine, 0.1% (w/v) SDS]. Gels were stained with Bio-Safe™ Coomassie stain (Bio-Rad) and imaged on a Molecular Imager® GS-800™ calibrated densitometer (Bio-Rad).

Results

Comparison of migration patterns between Mini-PROTEAN TGX and Laemmli gels

The choice of gel percentage is determined by the molecular weight range of interest. Mini-PROTEAN TGX gels are designed to have separation characteristics similar to the corresponding percentage of a standard Laemmli gel (Ready Gel precast gels). Similarity of separation behavior was evaluated by running natural protein standards on Mini-PROTEAN TGX gels and Laemmli gels of the same percentage. Whereas the mobilities of individual standards differ slightly between the two gel types, migration patterns and separation ranges were very similar (Figure 1).

Comparison of stability between Mini-PROTEAN TGX and Laemmli gels

Separation performance in standard Laemmli gels degrades over time due to decomposition of the polyacrylamide matrix. Mini-PROTEAN TGX gels have been designed to address this shortcoming and have a significantly longer shelf life. The effect of prolonged storage was modeled by incubation at 37°C and stability was assessed by running samples after 0, 6, and 12 days of incubation. There was no discernible effect of 37°C treatment on the Mini-PROTEAN TGX gels even after

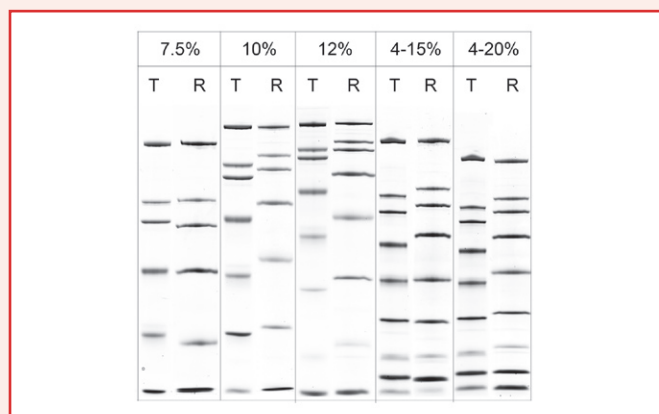


Figure 1. Comparison of migration patterns of natural protein standards between Mini-PROTEAN TGX (T) and Laemmli gels (R). Broad range SDS-PAGE standards were run on Mini-PROTEAN TGX and Ready Gel Tris-HCl gels (Laemmli gels) of the percentages indicated. From top to bottom the MW of the proteins standards are: 200, 116, 97, 66, 45, 31, 21.5, 14.4, and 6.5 kD. The top of the gels were lined up for the comparison, the smaller protein bands may not be visible on all gels.

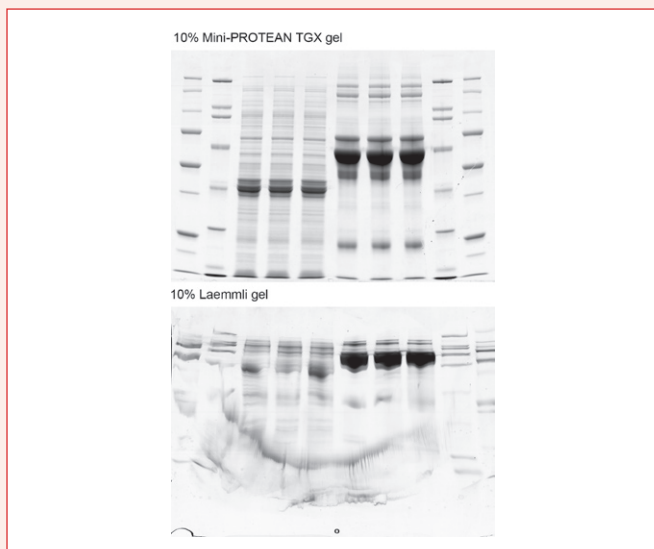


Figure 2. Comparison of separations on Mini-PROTEAN TGX and Laemmli gels following storage at 37°C. Freshly prepared 10% Mini-PROTEAN TGX gels and Ready Gel Tris-HCl gels (Laemmli gels) were incubated at 37°C for 12 days prior to running Precision Plus Protein unstained standards (lanes 1 and 9), broad-range SDS-PAGE standards (lanes 2 and 10), *E. coli* lysate (lanes 3-5), and mouse serum (lanes 6-8).

12 days, but the performance of the Laemmli system gels was greatly affected (Figure 2). A Mini-PROTEAN TGX gel stored under recommended conditions (4°C) for 12 months still exhibits high-resolution separation (data not shown).

Comparison of band sharpness between Mini-PROTEAN TGX and Laemmli gels

Accuracy of molecular weight estimation and quantitative densitometry are best when samples run as straight consistent lanes and form regular symmetrical bands. When an identical sample was run on both Mini-PROTEAN TGX and Laemmli system gels, the protein bands on the Mini-PROTEAN TGX gel were consistently about the same width as the loading well, whereas band spreading was observed with the Laemmli gel. In general, bands were straighter and more regular with the Mini-PROTEAN TGX gel than with the Laemmli gel (Figure 3).

Accuracy of molecular weight estimation on Mini-PROTEAN TGX and Laemmli gels

To evaluate the accuracy of molecular weight estimation of the two gel systems, 18 different purified proteins were run on both TGX and Laemmli gels of two percentages (10% and 4–20%). A lane of Precision Plus Protein unstained standards (Bio-Rad) was also run on each gel. Standard curves were generated by plotting the logarithm of the molecular weight of each standard versus its R_f . The molecular weight of each purified protein was estimated using a linear fit to the standard curve for each gel. With both gel percentages,

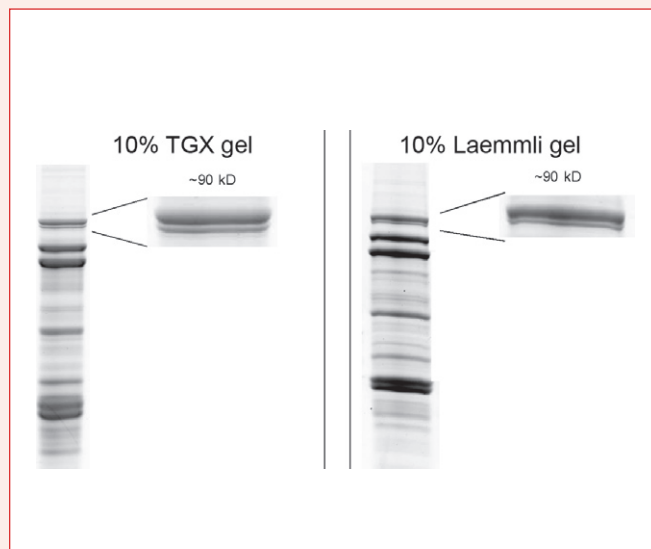


Figure 3. Comparison of soybean extract separation between Mini-PROTEAN TGX and Laemmli system gels. A protein doublet of ~90 kD was selected from each gel to illustrate the difference in band appearance.

Table 1. Accuracy of molecular weight estimation between Mini-PROTEAN TGX and Ready Gel precast gels

Gel %	Mini-PROTEAN TGX gels		Ready Gel TrisHCl gels	
	Correlation* coefficient	Regression coefficient	Correlation* coefficient	Regression coefficient
10%	0.994	1.010	0.972	0.867
4–20%	0.994	1.045	0.974	0.708

*Correlation coefficient between the molecular weight estimated by SDS-PAGE and the true molecular weight

molecular weight estimated by SDS-PAGE was more accurate using Mini-PROTEAN TGX gels than using Laemmli gels, as judged by the correlation coefficient between the molecular weight estimated by SDS-PAGE and the true molecular weight (Table 1).

Conclusions

Mini-PROTEAN TGX gels are based on a modification of the Laemmli system for SDS-PAGE. These gels retain Laemmli-like separation characteristics and use the same running and sample buffers, which should simplify adoption of this system by users familiar with the Laemmli system. The key feature of the TGX gel is superior stability, which reduces the need to order gels as frequently and confers better reproducibility over a longer period. Mini-PROTEAN TGX gels also demonstrate advantages in terms of both quality of separation and the ability to estimate molecular weight accurately.

Sponsored Paper. *BioTechniques* 48:156-157 (February 2010)
doi 10.2144/000113372