Mini-PROTEAN® TGX™ Precast Gel: A Gel for SDS-PAGE with Improved Stability — Comparison with Standard Laemmli Gels

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Introduction

SDS-PAGE is a widely used and versatile method that separates proteins according to size and allows estimation of molecular weight. SDS-PAGE involves treatment of the sample with SDS and a thiol reductant (usually β-mercaptoethanol or dithiothreitol). SDS is an anionic detergent which forms complexes with proteins giving them a rod-like shape and a uniform charge to mass ratio. The reductant cleaves disulfide bonds between and within proteins allowing complete denaturation and dissociation. Heat treatment in the presence of SDS and reductant effectively eliminates the effects of protein conformation and native charge on electrophoretic mobility so the migration rate depends primarily on molecular weight. Molecular weight is estimated by comparison to standards of known molecular weight.

The most commonly used SDS-PAGE method is the so-called 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. The 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.

The percentage of polyacrylamide controls the separation characteristics of the gel. Higher percentage gels have a more pronounced sieving effect and are best suited for separations of relatively low molecular weight proteins. Lower percentage gels are better suited for separations of larger proteins. Gels can also be cast with a polyacrylamide percentage gradient to give a broader separation range.

Commercially prepared Laemmli system gels typically have a shelf life of only a few months and separation performance degrades steadily over time. Despite the short shelf life of most precast gels, Laemmli system SDS-PAGE is very widely used. It is regarded as the "gold standard" of SDS-PAGE techniques due to its resolution, robustness, ability to accurately estimate molecular weight and compatibility with a wide variety of sample types and sample solution compositions. Laemmli system SDS-PAGE also has the advantage of only requiring relatively common and inexpensive buffer components (Tris and glycine).

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 37ºC to mimic accelerated assay conditions, and the separation performance and accuracy of molecular weight estimation of both gel systems are presented.

Table 2. Proteins used for molecular weight estimations.

Protein	Size*, kD	Protein	Size*, kD
Rabbit myosin	200	Equine alcohol dehydrogenase	40
Human α-macroglobulin	161	Rabbit aldolase	39
E. coli β-galactosidase	116	Enterobacter β-lactamase	39
Rabbit phosphorylase b	97	Rabbit glyceraldehyde phosphate dehydrogenase	36
Human apotranferrin	77	Bovine carbonic anhydrase	31
Chicken conalbumin	76	Bovine trypsinogen	24
Bovine serum albumin	66	Bovine β-casein	23.6
Bovine glutamic dehydrogenase	55	Bovine myelin basic protein	18.3
Chicken ovalbumin	45	Bovine α-lactalbumin	14.2

* The indicated molecular weights were either derived from gene sequence data or provided by the supplier.

To evaluate accuracy of molecular weight estimation of the two gel systems, eighteen different purified proteins (Table 2) 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 vs. its R_f and the molecular weight of each purified protein was estimated using a linear fit to the standard curve for each gel. With both gel percentages, 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 (Figure 6).

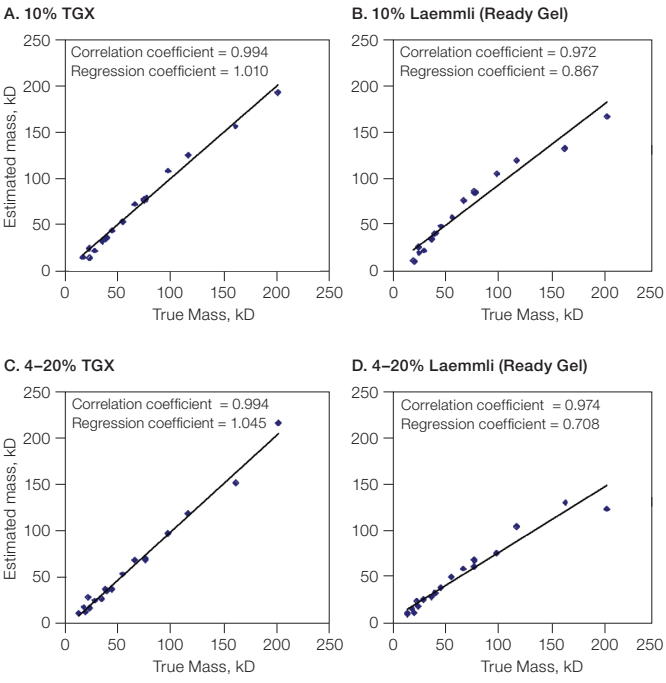

Fig. 6. Accuracy of molecular weight estimation between Mini-PROTEAN TGX and Laemmli gels. Eighteen different purified proteins (Table 2) were run on Mini-PROTEAN TGX gels and Ready Gel Tris-HCl gels (Laemmli gels) along with Precision Plus Protein unstained standards. Both 10% and 4–20% gels were evaluated. The molecular weight of each purified protein was estimated from plots of Log MW vs. molecular weight of the standards after SDS-PAGE. This estimated molecular weight was plotted against the actual mass.

Conclusions

Mini-PROTEAN TGX gels are based on a modification of the Laemmli system for SDS-PAGE. The gel chemistry was designed to have similar separation characteristics to the Laemmli gel and to use the same running and sample buffers. Similar separation patterns should simplify adoption of the Mini-PROTEAN TGX system by users familiar with the Laemmli system.

The key feature of the TGX gel is better stability than standard Laemmli system gels. The effect of incubation at 37ºC well illustrates the superior stability and shelf life of the Mini-PROTEAN TGX gel. A longer shelf life confers benefits beyond not needing to order gels as frequently. Reproducibility of separation is also enhanced. The separation characteristics of Laemmli gels change continually, and this change is not observed in Mini-PROTEAN TGX gels that are used before their expiration date.

In addition to improved stability, Mini-PROTEAN TGX gels have demonstrable advantages in terms of the both quality of separation and the ability to estimate molecular weight accurately. More consistent band width and shape allow better quantitation by densitometry. Since one of the primary purposes of SDS-PAGE is to estimate the molecular weight of proteins, any improvement in the linearity of separation behavior and the accuracy of molecular weight estimation is also a significant advantage.

References

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-185.

Information in this tech note was current as of the date of writing (2009) and not necessarily the date this version (rev A, 2009) was published.

Methods

Samples

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

Purified proteins used in this study were purchased from Sigma Aldrich Co.

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. Precision Plus Protein unstained standards were loaded as provided. 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) and therefore offer a predictable alternative. Similarity of separation behavior was evaluated by running natural protein standards on Mini-PROTEAN TGX gels and Laemmli gels of the same percentage. Migration patterns were indeed found to be very similar between the two different gel types (Figure 1). Whereas the mobilities of individual standards differ slightly between the two gel types, separation ranges were very similar between Mini-PROTEAN TGX gels and Laemmli gels of the same percentage (Figure 1).

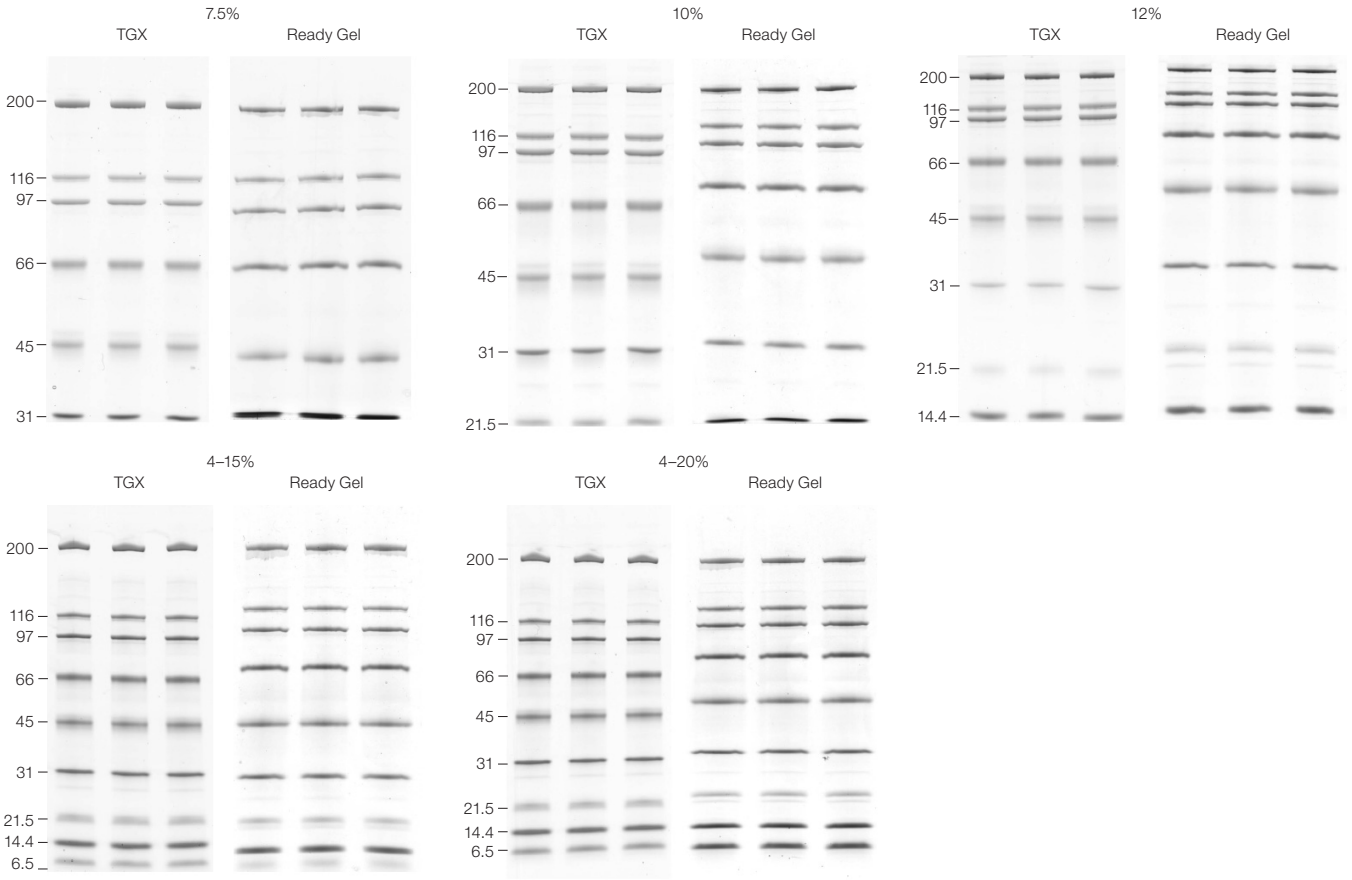

Fig. 1. Comparison of migration patterns of natural protein standards between Mini-PROTEAN TGX and Laemmli gels. Broad range SDS-PAGE standards were run in triplicate on Mini-PROTEAN TGX gels and Ready Gel Tris-HCl gels (Laemmli gels) of the percentages indicated. The standards used are: rabbit myosin (200 kD), *E. coli* β-galactosidase (116 kD), rabbit phosphorylase b (97 kD), bovine serum albumin (66 kD), chicken ovalbumin (45 kD), bovine carbonic anhydrase (31 kD), soybean trypsin inhibitor (21.5 kD), chicken lysozyme (14.4 kD), bovine aprotinin (6.5 kD). Gel migrations were aligned at the 200 kD to demonstrate the migration pattern.

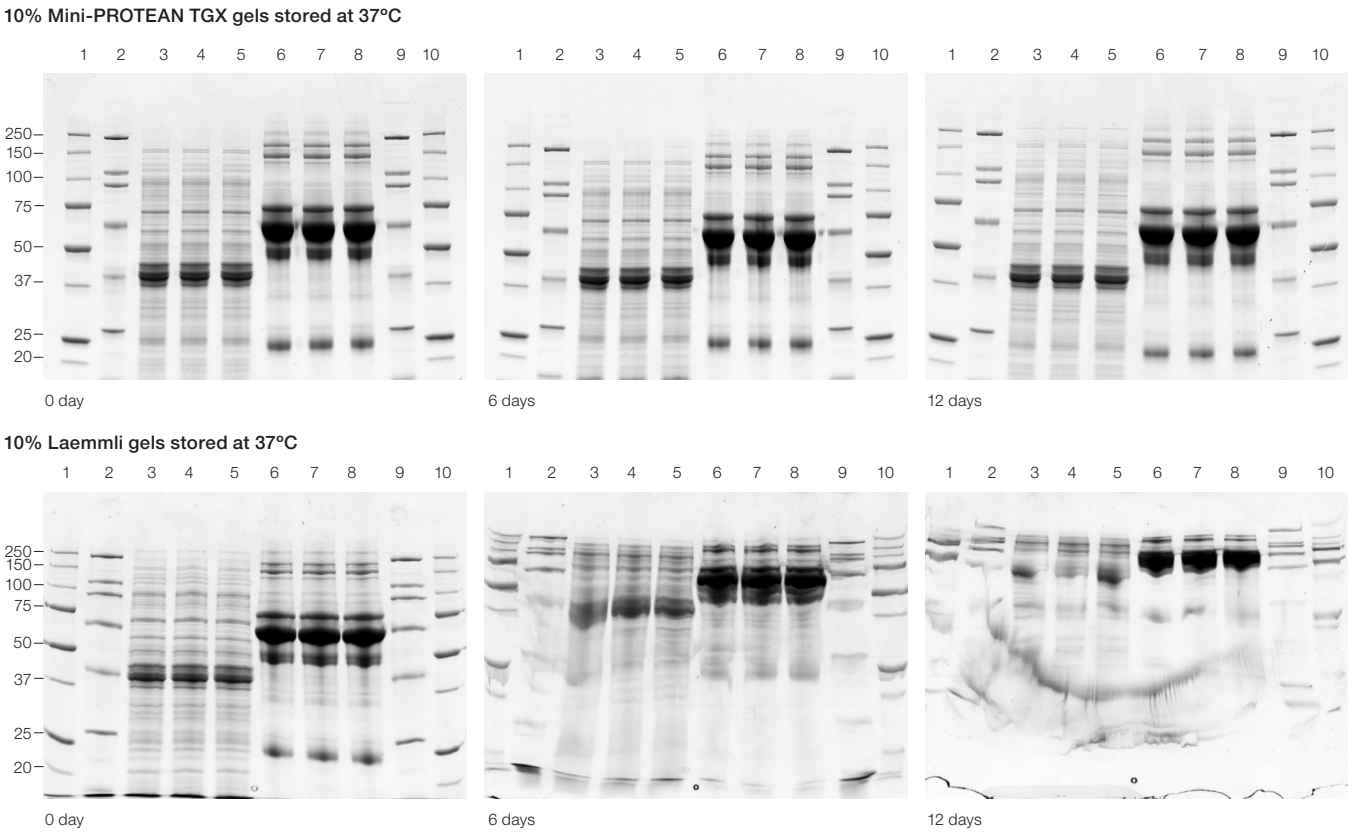

Fig. 2. Comparison of separations on Mini-PROTEAN TGX and Laemmli gels following storage at 37°C. Freshly prepared 10% Mini-PROTEAN TGX gels and Ready Gel Tris-HCl gels (Laemmli gels) were incubated at 37°C for the amount of time indicated. Following the treatment, they were loaded as follows: Lanes 1 and 9, Precision Plus Protein unstained standards; lanes 2 and 10, broad range SDS-PAGE standards; lanes 3-5, *E. coli* lysate; lanes 6-8, mouse serum.

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 accelerated by incubation at 37°C and stability was assessed by running standards and samples following 0, 6, and 12 days of incubation. There was no discernible effect of 37°C treatment on the Mini-PROTEAN TGX gels, but storage at elevated temperature had a marked negative effect on the performance of the Laemmli system gels (Figure 2). A Mini-PROTEAN TGX gel stored under recommended conditions (4°C) for 12 months still exhibits high resolution separation (Figure 3).

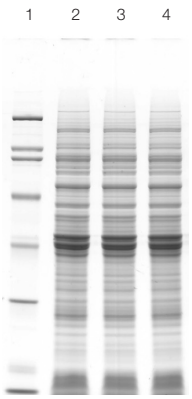

Fig. 3. Separation on Mini-PROTEAN TGX gel stored for 12 months at 4°C. A 4–20% Mini-PROTEAN TGX gel was kept at 4°C prior to running standards and samples. Lane 1, Broad range SDS-PAGE standards; lanes 2–4, *E. coli* lysate.

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 4).

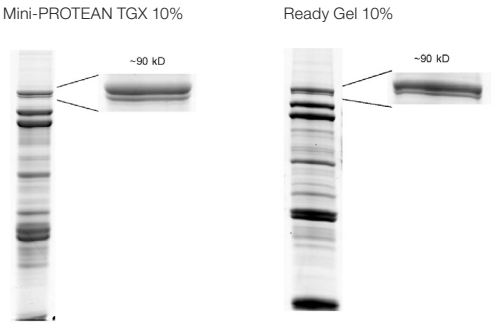

Fig. 4. Comparison of soybean extract separation between Mini-PROTEAN TGX and Laemmli system gels. Soybean extract (~10 µg) was run on 10% Mini-PROTEAN TGX and 10% Ready Gel Tris-HCl gel. A single lane from each gel was compared. A protein doublet of ~90 kD was selected from each gel and its image was enlarged to illustrate the difference in band appearance.

Linearity and Accuracy of Molecular Weight Estimation on Mini-PROTEAN TGX and Laemmli Gels

Molecular weight is estimated using a standard curve that is generated by plotting the logarithm of the molecular weight of each standard vs. its R_f (R_f is the ratio of the distance traveled by the protein to the distance traveled by the electrophoretic front). The standard curve approximates a straight line and molecular weight is most conveniently estimated using a linear fit to the standard curve. The linearity of the standard curve will therefore influence how accurately molecular weight may be estimated. Mini-PROTEAN TGX gels and Laemmli gels were evaluated with respect to linearity by running standards made of natural proteins and comparing curves of Log MW vs. R_f between same percentage gels of each gel type. Regression lines were drawn and R^2 values were calculated (Figure 5 and Table 1). In all cases except for the 4–15% gradient gel, the Mini-PROTEAN TGX gels exhibited better linearity than the Laemmli gels. The difference in linearity was more significant among the single percentage gels (7.5%, 10%, and 12%) than among the gradient gels.

Table 1. Linearity between Mini-PROTEAN TGX and Laemmli gels using natural protein standards.

	R^{2*}	
Gel Percentage	Mini-PROTEAN TGX gels	Ready Gel precast gels
7.5%	0.9894	0.9621
10%	0.9847	0.9527
12%	0.9815	0.9277
4–15%	0.9928	0.9957
4–20%	0.9974	0.9902

* R^2 values from the data shown in Figure 5 are compared.

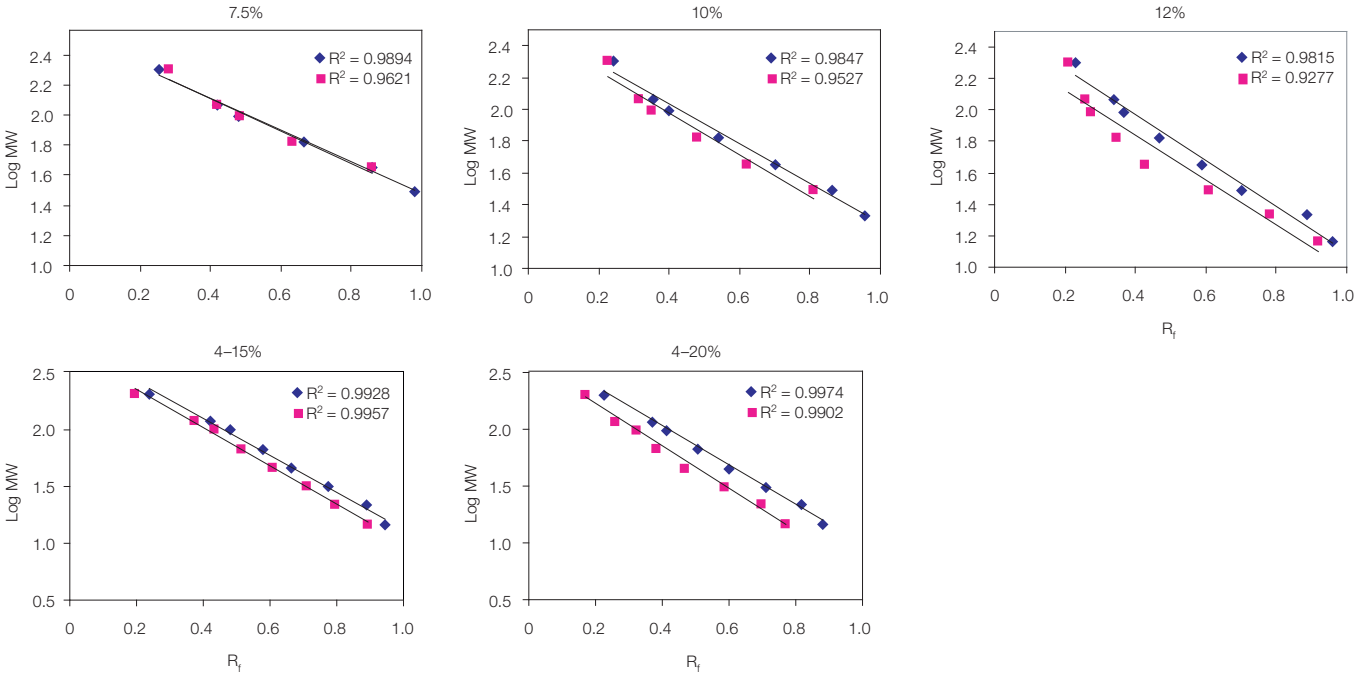

Fig. 5. Linearity between Mini-PROTEAN TGX and Laemmli system gels. Broad Range SDS-PAGE standards were run on Mini-PROTEAN TGX gels and Ready Gel Tris-HCl gels (Laemmli system) of the percentages indicated. The standards are: rabbit myosin (200 kD), *E. coli* β-galactosidase (116 kD), rabbit phosphorylase b (97 kD), bovine serum albumin (66 kD), hen ovalbumin (45 kD), bovine carbonic anhydrase (31 kD), soybean trypsin inhibitor (21.5 kD), hen lysozyme (14.4 kD), bovine aprotinin (6.5 kD). Log MW vs. R_f is compared between gel types of the same percentage on the same set of axes. Each value is an average of nine R_f values (from three lanes run on three gels). The R_f of bovine aprotinin was not used in this study because this small protein is consistently observed to run anomalously. ◆, Mini-PROTEAN TGX gels; ■, Ready Gel Tris-HCl gels.

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