Precision Plus Protein™ Dual Xtra Standards — New Protein Standards with an Extended Range from 2 to 250 kD

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Introduction

The Precision Plus Protein* Dual Xtra standards are expanded range molecular weight standards for protein electrophoresis composed of 12 recombinant proteins and peptides with molecular weights ranging from 2 to 250 kD. The Dual Xtra standards are prestained with two colors for easy band referencing during electrophoresis and blot transfer.

Protein standards are tools commonly used with one-dimensional electrophoresis to determine the molecular weight of unknown proteins (Bio-Rad Bulletin 3133). In polyacrylamide gel electrophoresis (PAGE), proteins are driven through the gel under an electric field, during which the gel matrix acts as a sieve to separate proteins based on size (SDS-PAGE), or charge and shape (native PAGE). The pattern of bands resulting from the electrophoretic separation of a standard, known as a protein ladder, is used to generate a standard curve that allows the estimation of the molecular weights of unknown proteins.

Reliable estimation of molecular weight depends upon the use of high-quality protein standards. Likewise, the appropriate choice of gel formulation for separation of proteins within a given molecular weight range is critical, as observed migration patterns differ according to gel composition. For example, low percentage polyacrylamide gels are better suited for separation of high molecular weight proteins than high percentage polyacrylamide gels, whereas gradient gels permit separation of a broad range of protein sizes.

Gel chemistry also contributes to protein migration behavior. For example, Bio-Rad's Tris-Glycine Mini-PROTEAN® TGX™ gels and Criterion™ XT Bis-Tris gels are well suited to resolve protein samples across a broad range of molecular weights. Criterion Tris-Tricine gels, however, are specifically formulated to visualize low molecular weight proteins and peptides.

In this study, the migration patterns of the Precision Plus Protein Dual Xtra standards were examined on a wide range of gel formulations. The relative migrations of bands within the standard were used to estimate the molecular weights of five known sample proteins. The results presented here demonstrate that the Precision Plus Protein Dual Xtra standards offer a convenient and reliable method to estimate the molecular weight of proteins on a variety of gel types.

Methods

Sample Preparation

Precision Plus Protein Dual Xtra standards were used according to manufacturer's specifications. A total of 10 µl of Dual Xtra standards was loaded onto each lane. All other purified proteins were purchased from Sigma-Aldrich. Sample proteins were suspended in Laemmli sample buffer containing β-mercaptoethanol (Bio-Rad Laboratories, Inc.) and heated at 85°C for 6 min prior to loading.

Gel Electrophoresis and Molecular Weight Analysis

For migration analysis a total of 10 µl of Precision Plus Protein Dual Xtra standards was loaded onto a range of gel types. Criterion precast gels were run in Criterion cells while Ready Gel® precast gels, Mini-PROTEAN TGX precast gels, and hand-cast gels were run using Mini-PROTEAN Tetra electrophoresis cells. Gels were run until the 2 kD band reached 2 cm from the bottom of the gel (30–95 min depending on gel type). All gels were run in duplicate or triplicate.

For molecular weight (MW) estimation a mixture of sample proteins (Table 1) and 10 µl of Precision Plus Protein Dual Xtra standards were loaded onto Criterion Tris-HCl 4–20%, XT Bis-Tris 4–12%, Tris-Tricine 10–20% linear gradient and Mini-PROTEAN® TGX Any kD™ gels. All gels were run until the 2-kD band reached 2 cm from the bottom of the gel (30–85 min depending on gel type).

Table 1. Sample proteins for molecular weight estimation.

Protein	Molecular Mass, kD	Quantity Loaded, µg
α2-Macroglobulin, human plasma	180	6
Apotransferrin, human	78.5	1.6
Ovalbumin, chicken egg white	44.3	1.6
α-Lactalbumin, bovine milk	14.2	1.6
Insulin B-chain, bovine pancreas	3.5	3.2

Following electrophoresis, gels were imaged immediately on a Molecular Imager® GS-800™ calibrated densitometer using Quantity One® analysis software (Bio-Rad) and MagicScan (UMAX Data Systems, Inc.) to obtain color images.

For staining, gels were fixed in a solution of 40% methanol/ 10% acetic acid for 3 hr. Fixed gels were then stained with BioSafe™ Coomassie stain (Bio-Rad) for 1 hr and destained in deionized water overnight.

Two methods were used to estimate the MW of the sample proteins: point-to-point semi-log interpolation and conventional linear regression. In both methods, Quantity One analysis software was used to measure the migration of the standard and sample protein bands (relative front). In point-to-point semi-log interpolation method Quantity One software creates a "standard curve" based on a defined log molecular weight and measures the relative front of the "standard", and uses the standard curve to automatically estimate the molecular weights of the known sample proteins. The linear regression method plots the relative front data against log MW to estimate the MW.

Results and Discussion

Precision Plus Protein Dual Xtra Standard Band Migration Pattern

In order to characterize the migration pattern of the Dual Xtra standards on a wide range of gel formulations, electrophoresis was performed on 11 gel types listed in Table 2. Running buffers, run times, and voltages for all gel types tested are also listed in Table 2. Band migration patterns were generated by loading 10 µl of Dual Xtra standards on 3 lanes for each gel type tested. A total of 3 replicates for each gel type were run under identical conditions. Average band migration distances were used to plot the migration data displayed in Figure 1.

As shown in Figure 1, the migration patterns vary with different gels. A Criterion Tris-HCl 4–20% gel, the most commonly used gel among the Criterion precast gels, gives good separation of all protein bands (Figure 2A). Bio-Rad's Mini-PROTEAN TGX 4–20% gels are also well suited to resolve protein samples across a broad range of molecular weights (Figure 2F). Criterion XT Bis-Tris 12% gel shows excellent separation of all 12 bands, with particularly sharp resolution of the 2 kD band (Figure 2B). It is a feature of the XT gel system that lower molecular proteins near the buffer front do not accelerate towards the end of the run to the same degree as in other buffer systems. The result is sharper low MW bands, higher resolution, and a broad range band distribution similar to a gradient gel (Figure 2B and 2C). For accurate estimation of low molecular weight proteins or peptides, Tris-Tricine gels outperform Tris-Glycine gels. Replacing glycine with tricine in the gel running buffer results in more efficient stacking and higher resolution of small proteins and peptides (Schaegger and von Jagow 1987). As shown in Figure 2D and 2E, lower molecular mass bands (<20 kD) exhibit excellent separation in both 10–20% and 16.5% Tris-Tricine gels.

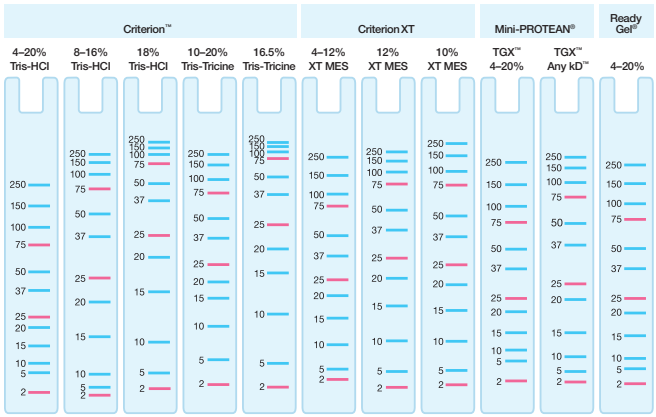

Fig. 1. Migration patterns of Precision Plus Protein Dual Xtra standards. The standards consist of 12 recombinant proteins. The MW of the proteins are: 2, 5, 10, 15, 20, 25, 37, 50, 75, 100, 150, and 250 kD. The 2, 25 and 75 kD proteins are stained pink. The gel types used are indicated.

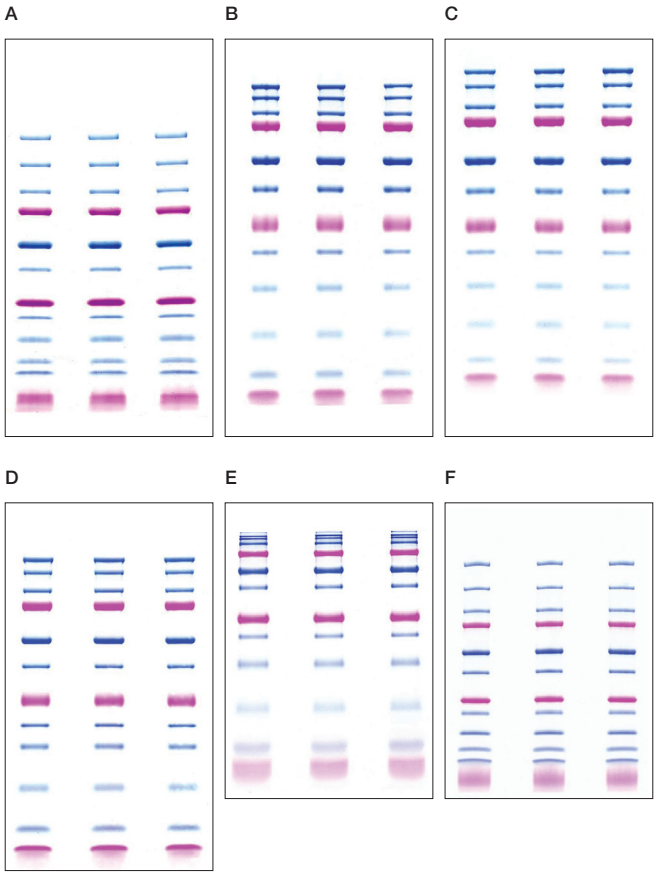

Fig. 2. Gel images of Precision Plus Dual Xtra protein standard run on different gel types. A, Criterion Tris-HCl 4–20%; **B**, Bis-Tris XT 12%; **C**, Bis-Tris XT 10%; **D**, Tris-Tricine 10–20%; **E**, Tris-Tricine 16.5% (mini-gel); **F**, Mini-PROTEAN TGX 4-20% (mini gel). All gels are Bio-Rad precast gels except E, which is a hand-cast mini-gel.

Table 2. Gel running conditions.

Gel	Running Buffer	Voltage, V	Run Time, min
Criterion			
Tris-HCl 4–20%	Tris/Glycine/SDS	200	60
Tris-HCl 8–16%	Tris/Glycine/SDS	200	57
Tris-HCl 18%	Tris/Glycine/SDS	200	60
Tris-Tricine 10–20%	Tris/Tricine/SDS	125	90
Criterion XT			
Bis-Tris XT 4–12%	MES	200	35
Bis-Tris XT 12%	MES	200	41
Bis-Tris XT 10%	MES	200	38
Ready Gel/Mini-PROTEAN			
Tris-HCl 4–20%	Tris/Glycine/SDS	200	43
TGX 4–20%	Tris/Glycine/SDS	200	30
TGX Any kD	Tris/Glycine/SDS	200	30
Hand-Cast			
Tris-Tricine 16.5%[1]	Tris/Tricine/SDS	150	95

[1] All gels are Bio-Rad precast gels except for Tris-Tricine 16.5% which is a hand-cast gel.

Molecular Weight Analysis

A mixture of five known sample proteins (Table 1) and 10 µl Dual Xtra standards were separated on Criterion Tris-HCl 4–20%, XT Bis-Tris 4–12%, Tris-Tricine 10–20% linear gradient and Mini-PROTEAN TGX Any kD gels. Standard and sample band migration relative front values were measured using Quantity One analysis software. The measured relative front values for each protein are shown in Table 3. The MW for the sample proteins estimated by both point-to-point and linear methods are shown in Table 4.

For the linear regression method, the accuracy of the calculated MW for the sample proteins is dependent on the linearity of the standard curve generated from the Dual Xtra standard, represented by the R^2 value. The values of the calculated MW for the sample proteins are shown in Table 4. The closer the R^2 value to 1, the more accurate the estimation of molecular weights over the given range of the standard. The range of linearity for the Dual Xtra standard is dependent on the gel type used (Neville 1971). For example, the Criterion Tris-HCl 4–20%, XT Bis-Tris 4–12%, and Tris-Tricine 10–20% gels produce excellent linear fits ($R^2 > 0.99$) for the Dual Xtra standards over the molecular weight ranges of 10–250, 5–250, and 5–150 kD, respectively. Likewise, the TGX Any kD mini-gel exhibits good linearity ($R^2 > 0.98$) over a range of 10–150 kDa (Figure 3). Within the linear range of the various gel types tested, the calculated molecular weights are very close to reported values (Table 4).

Molecular weights were also estimated using the Quantity One point-to-point semi-log interpolation method. Over the entire range of the Dual Xtra standard this approach gave better estimations of protein molecular weight for all five proteins tested (Table 4). The high degree of agreement between known and calculated values of molecular weight for each sample protein confirms the Dual Xtra standards' ability to provide a robust and direct method for protein molecular weight estimation.

Conclusions

The performance of the Precision Plus Protein Dual Xtra standards has been tested on a wide variety of gel

Table 3. Band migration relative front values for the Dual Xtra standards and protein samples.

Dual Xtra Standards Band Migration Relative Front					Protein Sample Band Migration Relative Front				
MW	Criterion Tris-HCl 4–20%	Criterion XT Bis-Tris 4–12%	Criterion Tris-Tricine 10–20%	Mini-PROTEAN TGX Any kD	MW[1]	Criterion Tris-HCl 4–20%	Criterion Bis-Tris 4–12%	Criterion Tris-Tricine 10–20%	Mini-PROTEAN TGX Any kD
250	0.257	0.173	0.138	0.142	180.0	0.292	0.209	0.147	0.161
150	0.329	0.235	0.169	0.174	78.5	0.443	0.340	0.252	0.272
100	0.401	0.299	0.217	0.224	44.3	0.561	0.461	0.374	0.415
75	0.454	0.343	0.263	0.278	14.2	0.789	0.725	0.707	0.791
50	0.540	0.435	0.352	0.368	3.5	0.893	0.880	0.882	0.933
37	0.598	0.500	0.422	0.448					
25	0.679	0.582	0.532	0.577					
20	0.715	0.628	0.591	0.649					
15	0.768	0.699	0.651	0.752					
10	0.821	0.785	0.771	0.841					
5	0.849	0.862	0.866	0.911					
2	0.913	0.899	0.908	0.958					

[1] MW provided by supplier.

Table 4. Molecular weight estimation by point-to-point semi-log interpolation and linear regression fit.

Molecular Weight Estimation, kD									
Criterion Tris-HCl 4–20%		Criterion Bis-Tris 4–12%		Criterion Tris-Tricine 10–20%		Mini-PROTEAN TGX Any kD			
Point-to-Point	Linear	Point-to-Point	Linear	Point-to-Point	Linear	Point-to-Point	Linear	Reported MW[1]	Protein Sample
188.8	188.7	182.6	166.4	202.7	ND[2]	193.6	ND[2]	180.0	α2-Macroglobulin
77.0	82.7	76.3	87.1	78.7	86.7	77.8	93.0	78.5	Apotransferrin albumin
43.4	43.6	44.2	47.2	44.9	50.3	42.2	51.7	44.3	Ovalbumin
12.5	12.7	13.3	12.5	12.2	11.4	12.6	11.0	14.2	α-Lactalbumin
2.6	ND[2]	3.2	ND[2]	3.2	ND[2]	3.4	ND[2]	3.5	Insulin B-chain

[1] MW provided by supplier.
[2] ND = Not Determined. These proteins lie outside the linear range for the given gel type, and the estimated MW was not calculated using the linear regression fit (see Figure 3).

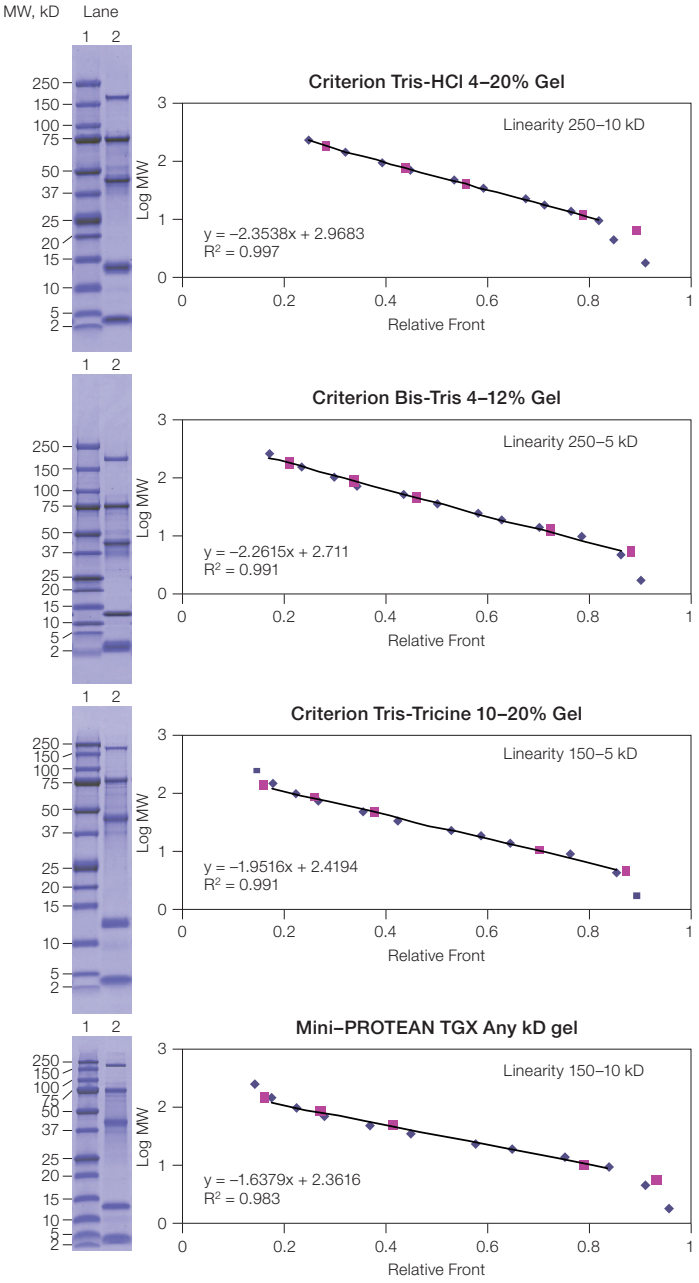

Fig. 3. MW determination of sample proteins using Precision Plus Protein Dual Xtra standards. Standard curves of log MW vs. relative front were generated using the Dual Xtra protein standards run on different gels as indicated. The linear fit equations were derived using the Dual Xtra standard's data points (◆), and were used to estimate the MW of five sample proteins (■). The corresponding gel images (Commassie blue stained) of the standards (Lane 1) and five sample proteins (Lane 2) are shown to the left of each plot.

formulations. Estimation of molecular weight is a technique commonly employed to characterize proteins and depends upon reliable markers to obtain accurate results. In this study, the Dual Xtra standards provided an accurate method of molecular weight estimation for five independent protein samples analyzed on four different gel types. While reliable estimation of molecular weight is a common attribute of all Precision Plus Protein standards, the principal benefit distinguishing the Dual Xtra standards is the capability to analyze proteins across the widest range of molecular masses, from 2 to 250 kD.

References

Schaegger H and von Jagow GV (1987). Tricine-sodium dodecyl-sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kD. Anal Biochem 166, 368-379.

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