Molecular Weight Estimation Using Precision Plus Protein™ WesternC™ Standards on Criterion™ Tris-HCl and Criterion XT Bis-Tris Gels

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Introduction

One-dimensional gel electrophoresis is frequently used to obtain information about the molecular weight and purity of proteins (Hames and Rickwood 1990). In PAGE, proteins migrate in response to an electric field, and the composition of the gel matrix acts as a sieve to separate proteins based on size, charge, and shape. The protein standards used in PAGE are fundamental tools of protein research and are critical in estimating the size of a protein of interest. Following electrophoresis on a gel, separated proteins are frequently blotted to a membrane and identified using antibodies and immunodetection reagents. Under these conditions, accurate and precise molecular weight estimation across a variety of different gel formulations depends upon the use of a high-quality protein standard.

Criterion and Criterion XT precast gels from Bio-Rad Laboratories provide convenient, high-resolution and high-throughput protein separation. The gels are available in a variety of single-percentage and gradient acrylamide concentrations, buffer compositions, and well configurations. In addition, Criterion XT precast gels utilize a neutral pH gel formulation to offer the same separation capabilities as other Criterion gels, but with an extended shelf life and room-temperature storage.

Precision Plus Protein* WesternC standards have ten prestained *Strep*-tagged protein bands that can be used to monitor migration during electrophoresis, and provide a simple method for assessing transfer efficiency after blotting. With the addition of StrepTactin horseradish peroxidase (HRP) during the secondary antibody incubation step, all ten bands of the standard can be detected by chemiluminescence. This capability provides an easy method for estimating the molecular weight of a protein of interest on gels and western blots (Urban and Woo 2007).

In this study, the migration patterns of the Precision Plus Protein WesternC standards on selected Criterion and Criterion XT gel types were compared. The relative migrations of protein bands in the standards were used to estimate the molecular weight of bovine serum albumin (BSA) on western blots generated from each gel type. The results demonstrate that the Precision Plus Protein WesternC standards can be used on a variety of Criterion and Criterion XT gel types and provide a convenient method to estimate the molecular weight of proteins directly from blots.

Methods

A total of 200 ng of BSA (Bio-Rad Laboratories, Inc.), and 5 µl of WesternC standards were loaded onto Criterion Tris-HCl 4–20% linear gradient, Criterion XT Bis-Tris 10%, and 4–12% resolving gels. Electrophoresis was performed using a Criterion cell at 200 V using the gel types listed in Table 1, until the dye front reached the bottom of the gel (35–60 min depending on gel type).

Table 1. Criterion and Criterion XT gels and buffers tested.

Gel Type	Buffer	Buffer Abbreviation
Criterion 4–20% Tris-HCl	Tris/Glycine/SDS	TGS
Criterion XT 4–12% Bis-Tris	2-(N-morpholino)ethanesulfonic acid	MES
Criterion XT 10% Bis-Tris	2-(N-morpholino)ethanesulfonic acid	MES
Criterion XT 4–12% Bis-Tris	3-(N-morpholino)propanesulfonic acid	MOPS

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For chemiluminescence detection of both Precision Plus Protein WesternC standards and BSA samples, the gels were blotted using a Criterion blotter onto 0.45 µm nitrocellulose membranes at 100 V for 30 min using Towbin buffer (25 mM Tris-HCl [pH 8.3], 192 mM glycine, 20% [v/v] methanol). The blots were blocked in 1% (w/v) casein in TBS (20 mM Tris-HCl [pH 7.5], 500 mM NaCl) for 1 hr and washed 3 x 5 min in TTBS (20 mM Tris-HCl [pH 7.5], 500 mM NaCl, 0.05% [v/v] Tween) before addition of a 1:200 dilution of primary BSA antibody (Sigma-Aldrich) in TTBS. After incubation for 1 hr, the blots were washed 6 x 5 min prior to incubation with a Bio-Rad HRP-conjugated goat anti-rabbit antibody (1:50,000) and Precision Plus Protein StrepTactin-HRP conjugate (1:10,000), both diluted in TTBS. Blots were washed in TTBS 6 x 5 min before the addition of Immun-Star™ WesternC™ chemiluminescent detection solutions, and were immediately imaged on a Molecular Imager® ChemiDoc™ XRS system. Relative migration of both standards and BSA were calculated using Quantity One® 1-D analysis software (Bio-Rad bulletin 3133). The actual molecular weight of BSA was determined by mass spectrometry using an autoflex MALDI-TOF system (Bruker Daltronik GmbH).

Results and Discussion

Figure 1 shows the migration patterns of the Precision Plus Protein WesternC standards and BSA on each of the four gel types following transfer and chemiluminescence detection on western blots. The relative front (R_f) values for the Precision Plus Protein WesternC standards and BSA determined for each gel type are shown in Tables 2 and 3.

By plotting the R_f versus the log molecular weight (MW) values, a standard curve for the Precision Plus Protein WesternC standards was generated to estimate the MW of BSA. Linear fits to the Precision Plus Protein WesternC standards data points were obtained for each gel type, and R^2 values were calculated to assess the accuracy of the fits. The molecular weight of each BSA sample was determined from the linear fits obtained from the standards. While all ten bands of the Precision Plus Protein WesternC standards were resolved on most Criterion gel types, the best linear fit with Criterion XT 10% and 4–12% gels run in a MES buffer was obtained after omitting the band at 250 kD due to the decreased linearity of the electrophoretic separation on these gel types for large molecular weight bands.

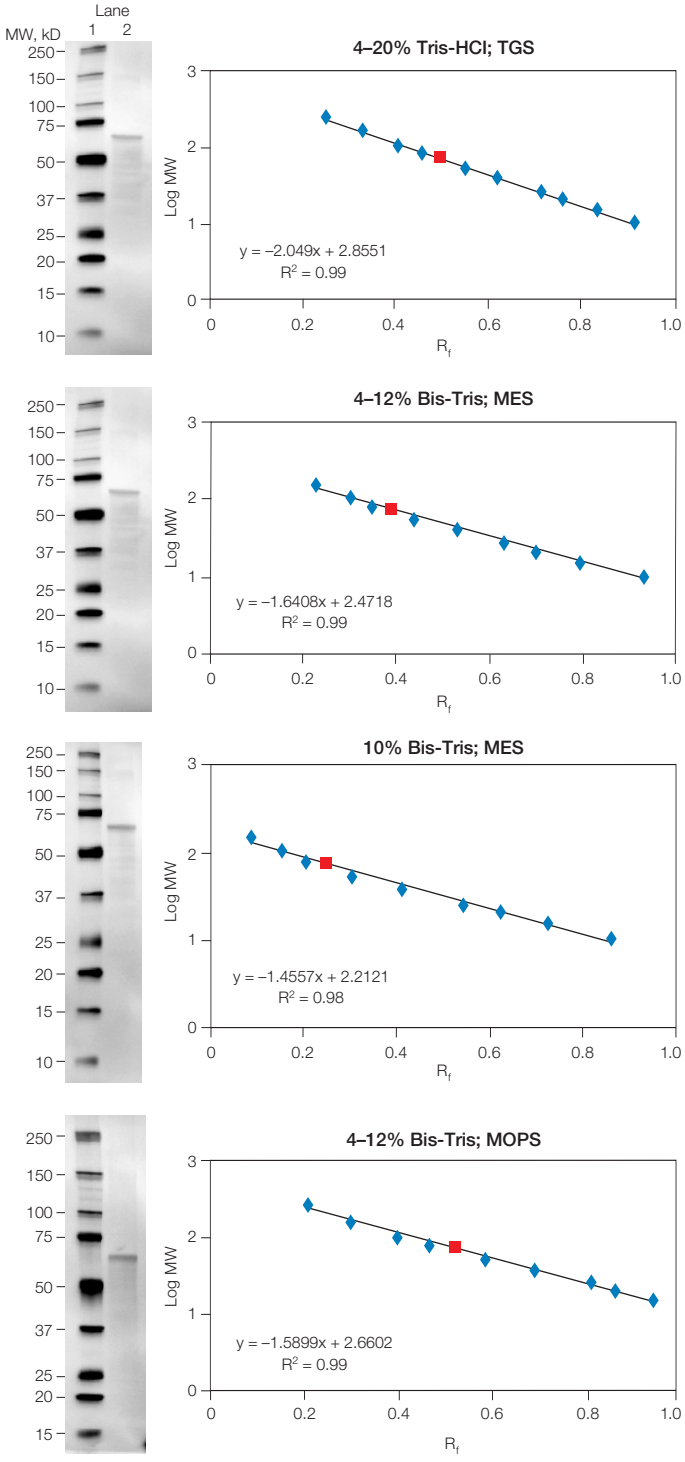

Fig. 1. Determining the MW of BSA using Precision Plus Protein WesternC standards on western blots obtained from different Criterion gel types. Standard curves of log MW versus relative front (R_f) were generated using Precision Plus Protein WesternC standards from chemiluminescence-detected western blots. Linear fits to the standards data points (♦) were used to estimate the MW of BSA (■) on each Criterion gel type tested based on the equations calculated from the standard curves. The corresponding gel migration patterns of the standards (lane 1) and BSA (lane 2) are shown on chemiluminescent detected blots to the left of each standard curve plot for the four gel types. The high R^2 values (>0.98) obtained from the linear fits demonstrate the exceptional linearity of the Precision Plus Protein WesternC standards.

Table 2. Measurement of R_f values for the Precision Plus Protein WesternC standards from western blots. Each blot was independently analyzed.

MW of Standards		R_f Value			
MW	Log MW	4–20% Tris-HCl	4–12% MES	10% MES	4–12% MOPS
250	2.398	0.256	omitted	omitted	0.209
150	2.176	0.329	0.226	0.091	0.302
100	2.000	0.407	0.304	0.157	0.399
75	1.875	0.460	0.350	0.206	0.461
50	1.699	0.548	0.437	0.305	0.582
37	1.568	0.621	0.531	0.415	0.688
25	1.398	0.712	0.631	0.543	0.805
20	1.301	0.759	0.697	0.624	0.858
15	1.176	0.833	0.798	0.725	0.948
10	1.000	0.912	0.934	0.860	unresolved

Table 3. Measurement of R_f and comparison of actual versus calculated MW of BSA from western blots using Precision Plus Protein WesternC standards.

	BSA, ng	R_f	Calculated MW WesternC Standards	Actual MW Mass Spectrometry	Difference
4–20% Tris-HCl	200	0.494	69.65	66.43	5%
4–12% Bis-Tris MES	200	0.388	68.42	66.43	3%
10% Bis-Tris MES	200	0.253	69.79	66.43	5%
4–12% Bis-Tris MOPS	200	0.521	67.90	66.43	2%

The 10 kD band was unresolved on Criterion XT 4–12% gels with a MOPS running buffer. MOPS provides greater separation of the large molecular weight bands at the expense of resolution of the low molecular weight bands. For all gel types tested, the R^2 values for the linear fit of the Precision Plus Protein WesternC standards were >0.98. Based on these fits, the molecular weight calculations of BSA were within 5% or less of the actual mass of the protein as determined by mass spectrometry (Table 3).

Conclusions

The results demonstrate that Precision Plus Protein WesternC standards provide an accurate method for estimating protein molecular weight on blots obtained using either Criterion or Criterion XT gels.

References

Hames BD and Rickwood D (1990). Gel electrophoresis of proteins, a practical approach, 2nd edition. Oxford University Press, 1-147.

Urban M and Woo L (2007). Molecular weight estimation and quantitation of protein samples using Precision Plus Protein WesternC standards, the Immun-Star WesternC chemiluminescent detection kit, and the Molecular Imager ChemiDoc XRS imaging system. Bio-Rad Bulletin 5576.

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