Comparison of the Criterion™ TGX Stain-Free™ Precast Gel System and Standard Coomassie Staining Procedures for Running and Imaging Protein Gels

Xuemei Yang, Kate Smith, and Lee Lomas
Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

SDS-PAGE is widely used for analyzing protein mixtures, and the Laemmli system is regarded as the gold standard SDS-PAGE technique due to its ability to cleanly resolve complex samples from a wide variety of sources in a wide array of sample backgrounds. Following SDS-PAGE separation, Coomassie staining is a standard yet time-consuming method used to visualize proteins in the gel.

The new Criterion TGX (Tris-Glycine eXtended) Stain-Free precast polyacrylamide gels are based on the long shelf life TGX formulation and include unique trihalo compounds that allow rapid fluorescent detection of proteins using the Gel Doc™ EZ imaging system. TGX Stain-Free gels retain Laemmli-like separation characteristics using standard sample and Tris/Glycine running buffers while providing an extended shelf life of up to 1 year when stored at 4°C. Proteins can be separated in as little as 20 min when using this gel format and then visualized using the Gel Doc EZ imager in as little as 2.5 min. The trihalo compounds react with tryptophan residues in a UV-induced reaction to produce fluorescence, which can be detected by the Gel Doc EZ imager within gels or on blotting membranes (PVDF) after the transfer of proteins. Activation of the trihalo compounds in the gels adds a 58 Da adduct to available tryptophan residues and is required for protein visualization. Proteins with a tryptophan content of 1.5% or greater show detection sensitivities comparable to Coomassie staining, while proteins with tryptophan contents greater than 3% show superior sensitivity. The current study was designed to evaluate the sensitivity, linearity, and precision of the Criterion Stain Free™ imaging system*, as compared to Bio-Safe™ Coomassie staining for proteins with varying tryptophan (Trp) content (0.3–3%) under recommended running conditions.

*The experiments completed for this tech note were done with the Criterion Stain Free imaging system. The Criterion Stain Free imaging system has been replaced by the Gel Doc EZ imager which has equivalent performance for imaging stain-free gels but has a broader range of imaging applications.

Methods

Samples

Samples used included Precision Plus Protein™ Dual Color standards (Bio-Rad Laboratories, Inc.), broad range, unstained SDS-PAGE standards (Bio-Rad), *E. coli* lysate (Bio-Rad), and mouse serum (Sigma). Purified proteins used in this study, including β-galactosidase (3.8% Trp), phosphorylase B (1.4% Trp), BSA (0.3% Trp), carbonic anhydrase (2.3% Trp), and lysozyme (3.4% Trp), were purchased from Sigma.

Electrophoresis and Imaging

Electrophoresis was performed using 4–15%, 4–20%, and Any kD™ Criterion TGX Stain-Free precast gels and compared to corresponding Criterion Tris-HCl precast gels. Samples were prepared in Laemmli sample buffer containing 5% β-mercaptoethanol. Precision Plus Protein Dual Color or unstained standards were loaded as provided. Gels were run using the Criterion electrophoresis cell at 200 or 300 V until the dye front reached the bottom of the gel. All gels were run using Tris/Glycine/SDS running buffer (25 mM Tris, 192 mM glycine, 0.1% [w/v] SDS). Criterion TGX Stain-Free gels were first imaged on the Criterion Stain Free imager (UV exposure time of 5 min followed by system default integration time). Criterion TGX Stain-Free gels and Criterion Tris-HCl gels were additionally stained with Bio-Safe Coomassie stain (Bio-Rad) and imaged on a GS-800™ calibrated densitometer (Bio-Rad).

Results

Reduced Gel Run Times

The Criterion TGX Stain-Free gels allowed faster run times as compared to standard Tris-HCl gels (Table 1). At 200 V, run times are 55–60 min for Criterion Tris-HCl gels and 42–50 min for Criterion TGX Stain-Free gels. However, Criterion TGX Stain-Free gels can also be run at higher voltages with little impact on gel performance (Figure 1);

Table 1. Comparison of running times for Criterion TGX Stain-Free gels and Criterion Tris-HCl gels.

Criterion TGX Stain-Free gel			
200 V		**300 V**	
Run time:	42–50 min	Run time:	20–26 min
Initial current:	45–50 mA	Initial current:	89–135 mA
Final current:	38–42 mA	Final current:	66–99 mA
Criterion Tris-HCl gel			
200 V		**300 V**	
Run time:	60 min	Not recommended	

when run at 300 V the run time can be as little as 20 min, whereas running a Tris-HCl gel at 300 V is not recommended.

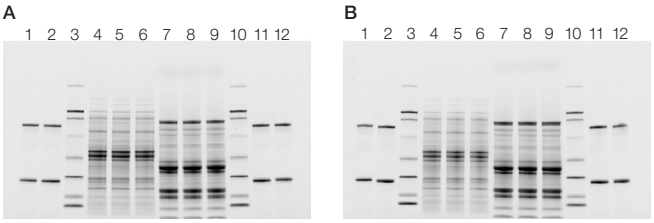

Fig. 1. Criterion TGX Stain-Free gels run at 200 V (A) and 300 V (B). Precision Plus Protein Dual Color standards* (lanes 1, 2, 11, and 12), broad range, unstained SDS-PAGE standards (lanes 3 and 10), *E. coli* lysate (50 ng/well, lanes 4–6), and mouse serum (50 ng/well, lanes 7–9) were loaded onto 4–20% Criterion TGX Stain-Free gels and run at 200 V (42 min) and 300 V (20 min) and then imaged on the Criterion Stain Free imager. At 300 V, no noticeable decrease in band resolution or band sharpness was observed as compared to the 200 V condition.

Rapid Protein Visualization

Standard Tris-HCl gels require staining and destaining prior to imaging. Stain-free detection technology allows rapid protein visualization by eliminating the need for staining and destaining. The ability of the trihalo compounds within the gel to react with tryptophan residues in a UV-induced reaction to produce fluorescence permits direct in-gel detection of proteins by the Criterion Stain Free imager. Activation of the trihalo compounds with tryptophan occurs in 2.5–5 min.

In-Gel Protein Quantitation

The limit of detection, linearity, and precision when quantifying proteins from polyacrylamide gels depends on a number of factors, including the quality of the staining reagent used to visualize the protein and variability in the staining and destaining conditions. Criterion TGX Stain-Free gels do not require staining or destaining steps and consequently have a uniform, low background that yields consistent and reproducible results. To assess the sensitivity, linearity, and reproducibility of the stain-free system, Criterion TGX Stain-Free gels were processed and imaged entirely on the Criterion Stain Free imaging system, or stained with Bio-Safe Coomassie stain and imaged on the GS-800 calibrated densitometer. A mixture of proteins spanning wide molecular weight and % tryptophan content ranges was prepared in serial dilutions ranging from >300 to 2.6 ng per band. The protein mixture was chosen because it was composed of a variety of proteins with diverse amino acid compositions. Dilution series were run in quadruples on each gel type to establish the reproducibility of each method. By visually establishing the concentration of the lowest detectable band, it is apparent that the sensitivity of the stain-free system is comparable to that of Bio-Safe Coomassie staining and for some protein bands shows a greater level of sensitivity (Figure 2). The exception is BSA, which has only 0.3% tryptophan content. For proteins with

* Note: Some of the bands of the prestained protein standards are undetected using stain-free technology due to interference from the dye. It is recommended to use unstained protein standards with stain-free technology.

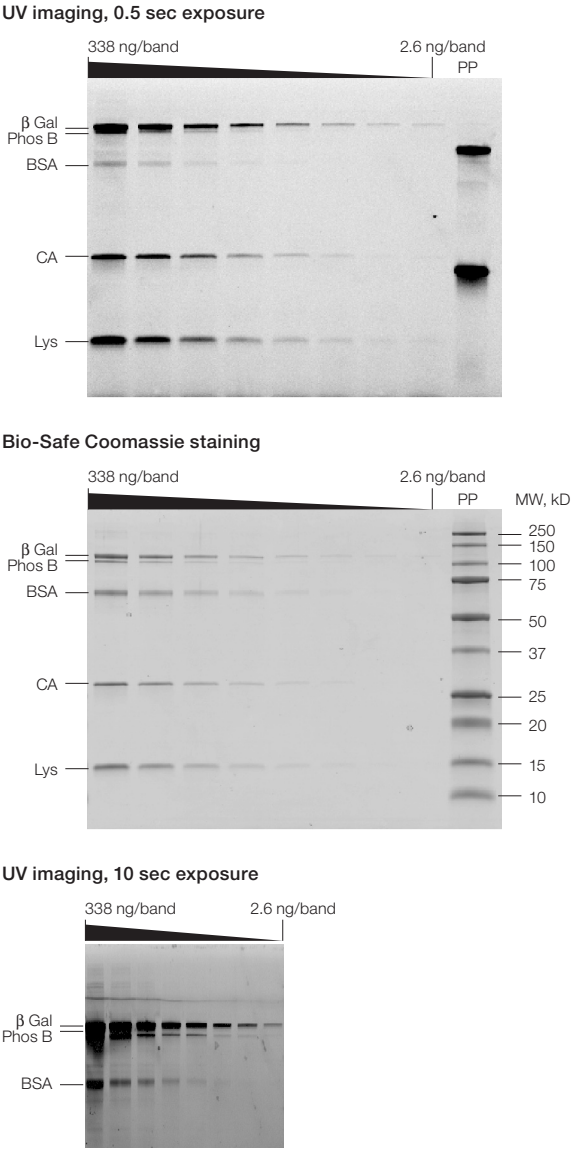

Fig. 2. Limit of detection comparison between Criterion TGX Stain-Free gels imaged on a Criterion Stain Free imager or stained with Bio-Safe Coomassie stain. Serial dilutions of a protein mixture were run on Criterion TGX Stain-Free gels and UV-imaged on a Criterion Stain Free imager or visualized using Bio-Safe Coomassie stain. PP, Precision Plus Protein Dual Color standards*. The dilution series ranged from 338 ng/band down to 2.6 ng/band of β-galactosidase (3.8% Trp), phosphorylase B (1.4% Trp), BSA (0.3% Trp), carbonic anhydrase (2.3% Trp), and lysozyme (3.4% Trp). % Trp represents the percent tryptophan content for each protein.

Table 2. Limit of detection on Criterion TGX Stain-Free gels.

	UV detection, ng/band	Bio-Safe Coomassie staining, ng/band
β-galactosidase (3.8% Trp)	2	4
Phosphorylase B (1.4% Trp)	16	14
BSA (0.3% Trp)	32*, 4**	8

* 0.5 and ** 10 sec integration time of image capture.

low tryptophan content, the signal can be enhanced by simply increasing the integration time of the image capture step. By increasing the integration time from 0.5 sec to 10 sec, the sensitivity of the Criterion Stain-Free system for proteins with low tryptophan content can be made comparable to that of Bio-Safe Coomassie staining. However, it should be noted that proteins that lack tryptophan residues cannot be imaged.

Generally for proteins containing tryptophan, the sensitivity of the Criterion Stain Free detection system is comparable

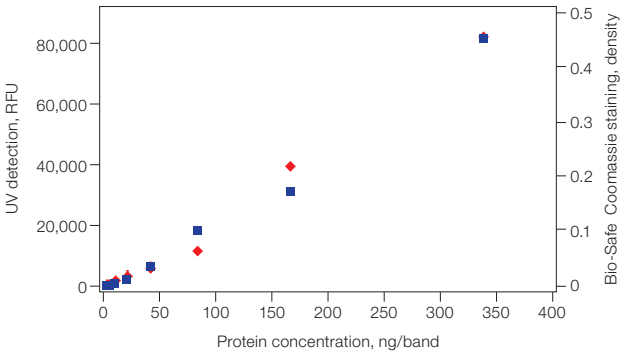

Fig. 3. Detection of a protein with low tryptophan content using Bio-Safe Coomassie stain or the Criterion Stain Free system. UV detection occurred on the Criterion Stain Free imager; Bio-Safe Coomassie staining density was measured on a GS-800 calibrated densitometer. ■, UV detection; ◆, Bio-Safe Coomassie staining.

to Bio-Safe staining and is linear over at least two orders of magnitude with an R^2 >0.99 (Figure 3). The overall precision of the measurement was also better with the stain-free system as compared to conventional Coomassie staining. The average %CV across all concentrations for the samples quantified with the Criterion Stain Free imager was less than 14%, while the average for the gels stained with Bio-Safe Coomassie stain was 35% (data not shown).

Increased Gel Shelf Life

The introduction of TGX chemistry in the Criterion format allowed for both an increased shelf life of at least 1 year and short run times, using standard Laemmli running buffers, in addition to rapid protein visualization. Figure 4 depicts TGX stain-free gel chemistry performance after storage at elevated temperature. When maintained at 37 °C for 0 and 12 days, no discernible effect on performance was observed. Twelve days of storage at 37 °C is equivalent to 12 months at 4 °C. The storage of Criterion Tris-HCl precast gels for more than 12 weeks is not recommended; therefore, Criterion TGX Stain-Free gels provide greater flexibility with a 1 year shelf life.

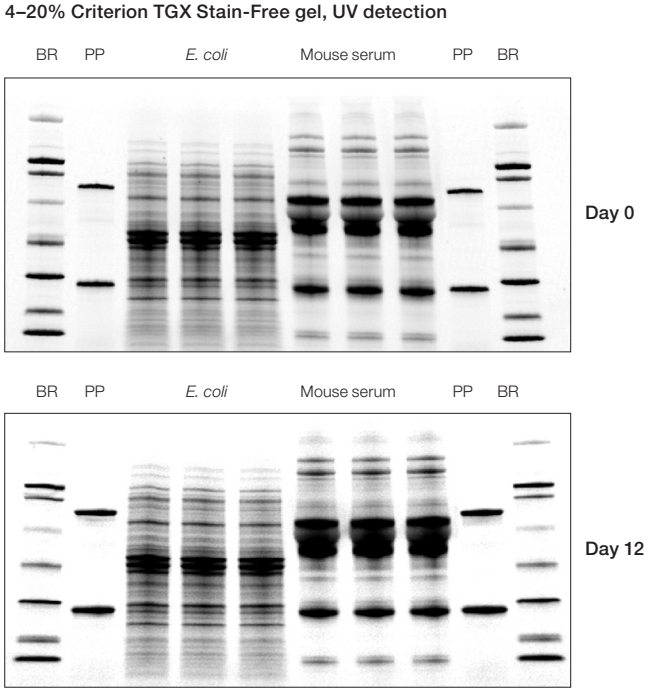

Fig. 4. Performance of Criterion TGX Stain-Free gels following storage at 37°C. Freshly prepared 4–20% Criterion TGX Stain-Free gels were maintained at 37°C for 0 and 12 days prior to performing PAGE of *E. coli* lysates and mouse serum. Twelve days of storage at 37°C is equivalent to 12 months at 4°C. BR, broad range, unstained SDS-PAGE standards; PP, Precision Plus Protein Dual Color standards.

Conclusions

Criterion TGX Stain-Free precast gels have the potential to substantially improve and streamline the SDS-PAGE workflow. They can be run at 300 V without overheating, allowing run times as short as 20 min, and can be visualized directly without the need for staining and destaining. Proteins can now be separated and visualized in as little as 25 min, saving considerable time over the traditional workflow using Tris-HCl gels followed by staining, which requires at least 3 hr. This time savings can be achieved while maintaining the sensitivity and reproducibility achieved with Coomassie staining. Furthermore, Criterion TGX Stain-Free precast gels maintain their performance after 12 days at 37°C, which is equivalent to 1 year at 4°C.

Information in this tech note was current as of the date of writing (2010) and not necessarily the date this version (rev A, 2010) was published.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 31 3689 6600* ***Canada*** *905 364 3435* ***China*** *86 20 8732 2339* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666* ***New Zealand*** *0508 805 500* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189* ***United Kingdom*** *020 8328 2000*

Bulletin 6008 Rev A US/EG *10-0998 1010 Sig 1109*