In-Gel Protein Quantitation Using the Criterion Stain Free™ Gel Imaging System

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Introduction

Since the first descriptions of the use of Coomassie Brilliant Blue dye to stain proteins separated electrophoretically (Fazekas De St Groth et al. 1963, Meyer and Lamberts 1965), the technique has been widely adopted for the detection of proteins separated by polyacrylamide gel electrophoresis (PAGE). Further research on the colloidal properties of these dyes has led to easier, more sensitive, and shorter procedures (Neuhoff et al. 1985). However, because of the user-dependent nature of the staining/destaining process, variability becomes a problem when different users compare data.

The mechanisms by which Coomassie stain binds to proteins are not well understood. Proteins that are rich in the basic amino acids arginine, histidine, and lysine bind the dye strongly, whereas others do not (Tal et al. 1985). In addition, glycoproteins, which make up more than half of all proteins, stain poorly with Coomassie dye (Moller et al. 1993, Osset et al. 1989, Van den Steen et al. 1998). At least one publication has reported that Coomassie staining may overestimate relative protein quantities in gels (Ownby et al. 1993).

The Criterion Stain Free gel imaging system is an alternative to Coomassie staining for protein visualization on SDS polyacrylamide gels after electrophoresis. The system consists of three components: Criterion Stain Free gels, the Criterion Stain Free imager, and Image Lab™ software. The Criterion Stain Free technology is based on a UV-induced trihalocompound modification of tryptophan residues contained in proteins after separation by electrophoresis (Kazmin et al. 2002) on Criterion Stain Free gels. The system uses standard reagents, sample preparation methods, and electrophoresis protocols. After electrophoresis, the gel is removed from the cassette and placed into the Criterion Stain Free imager where the separated proteins are activated by UV irradiation and produce a fluorescent signal that is detected and captured by a CCD camera. In 2.5–5 min, the system provides an image of the proteins, and using the molecular weight standard lane, it automatically estimates the molecular weight and quantity for each detected protein band. After activation, the gel remains compatible with downstream applications such as western blotting (Bio-Rad bulletin 5781; Ladner et al. 2004) or staining with Coomassie stain, silver stain, or fluorescent dyes.

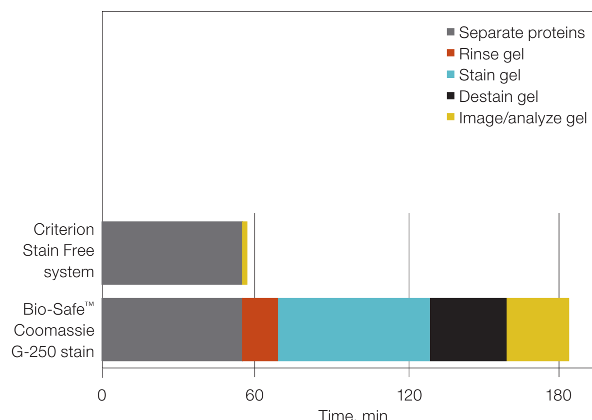

Fig. 1. Comparison of Criterion Stain Free system and Bio-Safe Coomassie staining workflows. The electrophoresis run time for both Criterion Stain Free system and Coomassie-stained gels is the same at 55 min. After electrophoresis, Criterion Stain Free gels take 2.5–5 min to generate results, while Coomassie staining takes at least 2 hr to generate the same level of sensitivity (the graph does not include times for changing solutions).

A comparison of standard SDS-PAGE/Coomassie staining and Criterion Stain Free system workflows shows a 70% reduction in experiment time with the Criterion Stain Free system (Figure 1). Rinsing, staining, and destaining steps are eliminated from the workflow when using the Criterion Stain Free system, and the imaging and analysis steps are automated, thus reducing post-electrophoresis time from more than 2 hr to about 2.5 min.

This study compares the reproducibility of protein quantitation results and sensitivity of the Criterion Stain Free system to those of two available Coomassie stains (Bio-Safe™ Coomassie G-250 stain and Coomassie Brilliant Blue R-250 stain, Bio-Rad Laboratories, Inc.). Results indicate superior to equivalent performance of the Criterion Stain Free system compared with traditional Coomassie staining.

Methods

Electrophoresis was performed on 4–20% gradient Criterion Stain Free Tris-HCl gels (Bio-Rad). Samples were prepared in Laemmli buffer containing 5% β-mercaptoethanol and heated at 95°C for 5 min.

The broad range unstained SDS-PAGE molecular weight standards (Bio-Rad) were diluted 1:40 in Laemmli sample buffer containing 5% β-mercaptoethanol, and 2-fold serial dilutions were made in the same sample buffer for a total of 13 protein concentrations. A volume of 10 µl of each dilution was loaded onto 26-well gels and electrophoresed for 55 min at 200 V.

Coomassie staining was performed with Bio-Safe Coomassie G-250 and Coomasie Brilliant Blue R-250 (CBB R-250) stains. Bio-Safe G-250 staining was performed following manufacturer's instructions. Gels were rinsed 3 x 5 min in deionized water before being placed in 200 ml of Bio-Safe G-250 stain for 1 hr under agitation. The gels were rinsed 3 x with 200 ml water and destained for 3 x 30 min in 200 ml water with gentle rocking at room temperature. CBB R-250 staining was done with a premixed stain solution. The gels were submerged in 40 ml of staining solution for 1 hr on a rocking platform. Destaining was done using 50 ml of Coomassie Brilliant Blue R-250 destaining solution (Bio-Rad) on a rocking platform for 15 hr followed by 2 washes of 2 hr each.

Mouse and rat liver total protein, mouse and rat thymus extracts, and HeLa whole cell lysate were purchased from Santa Cruz Biotechnology, Inc. *E. coli* protein was prepared from a lyophilized sample (Bio-Rad); human serum was diluted 1:80 prior to loading on gel. Human serum was depleted in high abundance proteins using the ProteoMiner™ protein enrichment kit (Bio-Rad) as recommended by the manufacturer. Depleted fraction was diluted 1:8 prior to loading on gel.

Imaging and Analysis

Criterion Stain Free gels were placed in the Criterion Stain Free imager, activated for 5 min, and imaged for 1.1 sec. Gel images were analyzed using Image Lab software. Gels stained with Coomassie stain were imaged on the Molecular Imager® GS-800™ calibrated densitometer (Bio-Rad) at 95 µm resolution to achieve resolution equivalent to that of the Criterion Stain Free imager and analyzed using Quantity One® 1-D analysis software (Bio-Rad).

Results and Discussion

Reproducibility of Protein Quantitation on Polyacrylamide Gels

Quantitation of protein bands on a polyacrylamide gel depends on the quality of the staining used to visualize the protein. Variables in staining/destaining conditions such as agitation times, volumes, solution changes, and temperature affect reproducibility of results. Uneven staining of the gels (Figure 2) may result in erroneous quantitation of the protein bands. In contrast, because they do not require staining and destaining steps, Criterion Stain Free gels have a uniform and low background level (Figure 2) and yield consistent and reproducible results.

To assess reproducibility of the Criterion Stain Free system compared with CBB R-250 staining, we quantitated the band corresponding to β-galactosidase in serial dilutions of the broad range SDS-PAGE standards. Thirteen serial dilutions of the standard, ranging from 500 ng to 0.12 ng per band, were run in triplicate on Criterion Stain Free gels. The gels were imaged using the Criterion Stain Free imager and subsequently stained using CBB R-250. Figure 2 shows representative images of a Criterion Stain Free gel and a CBB R-250–stained gel. The quantity of protein present in the band corresponding to β-galactosidase (MW 116.000) was estimated in the three replicates using Image Lab software and Quantity One software after scanning the CBB R-250–stained gels using a densitometer. Only the first eight dilutions were compared, because they were detected in all of the images. Figure 3 shows a greater variability in the quantitation results when Coomassie staining was used. The average %CV across all concentrations for the samples quantified with the Criterion Stain Free imager was 4.85%, while the average for the gels stained with CBB R-250 was 24%. A similar trend was observed for all other proteins (data not shown).

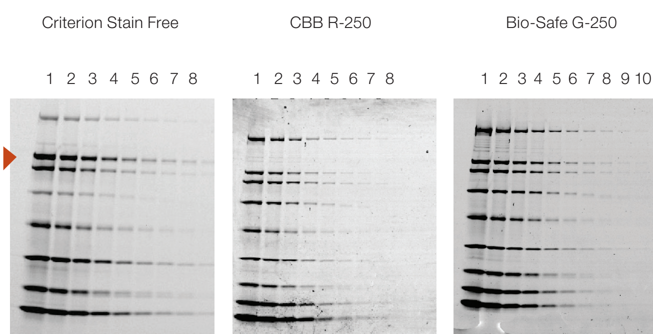

Fig. 2. Comparison of a Criterion Stain Free gel image and CBB R-250– and Bio-Safe G-250–stained gel images. Serial 1:2 dilutions of broad range unstained molecular weight standards were separated on a 4–20% Criterion Stain Free Tris-HCl gel. The gel was imaged with the Criterion Stain Free system, then stained with Coomassie stain and imaged on a Molecular Imager GS-800 calibrated densitometer. Arrowhead indicates β-galactosidase.

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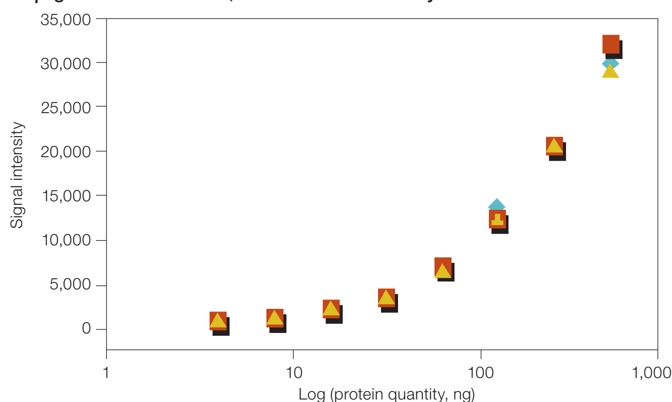


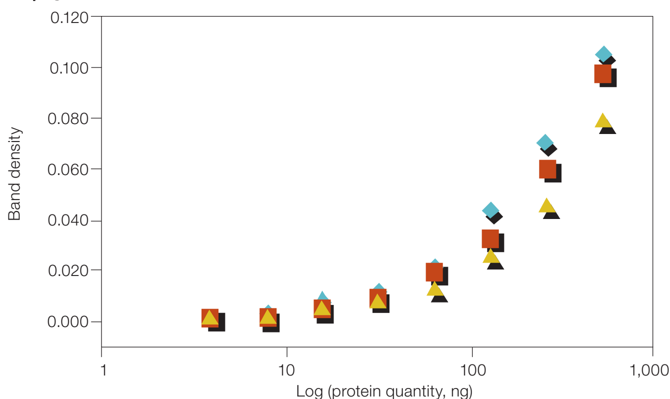
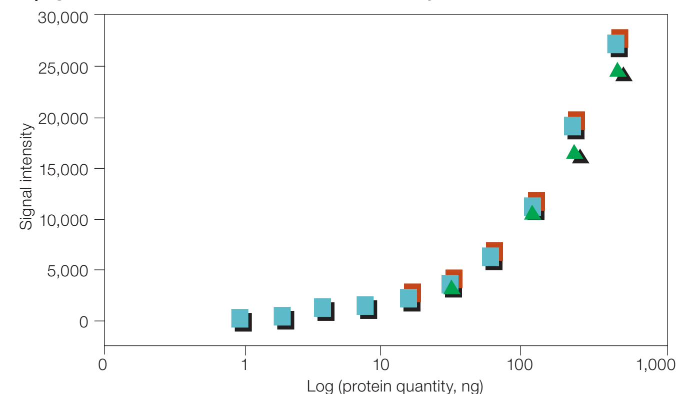


Fig. 3. Reproducibility of protein quantitation in Criterion Stain Free gels (A) and CBB R-250 stained gels (B). Quantitation of the β-galactosidase bands of the broad range standards was performed for different dilutions in three replicate gels. Higher reproducibility is observed with the Criterion Stain Free system compared with Coomassie staining. Average %CV for Criterion Stain Free gels is 4.85%; for CBB R-250–stained gels, it is 24.08%. The band detection sensitivity of the Image Lab software was set to high.

Similar results were obtained when comparing Criterion Stain Free gel images with Bio-Safe G-250–stained gel images (Figure 2). Quantitation of the β-galactosidase bands was done for the first ten dilutions on four gels. Figure 4 shows higher variability with Coomassie staining with a %CV of 19.7% compared with 7.8% for Criterion Stain Free gels. The quantitation data for the other proteins of the molecular weight standards showed a lower %CV for the Criterion Stain Free system detection as well.

Our results show that both Coomassie staining methods yield less consistent quantitation results than those of the Criterion Stain Free system. There is some difference in %CV between the Criterion Stain Free system data sets, but it is significantly less than the 20% range seen with the Coomassie stains.



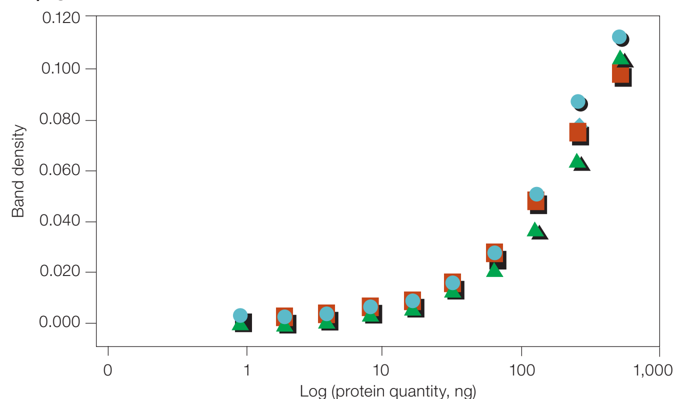


Fig. 4. Reproducibility of protein quantitation in Criterion Stain Free gels (A) and Bio-Safe G-250 stained gels (B). Quantitation of the β-galactosidase bands of the broad range standards was performed for different dilutions of the protein in four replicate gels. Higher reproducibility is observed with the Criterion Stain Free system compared with Coomassie staining. Average %CV is 7.8% for Criterion Stain Free gels and 19.67% for Bio-Safe G-250 staining. The band detection sensitivity of the Image Lab software was set to high.

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Table 1. Tryptophan content of the proteins in Bio-Rad broad range protein standards.*

	Number of Residues	Number of Tryptophans	%Tryptophan
Myosin	1,938	9	0.5
β-galactosidase	1,024	39	3.8
Phosphorylase B	843	12	1.4
BSA	583	2	0.3
Ovalbumin	386	3	0.8
Carbonic anhydrase	261	6	2.3
Trypsin inhibitor	216	2	0.9
Lysozyme	148	5	3.4
Aprotinin	58	0	0

* Protein sequence data from NCBI Entrez.

The broad range SDS-PAGE standards were chosen because they are composed of a variety of proteins with diverse amino acid composition that may affect their staining abilities. Myosin and BSA had better average sensitivity for detection with Bio-Safe Coomassie stain. Aprotinin was not detected using the Criterion Stain Free system because it lacks the tryptophan residues required for detection by the Criterion Stain Free system. Myosin and BSA are examples of proteins with extremely low percentages of tryptophan (Table 1; Figure 5), which explains their higher limit of detection (LOD). Comparing the sensitivity results (Figure 5) with the tryptophan content (Table 1) shows a correlation between higher tryptophan content and improved LOD using the Criterion Stain Free system.

In most organisms, proteins without tryptophan represent less than 10% of the proteins from 10–260 kD, the separable range for most PAGE. Examination of predicted proteomes for common experimental organisms shows that proteins lacking tryptophan are biased towards small molecular weight. If proteins below 10 kD are excluded from the list of proteins, the percent that lack tryptophan falls to less than 9% for the common organisms shown in Table 2.

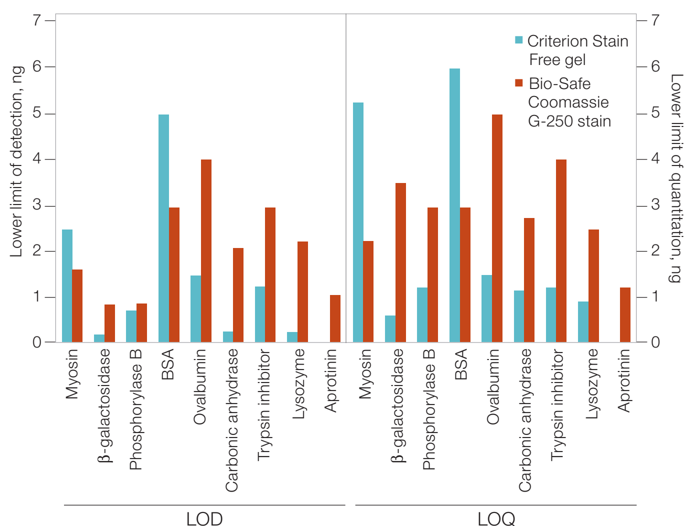

Fig. 5. Limits of detection (LOD) and limits of quantitation (LOQ) of proteins on Criterion Stain Free and Bio-Safe G-250–stained gels. Individual protein bands from broad range unstained standards from four replicate gels were used to determine visual LOD and LOQ. Averaged numbers were used to generate the graph.

Examples of complex protein samples or cell lysates detected by the Criterion Stain Free system are shown in Figure 6. A comparison of protein profiles from a number of tissues and organisms shows that detection by the Criterion Stain Free system and by Coomassie staining are visually equivalent.

Table 2. Tryptophan content of the predicted proteomes of several model organisms.*

Species	Total Number of Proteins	Number of Proteins Lacking Tryptophan	% of Proteins Lacking Tryptophan	Number of Proteins >10 kD	Number of Proteins >10 kD Lacking Tryptophan	% of Proteins >10 kD Lacking Tryptophan
Homo sapiens	40,827	4,209	10.31	37,548	2,754	7.33
Escherichia coli O1:K1 / APEC	4,865	458	9.41	4,754	408	8.58
Escherichia coli (strain K12)	4,181	456	10.91	3,879	325	8.38
Escherichia coli O6:K15:H31	4,604	562	12.21	4,147	365	8.80
Rattus norvegicus	12,022	1,081	8.99	11,421	745	6.52
Mus musculus	35,344	3,435	9.72	33,262	2,480	7.46
Saccharomyces cerevisiae	5,815	648	11.14	5,563	491	8.83

* Sequence data was obtained from UniProt (http://www.ebi.ac.uk/uniprot/database/Databases.html).

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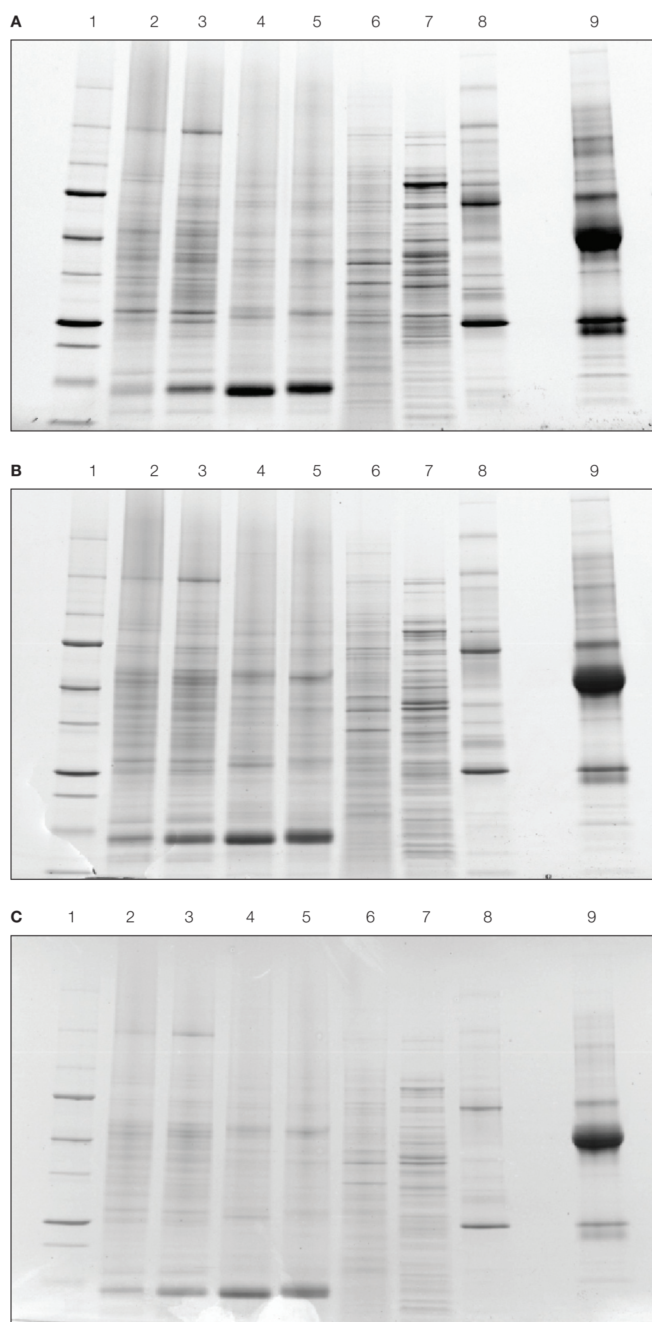

Fig. 6. Criterion Stain Free gel images of complex protein samples: comparison with Bio-Safe G-250 and CBB R-250 stained gels. Lane 1, Precision Plus Protein™ unstained standards; lane 2, mouse liver extract (12.5 µg); lane 3, rat liver (12.5 µg); lane 4, mouse thymus (12.5 µg); lane 5, rat thymus (12.5 µg); lane 6, HeLa cell lysate (12.5 µg); lane 7, *E. coli* lysate (67.5 µl); lane 8, ProteoMiner kit—treated human serum (5 µl of a 1:8 dilution); lane 9, untreated human serum (5 µl of a 1:80 dilution). Samples were run on a 12+2 well, 4–20% Criterion Stain Free Tris-HCl gel. The gels were activated and imaged on the Criterion Stain Free system (**A**), and then stained with Bio-Safe G-250 stain (**B**), or CBB R-250 (**C**), followed by imaging on a Molecular Imager GS-800 calibrated densitometer. Visible bands in the complex protein samples are comparable.

Conclusions

The Criterion Stain Free system is a fast and easy-to-use protein visualization system that utilizes the standard conventions of SDS-PAGE. Consisting of a new formulation of Bio-Rad's Criterion precast gels, a Criterion Stain Free imager, and Image Lab software, the system triggers protein fluorescence and generates a digital image of the gel after electrophoresis in as little as 2.5 min. Maximum sensitivity is reached in 5 min, with equal or better sensitivity than that of Coomassie staining.

The Criterion Stain Free gel imaging system is a tool for reproducible, fast, and environmentally friendly SDS-PAGE analysis that enables efficient protein purification workflows. Because of its ease of use and its protein quantification and data analysis capabilities, this system is the first serious alternative to the decades-old Coomassie Blue gel staining technique.

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