

Laser scanning quantification of 2-D gel spots using ChromaPhor™ Green Stain

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Protein spots stained with ChromaPhor Green Stain are much more sensitively detected and quantified by helium-neon laser scanners than are spots stained with Coomassie blue. This offers significant advantages for analysis of 2-D gel patterns.

Introduction

Kendrick Laboratories performs custom protein electrophoresis services, including 2-dimensional electrophoresis (1,2), SDS slab gel electrophoresis (3), transblotting (4), fluorography (5), and quantification of results (6). One ancillary service often used is quantification of proteins resolved on 2-D gels. For example, when evaluating 2-D gel results, a researcher may notice that a few polypeptides in a cell culture preparation appear to be increased in concentration by hormone treatment. To convert the visual scoring of spot densities to quantitative data, the stained 2-D gels are scanned by a laser densitometer and the resultant images are analyzed using a microcomputer. Integrated densities are determined for the spots of interest and for control spots. Once averages and standard deviations are obtained, statistics are used to determine the significance of the observation. A decision then may be made to proceed with partial sequencing and cloning to further characterize the spots of interest.

In cases where radiolabeling is not convenient or desirable, Coomassie blue staining often is used for 2-D quantification. One problem with Coomassie blue, however, is low sensitivity. Silver staining (7), a popular alternative, is more sensitive but difficult to quantify because of protein to protein and gel to gel variation in the colors and intensities of stained spots.

Absorbance spectra of ChromaPhor Green and Coomassie blue

We explored the use of ChromaPhor Green* Stain (8) as an alternative to Coomassie blue because its absorbance spectrum suggested that increased sensitivity might be gained when using laser densitometry. **Figure 1** shows the absorbance spectra of the two stains. A major problem in quantifying with Coomassie blue is that the peak absorbance of the stain is poorly matched to the monochromatic helium-neon beam of laser scanners (fixed at 633 nm). By contrast, the absorbance peak of ChromaPhor Green actually coincides with the wavelength of the laser beam. Thus, staining gels with ChromaPhor Green would be expected to increase the sensitivity of quantification, assuming that the protein binding capacity of ChromaPhor Green is similar to that of Coomassie blue.

*Patent pending

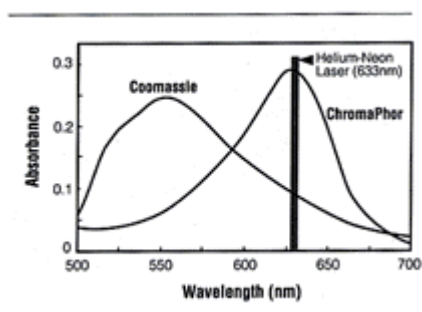


Figure 1. Absorbance spectra of the protein stains Chromaphor Green and Coomassie blue. Solutions of each stain were analyzed over the visible wavelength region of 500-700 nm using a Bausch & Lomb Spectronic 2000 recording scanning spectrophotometer.

Gel quantification with Coomassie blue becomes more reproducible if protein calibration strips are used (6). If a calibration strip containing known amounts of bovine serum albumin (BSA) polymerized within polyacrylamide layers is stained, destained and scanned concurrently with each 2-D gel (shown in [Figure 3](#)), the density values obtained may be used to construct a standard curve. The amount of protein in individual polypeptides then may be expressed relative to the standard curve, in ng protein per spot. [Figure 2](#) shows standard curves obtained from calibration strips stained with ChromaPhor Green and Coomassie blue. Both stains show a linear relationship between stain absorbance and ng protein per mm^2 . However, the ChromaPhor Green plot shows a substantially higher signal for each concentration of BSA, suggesting that the affinity of the ChromaPhor Green stain for protein is similar to that of Coomassie blue, and that increased sensitivity would be gained by using this stain.

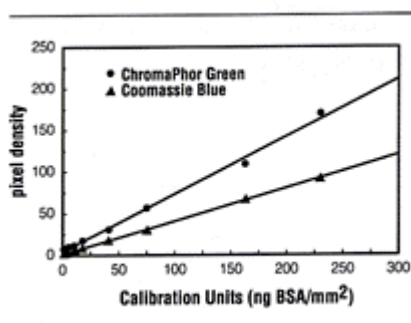


Figure 2. Calibration strip standard curves for Chromaphor Green and Coomassie blue stains. The calibration strips were made by polymerizing serial dilutions of BSA in consecutive layers of acrylamide and then slicing the layered gel vertically. The strips were stained and destained as described in the legend to [Figure 3](#) and then quantified by laser densitometry and computer analysis. The linearity of the curve and signal strength suggest that ChromaPhor Green binds stoichiometrically to protein, with a binding capacity similar to that of Coomassie blue.

2-D Gel quantification

[Figure 3](#) shows the patterns obtained from 2-D electrophoresis of two aliquots of the same preparation of *E. coli* lysate. The two 2-D gels were run in parallel and stained after electrophoresis. The gel in [Figure 3A](#) was stained with Coomassie blue while the gel in [Figure 3B](#) was stained with ChromaPhor Green. The BSA calibration strips at the tops of the gels were stained and destained with the gels. Although the

Coomassie blue stained pattern appears slightly darker to the eye than the pattern stained with ChromaPhor Green, in general the patterns are comparable.

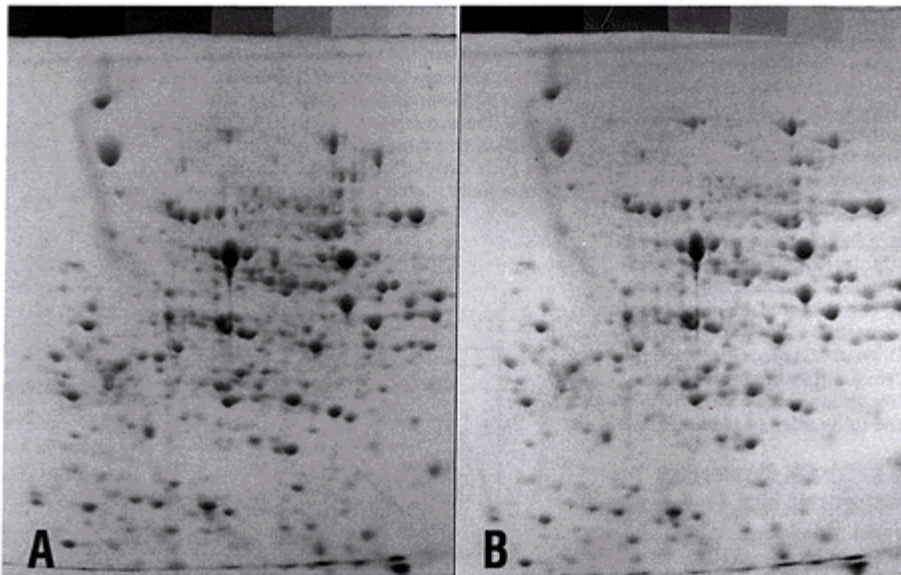


Figure 3. Comparison of Coomassie blue (Panel A) and ChromaPhor Green (Panel B) stained 2-D gels using identical *E. coli* samples. The two 2-D gels were run under identical electrophoresis conditions in one tank. The ChromaPhor Green gel and accompanying calibration strip were stained overnight with gentle shaking in a solution of 50% ethanol:10% acetic acid containing a 1:150 dilution of ChromaPhor Green. The next day, the gel and calibration strip were rehydrated for 3 hours in a solution of 10% acetic acid containing a 1:150 dilution of ChromaPhor Green, and then destained for several hours in a solution of 10% acetic acid. The Coomassie blue gel and calibration strip was similarly stained and destained except that the staining and rehydration solution containing 0.01% Coomassie blue and the rehydration time was 2 hours. Note that these staining conditions do not fix the ampholines, which migrate at the dye front of 2-D gels.

A different result is obtained, however, when laser-scanned, digitized images of the Coomassie blue (Panel A) and ChromaPhor Green (Panel B) stained gels are compared. [Figure 4](#) shows magnified areas of the computerized images of these two images. As might be predicted, virtually all of the spots are darker on the gel stained with ChromaPhor Green, and many more minor polypeptide spots are detected (see arrows). Thus, when a laser densitometer is used for quantification of 2-D gels, the ChromaPhor Green Stain substantially increases the sensitivity of detection of polypeptide spots.

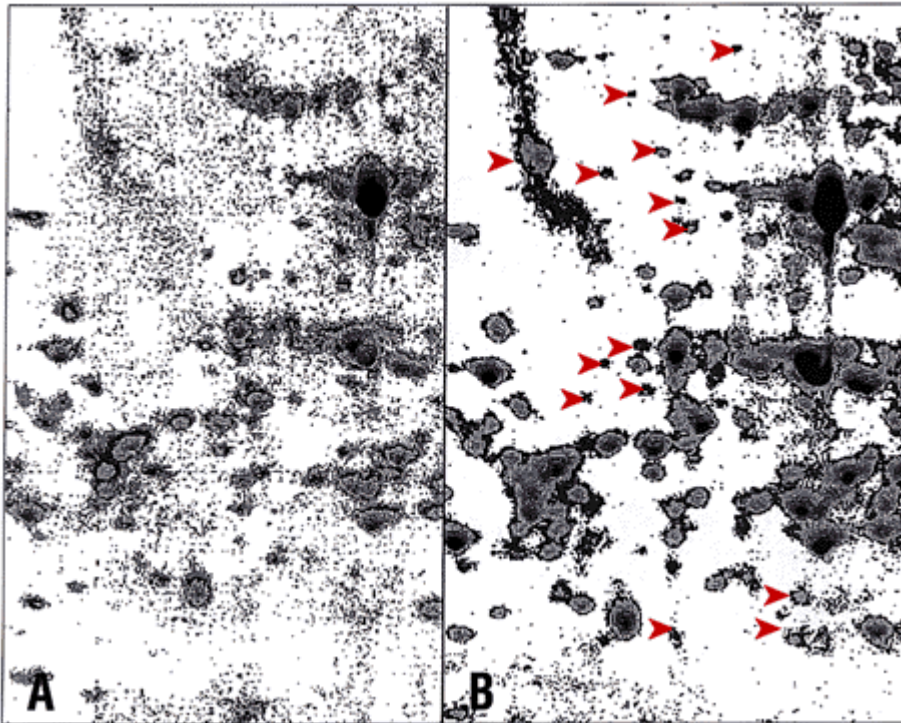


Figure 4. Comparison of laser-scanned, digitized images from the Coomassie blue (Panel A) and ChromaPhor Green (Panel B) stained 2-D gels shown in Figure 3. The gels were scanned with a Digital Instruments laser densitometer at 200 micron resolution. The images were transferred to an IBM compatible 386 computer for analysis with QGEL software (Kendrick Laboratories). Many more polypeptide spots are resolved on the image from the ChromaPhor Green stained gel (see arrows) than on the image from the Coomassie blue stained gel.

References:

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Ordering Information:

Product	Size	Cat.#
ChromaPhor TM		

Protein Visualization System	20 minigels	V7430
	40 minigels	V7420
ChromaPhor™ Protein Visualization System with Acrylamide	20 minigels	V7260
	40 minigels	V7270

The ChromaPhor Protein Visualization Systems contain all the reagents required to stain either 20 or 40 minigels (7 x 10cm).

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