Focal Points

Application Note FP-154

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Ultra Sensitive Protein Detection and Imaging with Lumitein™ Protein Gel Stain and GelDoc-It® Imaging System

Introduction

Imaging gels are an essential element in life science research. In particular, highly sensitive, user-friendly reagents are the building blocks of modern protein and DNA analysis. Biotium, Inc. has developed a unique fluorescent protein gel stain that is highly sensitive and simple to use. Unlike other fluorescent protein gel stains that require separate fixation, staining, and destaining steps, Lumitein™ stain combines fixation and staining in a single step with no or minimal destaining **(Table 1).**

Protocol Step	SYPRO® Ruby	Lumitein™
Fixation step 1	15 min, 50% MeOH + 7.5% acetic acid	None
Fixation step 2	15 min, 50% MeOH + 7.5% acetic acid	None
Staining	Overnight	90 min
Destaining	30 min, 10% MeOH + 7 % acetic acid	5 min, 30% MeOH + 15% acetic acid (or no destaining)
Rinse step 1	5 min water	Single 5 min water rinse (or 20 min water rinse without destaining)
Rinse step 2	5 min water	None

Table 1. Comparison of SYPRO® Ruby and Lumitein™ standard protocols.

In addition, because of its unique spectral properties, Lumitein can be used with a UV transilluminator, eliminating the need for an additional, more expensive LED light table or laser scanner. Laboratories that have UV imaging systems traditionally designed for viewing nucleic acids stained with ethidium bromide (EtBr), can easily use the same instruments for protein gel staining with Lumitein.

In this article, we demonstrate how Lumitein and the GelDoc-It® Imaging System (UVP, LLC) enable scientists to achieve highly sensitive imaging results.

GelDoc-It Imaging System

Materials and Methods

The following protocol is optimized for standard 1 mm thick, 8 cm x 10 cm SDS-PAGE minigels. Two 12% Precise™ protein gels (Thermo-Fisher) were loaded with 10 uL of 2 fold-dilutions of unstained standard in Laemmli SDS sample buffer, and electrophoresed in HEPES running buffer at 100 V in a mini gel electrophoresis system until the dye front reached the bottom of the gel. After electrophoresis, the gels were directly placed in separate clean plastic containers and incubated either with approximately 80 mL of Lumitein 1X staining solution or 100 mL of Coomassie Blue solution.

The gels were agitated on a rocking platform for approximately 90 minutes. The Lumitein stained gel was destained briefly in distilled water before visualization on the GelDoc-It system with a FirstLight® UV transilluminator (UVP, LLC) and EtBr filter. The Coomassie Blue gel was destained overnight with several changes of destaining solution (30% methanol). The gel was visualized using the high uniformity white light converter plate placed on top of the FirstLight UV illuminator. The Lumitein gel image was pseudocolored red while the Coomassie Blue gel image was pseudocolored blue using the VisionWorks®LS software (UVP, LLC).

Results

Lumitein has a UV excitation maximum at around ~280 nm and a broad visible excitation maximum centered around ~450 nm **(Figure 1).**

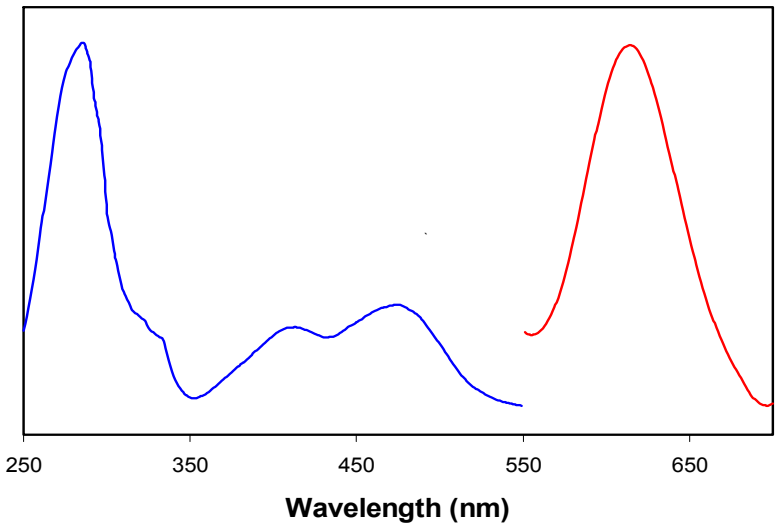


Figure 1. Excitation and emission spectra of Lumitein™ stain. Lumitein is compatible with excitation by 302 nm UV light, blue LED, or a UVP Biolite™ Light Source from 430 to 530 nm. The emission at 610 makes EtBr filters suitable for documentation.

It emits bright red fluorescence at around ~610 nm. As a result, gels stained with Lumitein can be viewed using a standard UV transilluminator with an EtBr emission filter or a Visi-Blue™ transilluminator (UVP, LLC). With the GelDoc-It system, FirstLight UV transilluminator and EtBr emission filter, visualization of the protein standard can be seen down to the last dilution **(Figure 2A).** In comparison, the Coomassie Blue stained gel is unable to detect any bands beyond the fifth lane, and the background color is high despite overnight destaining **(Figure 2B).**

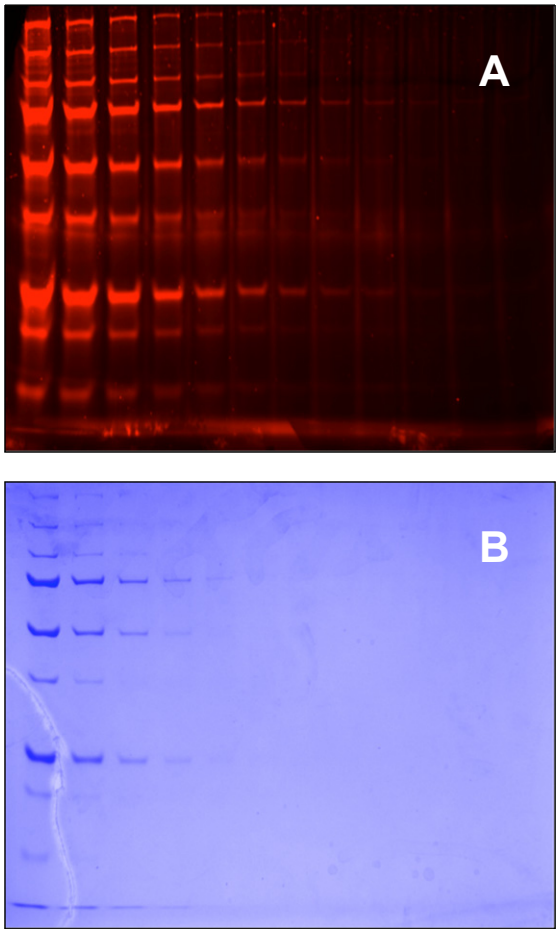
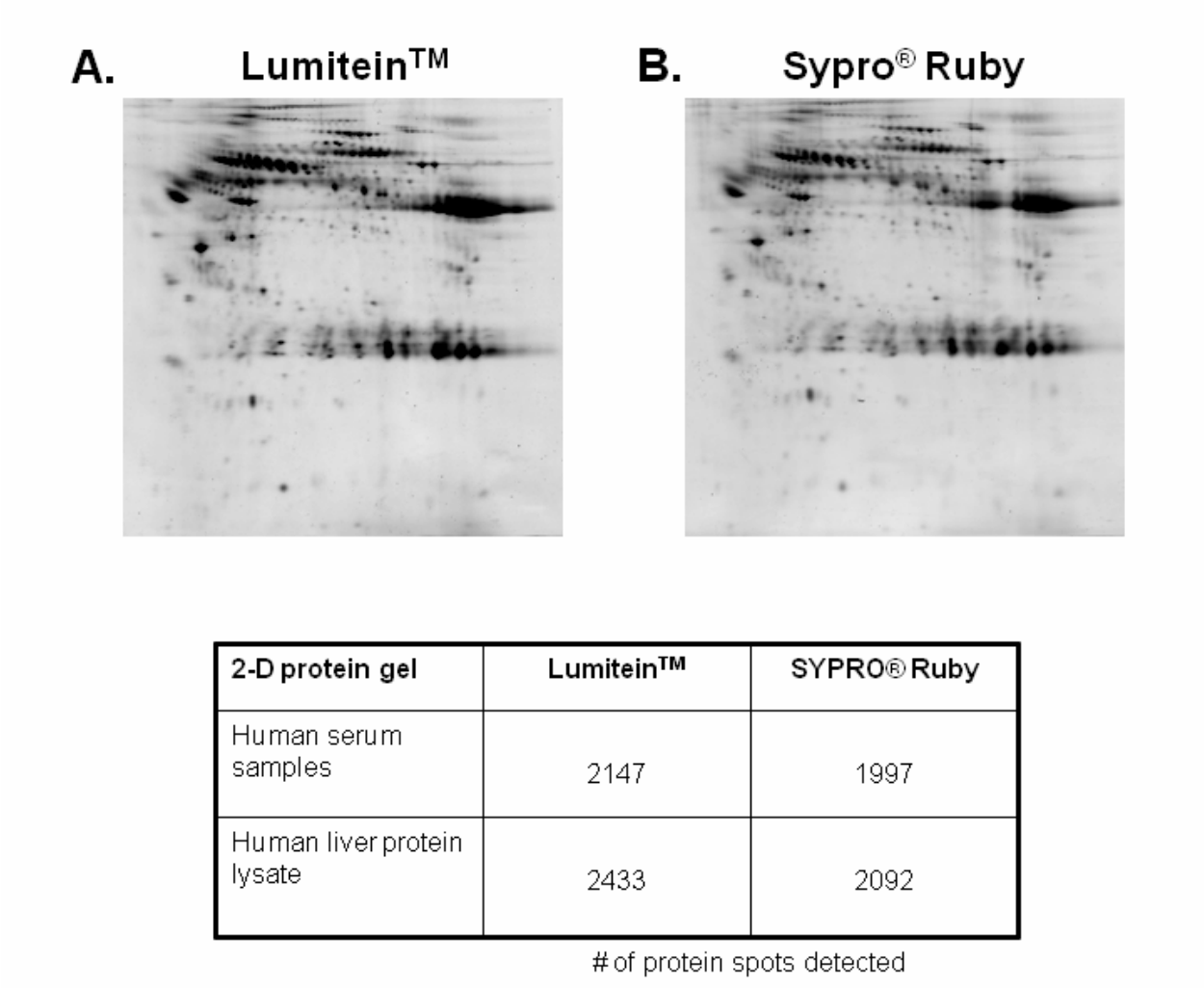


Figure 2. Need A and B. Lumitein™ stained protein gel.

(A) Unstained protein marker was loaded onto an acrylamide gel in two-fold dilutions. The gel was stained in 1X Lumitein™ for 90 minutes and destained briefly in distilled water. Image of the gel was captured using the GelDoc-It system equipped with a FirstLight UV transilluminator, GelCam 310 CCD camera, and EtBr filter.

(B) Coomassie Blue stained protein gel. Unstained protein marker was loaded onto an acrylamide gel in two-fold dilutions. The gel was stained in Coomassie Blue for 90 minutes and destained overnight. Image of the gel was captured using the GelDoc-It system with a white light converter on top of the FirstLight UV transilluminator, GelCam 310 CCD camera, and Vision Works LS software. The images were pseudocolored red and blue respectively using the VisionWorks LS Software.

An independent company performed side-by-side analysis of duplicate two-dimensional gels of human protein samples stained with Lumitein and SYPRO® Ruby (Invitrogen Corporation, **Figure 3**). Analysis of the stained gels revealed that on average, 10% more protein spots could be detected with Lumitein. (Images and analysis courtesy of Applied Biomics, Hayward, CA.)



2-D protein gel	Lumitein™	SYPRO® Ruby
Human serum samples	2147	1997
Human liver protein lysate	2433	2092

# of protein spots detected

Figure 3

Discussion

For ultimate sensitivity, it is best to incubate gels with 1X Lumitein for at least 90 minutes; however, a rapid staining protocol can also be performed in 30 minutes. For larger gels, it is best to scale-up the volume of staining solution accordingly using the 8 cm x 10 cm minigel as a reference (i.e., V (mL) = 80 mL x (S/64), where S is the size of the gel in cm^2). For large two-dimensional gels, use of a staining time longer than 90 minutes may yield a more sensitive detection of protein spots.

To destain gels, add 100 mL of destaining solution (30% methanol, 15% acetic acid) and agitate on a shaker for 5 minutes. Decant the destaining solution, add at least 100 mL deionized water and agitate for at least another 5 minutes before viewing/imaging. Alternatively, destaining and rinsing may be carried out in a single step by placing the stained gel in at least 100 mL deionized water on a shaker for 20 minutes. This single-step destaining/rinsing in water may produce slightly higher background fluorescence. However, considering that it avoids organic solvents, it is a good alternative to the organic solvent-based destaining method for most applications.

Lumitein protein gel stain is a fluorescent dye designed for detecting proteins in polyacrylamide gels that is superior to Coomassie staining and rivals that of other fluorescent protein gel stains such as SYPRO Ruby. Lumitein is the only protein gel stain that combines superior sensitivity, staining speed, ease of use and compatibility. Lumitein is among the simplest protein gel stains by staining protein in gels in 90 minutes or less time without a separate fixation step (see Table 1). It can detect 1 ng or less protein and permits quantitative detection of protein bands/spots with a linear detection range spanning at least three orders of magnitude (for more detailed information, please download the Lumitein flyer from the Biotium website: www.biotium.com). Note, however, that the data must be plotted in logarithm form [i.e. Log(Luminescence Intensity) vs. Log(Protein Amount)] for the best linear fit. Moreover, protein gel staining with Lumitein is compatible with downstream protein analyses such as mass spectrometry and Edman peptide sequencing. Finally, the exceptional photostability of Lumitein allows long exposure times for maximal sensitivity.

Innovative excitation sources such as the FirstLight Illuminator and imaging systems such as the GelDoc-It allow highly sensitive imaging documentation. The FirstLight illuminator offers a unique patented design emitting 302 nm ultraviolet excitation and combines a specially designed, high density grid array ultraviolet lighting configuration with a phosphor coating to generate exceptionally uniform ultraviolet illumination. It produces less than 5% coefficient of variance (CV) across the full imaging surface which is essential for capturing high quality images for documentation and quantitative analysis. The design assures consistent sensitivity and dynamic range for achieving accurate and reproducible gel analysis no matter where the gel is placed on the surface. Because of its unique spectral properties, Lumitein can be used with a UV transilluminator (Figure 1), eliminating the need for an additional, more expensive LED blue light transilluminator or laser scanner. Therefore, laboratories that have UV imaging systems such as the GelDoc-It can easily use the same instruments for protein gel staining with Lumitein.

Conclusion

Scientists can shorten the time it takes to analyze proteins and achieve more sensitive images using the Lumitein protein gel stain and the GelDoc-It imaging system.