Focal Points
Application Note FP-176

Multiplex Fluorescent Western Blot Detection Using the BioSpectrum Imaging System

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Introduction

Western blotting is a commonly used analytical technique for the identification and quantification of specific proteins in a biological sample. Traditionally, a target protein is interrogated by antigen-specific antibodies which are then probed by secondary antibodies conjugated to either HRP or ALP and followed by colorimetric or chemiluminescent detection.

Fluorescent western blotting employs secondary antibodies labeled with a fluorophore to perform direct, non-enzymatic detection of protein expression. On an immunoblot which is incubated with two different primary antibodies from different species and then probed with CyDye™-tagged secondary antibodies for detection (Figure 1), the two-color multiplexing abilities of the BioSpectrum® Imaging System with the BioLite™ Xe MultiSpectral Light Source allow for detection of multiple proteins.

Additional benefits of fluorescent blotting include excellent signal stability over time as well as accurate quantitative analysis with broader dynamic range and high linearity, reducing or eliminating the need to strip and re-probe.

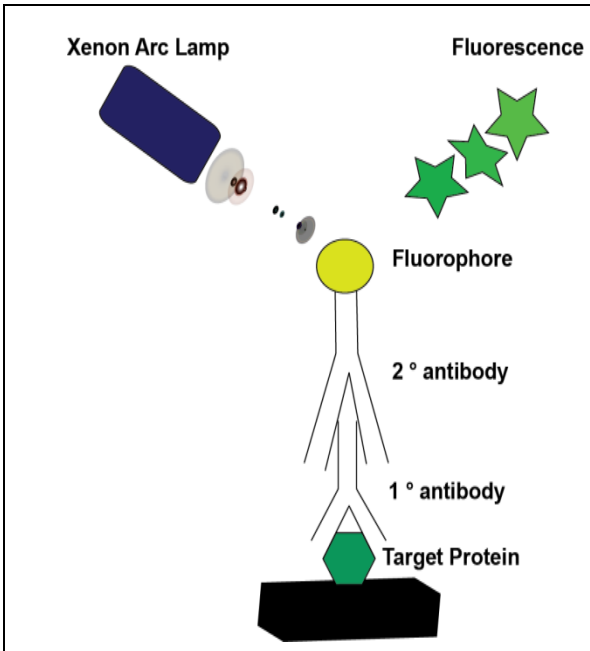

Figure 1. Schematic of Fluorescent Western Blotting

Materials and Methods

Sample Preparation and Western Blotting

Two-fold dilutions of normal rabbit and mouse serums (Jackson Immuno Research Laboratories) were separated by SDS PAGE on 4-12% acrylamide gels (Invitrogen). The separated proteins were then transferred to nitrocellulose membranes.[1]

Blots were simultaneously probed with goat-∝-rabbit IgG – Cy3® and goat-∝-mouse IgG – Cy5® (GE Healthcare Life Sciences) secondary antibodies at 1:1250 concentration in Western Breeze blocking buffer (Invitrogen) for one hour incubation at room temperature. Blots were washed in secondary antibody incubations with 4 x 5 minutes in tris-buffered saline (PBS) containing 0.1% Tween 20.

Imaging

The BioSpectrum Imaging System with the BioLite Xe MultiSpectral Light Source (UVP, LLC) was used for fluorescent imaging (Figure 3). Images were processed with VisionWorks®LS Image Acquisition and Analysis software (UVP, LLC) to remove background intensity and composite the pseudocolored images.

Briefly, the processed blot was positioned on the sample platen. Utilizing software presets, the excitation and emission wavelengths were selected, the lens aperture was set at f/1.2, and the image was focused. Exposure time was adjusted for maximal signal without saturation and ranged from 1 to 5 seconds, depending on the sample and filter set (Table 1).

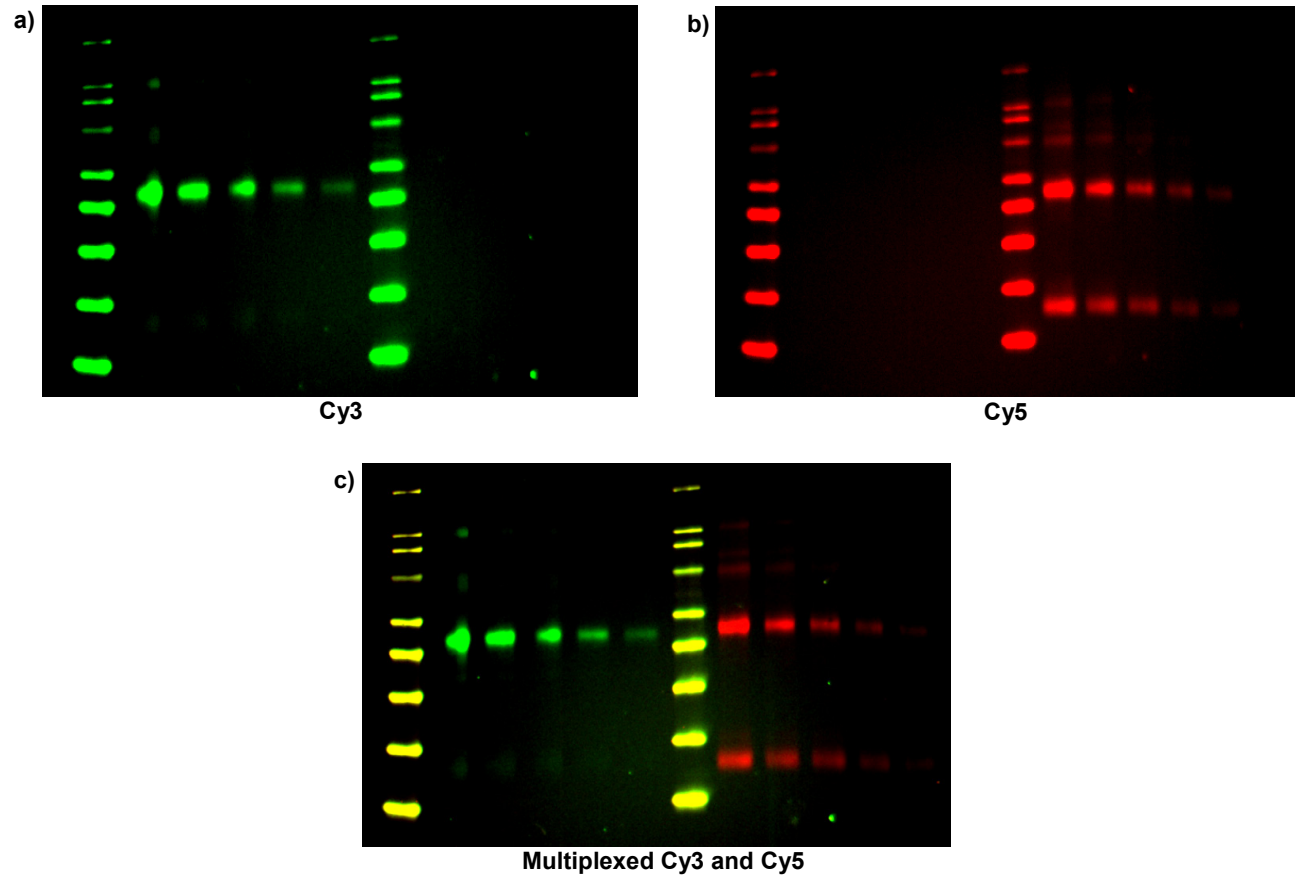

Figure 2. Multiplex Fluorescent Western Blot Detection with CyDyes™ using BioSpectrum® Imaging System

a) Rabbit IgG probed with Cy3–tagged goat anti-rabbit IgG **b)** Mouse IgG probed with Cy5-tagged goat anti-mouse IgG **c)** Multiplex fluorescent detection of two fold serial dilution of mouse and rabbit serum proteins probed with Cy3–tagged goat anti-rabbit IgG and Cy5-tagged goat anti-mouse IgG, respectively, on same immunoblot. CyDyes were imaged using a one second exposure with the BioSpectrum Imaging System.

Once acquired, the original unaltered image was archived and a copy was used for image analysis. The image was adjusted to globally remove background intensity and contrast, and was pseudocolored green and red using VisionWorksLS Software to indicate Cy3 and Cy5, respectively.

Dye	Excitation	Emission
Cy3	525BP45	605BP50
Cy5	630BP30	730BP40

Table 1. Filters used for NIR blotting with 680 and 770 to 800nm fluorescent tags.

Results and Discussion

Figure 2 illustrates the multiplex imaging capabilities of the BioSpectrum Imaging System, specifically separating out the signal of both Cy3 and Cy5 color channels.

Fluorescent western blotting applications offer many advantages over chemiluminescent or chromogenic visualization. Most significantly, fluorescent labels permit multiplexing so that several proteins in a sample can be detected and analyzed at the same time and on a single protein blot. Fluorescent labels in particular offer very low background and a high signal-to-noise ratio for quantitative imaging.

The combination of the BioSpectrum Imaging System and the BioLite MultiSpectral Light Source provides not only a full range of wavelengths for excitation light but also rapid high resolution image capture through the use of deeply cooled MegaCam 810 and OptiChemi™ 610 cameras and low light lenses.

Imaging Time

Compared to laser scanning imaging systems where imaging times range from 3 to 5 minutes, the BioSpectrum Imaging System provides a significant advantage with imaging times ranging from 1 to 5 seconds for fluorescent Western blotting applications.

Conclusion

Fluorescent Western blot imaging with the BioSpectrum Imaging System and BioLite MultiSpectral Light Source is a fast and efficient process for enabling researchers to achieve simultaneous detection of multiple proteins on the same immunoblot and generate high resolution, publication-ready images.

Figure 3. BioSpectrum Connected to the BioLite

Together, the BioSpectrum and BioLite are a powerful combination that are able to specifically excite and illuminate at wavelengths from 365 to 765nm and read emissions from 400 to 850nm. Use of up to eight excitation and five emission wavelengths are possible in a single experiment.

The BioSpectrum 810 and BioSpectrum 610 are recommended for imaging multiplex fluorescent Western blots.

References

1. Gallagher, S.R. and Wiley, E.A. Current Protocols: Essential Laboratory Techniques. Wiley, 2012