



Focal Points



Application Note FP-158

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Multiplex NIR Imaging of Protein Blots with the UVP BioSpectrum® System and BioLite™ MultiSpectral Source

Introduction

NIR imaging with the BioSpectrum Imaging System and BioLite MultiSpectral Light Source is fast, efficient, and straightforward.

With the extensive range of excitation and emission filters available, researchers can detect and quantify virtually any fluorescent dye, from visible to NIR. Protein blotting is a routine technique for determining the presence or absence of one or more proteins, and can provide additional information on quantity of protein (Gallagher and Wiley, 2008).

The process is straightforward (Figure 1), using SDS Page (Gallagher and Wiley, 2008) to first separate the proteins by size, followed by electrotransfer of the proteins onto nitrocellulose or PVDF membranes.

The membrane surface absorbs the protein, and the researcher probes the membrane with primary antibodies specific to the protein of interest. Enzyme or fluorescent dye tagged secondary antibodies are used to identify the primary antibody binding site. If only one protein species is being identified, then only one primary antibody and label is used. If multiple proteins are being identified on the same blot, then the analysis relies on probing each protein with a different primary antibody and secondary antibody with a different fluorescent tag, yielding a multiplexed result.



BioSpectrum Imaging System with BioLite MultiSpectral Light Source

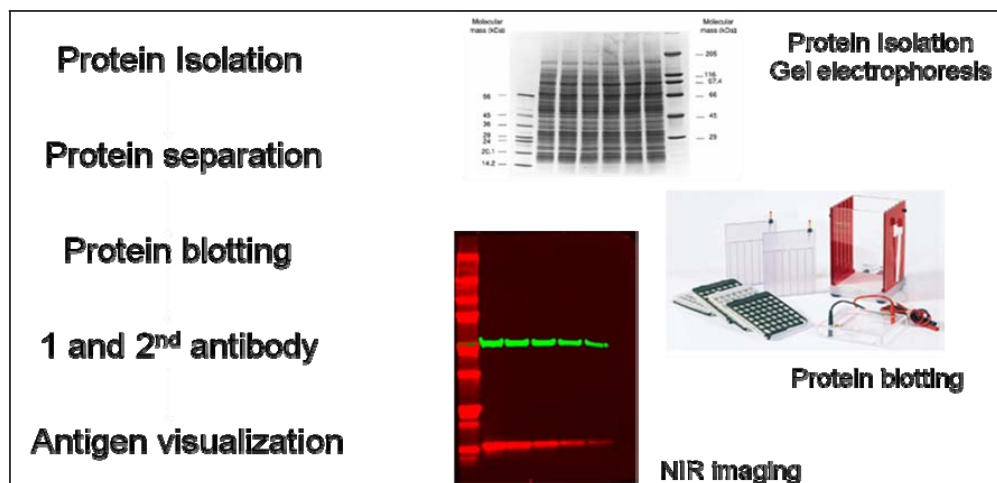


Figure 1. The immunoblotting process. Protein blotting is a mainstay in life science research laboratories, and starts with SDS PAGE separation followed by blotting and antibody probing and analysis.

For NIR blotting applications, two fluorescent dyes are typically, either alone or in combination used (Table 1).

Filter		Protein Tag Emission		Comments
Ex (nm) (29mm round, for BioLite)	Me (nm) (50mm square, for BioSpectrum)	680nm	770- 800nm	
720/40 38-0371-02	800LP 38-0368-01	X	X	Both bands visible
630/50 38-0359-02	720/40 38-0369-01	X		Only 680 band visible
765/30 38-0370-02	800LP 38-0368-01		X	Only 800 band visible

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

Detection of the primary antibody binding to the protein of interest is easily accomplished via a fluorescent label attached directly to the secondary antibody.

For fluorescent imaging, the membrane is illuminated with overhead monochromatic excitation light from the BioLite MultiSpectral Source, and the induced fluorescence at each of the tagged sites is recorded with a cooled CCD camera through an emission filter that selects for the fluorescent light while blocking the excitation light. For the results shown in Figure 2, an NIR blot was processed to identify two proteins in the sample.

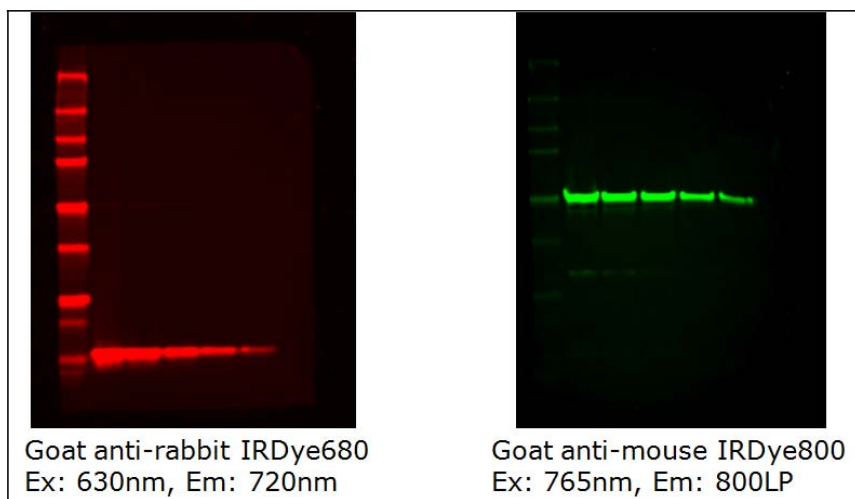


Figure 2. Multiplexed NIR blots of COX IV inhibitor (red) and tubulin (green) dilution series on the same immunoblot, imaging at in the NIR at emission wavelengths of 680 and 800 nm.

Materials and Methods

- BioSpectrum 600 System, with 3.2 mpx cooled (-60°C from ambient) camera and 5 emission filters
- BioLite MultiSpectral Light Source with 8 excitation filters
- NIR-specific excitation and emission filters (see Table 1)
- Reagents for NIR protein blotting and processing

Sample Preparation

Two-fold dilutions of HeLa cells were separated by SDS PAGE on 12% acrylamide gels. The separated proteins were transferred to nitrocellulose membranes according to Gallagher and Wiley (2008). Blots were probed with mouse anti- α -tubulin (Sigma) and rabbit anti-COX IV (Cell Signaling) primary antibodies in Licor blocking buffer containing 0.1% Tween-20 overnight at 4°C, followed by one hour incubation at room temperature goat anti-mouse IRDye800 and goat anti-rabbit IRDye680 (Licor) at 0.2ug/mL or 0.04 ug/mL final concentrations of secondary antibody in blocking buffer containing 0.1% Tween 20.

Blots were washed after the primary and secondary antibody incubations with 4 x 5 minutes in phosphate buffered saline (PBS) containing 0.1% Tween 20.

Imaging

The BioSpectrum system with the BioLite MultiSpectral Source was used for the NIR 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. The excitation and emission filters used are listed in Table 1.



Figure 3. BioSpectrum connected to the BioLite. Together, the BioSpectrum and BioLite are a powerful combination that, depending on the filters used, are able to specifically excite and illuminate at wavelengths from 365 to 765nm and read emissions from 400 to 850nm. UP to 8 excitation and 5 emission wavelengths are possible in a single experiment.

Briefly, the processed blot was positioned on the sample platen with the door open and camera preview running, to provide light for positioning and focus. Through software presets, the excitation and emission wavelengths were selected and the lens was set at f/1.2. Exposure, adjusted for maximal signal without saturation, ranged from 30 seconds to 2 minutes, depending on the sample and filter set.

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, colored red and green to indicate the IR680 and IR800, respectively, using VisionWorksLS Software (Figure 2).

Results and Discussion

Figure 3 illustrates the NIR multiplex imaging capabilities of the UVP BioSpectrum 600 system, clearly and specifically separating out the signal of both IRDye680 and 800 emission tags. NIR labels for protein blot applications offer many advantages over chemiluminescent or chromogenic visualization.

Most importantly, NIR and visible fluorescent labels permit multiplexing so that several proteins in a sample can be detected and analyzed at the same time on a single protein blot. NIR labels, in particular, offer very low background and high signal-to-noise ratio for quantitative imaging. Additional advantages include the high stability of the label on the processed and dried blot. Shelf stable for many months, the blot can be repeatedly imaged dry.

The combination of the BioLite MultiSpectral Light Source (UVP, LLC) and the BioSpectrum not only provides a full range of wavelengths for excitation light, but also rapid, high resolution image capture, through the use of cooled CCD 500 and 600 cameras and low light lenses. Typically, exposures are complete in 30 seconds to 2 minutes, much faster than laser scanning.

Conclusion

Routine NIR imaging with the BioSpectrum and BioLite MultiSpectral Source is fast, efficient, and straightforward, yielding full 16-bit images for quantification and publication.

References

1. Gallagher, S.R. and Wiley, E.A. Current Protocols: Essential Laboratory Techniques. Wiley, 2008

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