Focal Points

Application Note FP-159

UVP, LLC Upland, CA | (800) 452-6788 | (909) 946-3197 | info@uvp.com
Ultra-Violet Products Ltd. Cambridge UK | +44(0)1223-420022 | uvp@uvp.co.uk
Web Site: uvp.com

Digital Chemiluminescent Imaging of Protein Blots

No Film or Processing Chemicals for Green Imaging

Sean Gallagher, Ph.D.

Introduction

The BioSpectrum® Imaging System (UVP, LLC) is ideal for imaging chemiluminescent protein blots.

Protein blotting is a routine technique for determining the presence or absence of one or more proteins and can provide additional information on the antigen amount in the sample (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 protein is absorbed onto the membrane surface during transfer and probed 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.

BioSpectrum Imaging System

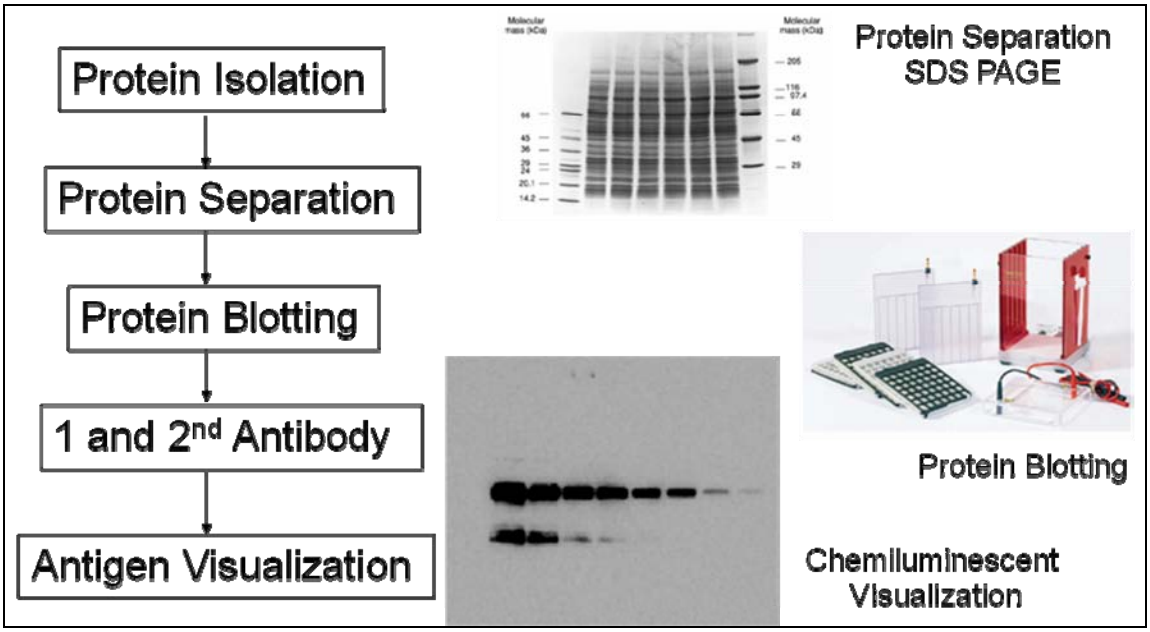

Figure 1. The immunoblotting process. Protein blotting is a mainstay in life science research laboratories, and starts with SDS PAGE separation followed by blotting and antibody probing and analysis.

Detection of the primary antibody binding to the protein of interest is accomplished via a secondary antibody that is tagged with an enzyme (either with peroxidase or alkaline phosphatase) or a fluorescent label. The enzyme reactions can be measured either with white light (via colored reaction precipitates on membrane), chemiluminescence, or fluorescence imaging.

For chemiluminescent imaging of protein blots, two enzymes-either peroxidase or alkaline phosphatase-are used as secondary antibody tags. The enzymes catalyze the chemiluminescent reactions in conjunction with luminescent substrates for those enzymes. There are several light emitting substrates available, with luminol based reagents most popular for peroxidase and dioxetane phosphates for alkaline phospahatase.

In this application, we describe the use of chemiluminescent imaging of protein blotting and the sensitivity of this procedure compared to film. The results show that the BioSpectrum with the cooled BioChemi 500 camera to be superior to film in sensitivity, accuracy, dynamic range, speed, and simplicity. Due to the high sensitivity and resolution of the BioSpectrum 500 system, the resultant image is both quantitative and publication quality.

Materials and Methods

- BioSpectrum 500 Imaging system
- Cooled (-60C from ambient) 4 megapixel camera
- Large area (no vignetting), low light f/1.2 lens (Figure 2)
- Glowell GTLS low light standard for film and CCD based sensitivity testing.
- Rabbit sera, peroxidase tagged secondary antibodies and FemtoMax™ visualization reagent (Rockland Immunologicals)

Figure 2. BioSpectrum System with Chemi Tray.

Sample Preparation

Rabbit sera was serial diluted in 1:1 in SDS sample buffer, reduced and separated on a mini SDS PAGE gel. Blots were prepared by transferring the separated proteins to a 10 x 10 cm nitrocellulose membrane using a tank protein transfer unit.

The resulting blots were processed and probed with peroxidase tagged goat anti-rabbit antibody (Gallagher and Wiley, 2008). After the final wash, the moist membrane was placed between two sheets of clear Mylar, and the visualization reagent (FemtoMax™, Rockland Immunology Inc.) was added to the membrane by lifting the top sheet back, pipetting the reagent in the center of the membrane, and then lowering the top sheet, avoiding the introduction of bubbles.

After addition of the reagent, due to rapid signal drop off, the blot should be imaged immediately if using peroxidase based reagents.

Imaging

The BioSpectrum 500 system with the f/1.2 lens was used for the chemiluminescent imaging (Figure 2). Images were processed with VisionWorks® LS image acquisition and analysis software (UVP, LLC) to remove background signal and quantitate the intensity of the bands. 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 use of software presets, the lens was set at f/1.2 throughout. Exposure, adjusted for maximal signal without saturation, ranged from 10 seconds to 2 minutes, depending on sample and binning. Once acquired, the original unaltered image was archived, and a copy used for image analysis.

With film, the membrane was sealed between plastic wrap immediately after visualization reagent addition and placed in film cassette and back closed for longer exposures, and pressed onto film while in the open cassette in a darkroom for precise short exposures. The exposures of the GTLS Glowell calibration standard were preformed in a darkroom, inverted onto the film and removed at timed intervals while in the open film cassette.

Once the GTLS exposure was complete, the visualization reagent was added to the membrane, wrapped in plastic and placed in film cassette for exposure. Film was processed with Kodak GBX developer and fixer at recommended dilutions.

Results

Immunoblotting reactions using chemiluminescent substrates are recorded with higher sensitivity using the Biospectrum 500 with cooled CCD cameras, as compared to using film (Figure 3).

This is confirmed using quantitative low light standards (Figure 4). The weak signals are poorly recorded or missed completely with film, while the BioSpectrum camera effectively records both weak and strong signals of both the blot and standard (Figures 3 and 4).

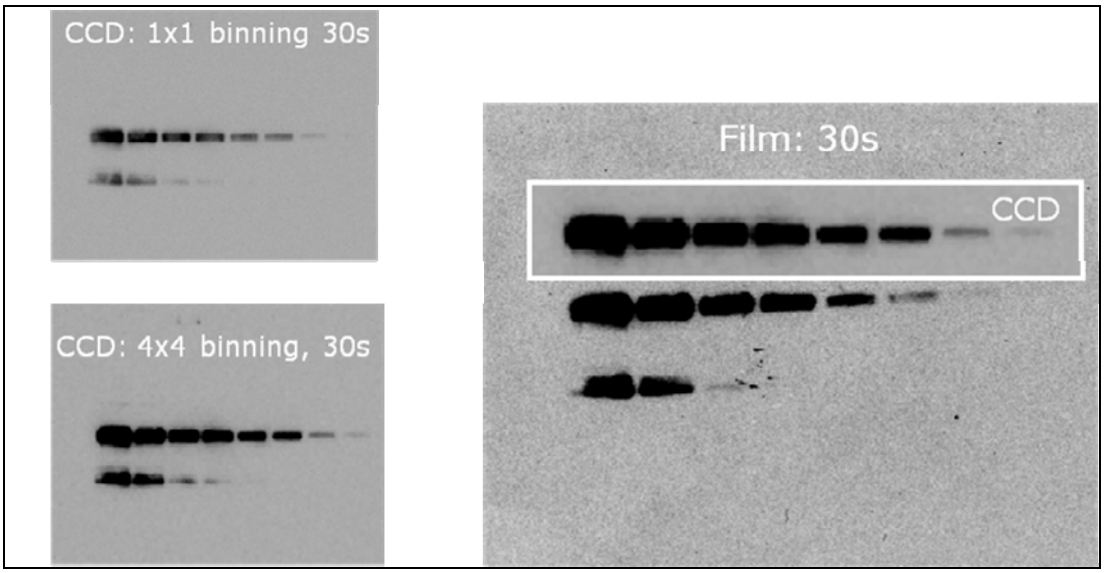

Figure 3. Chemiluminescent blots of rabbit sera dilution series comparing film to CCD capture with the BioSpectrum 500. The BioSpectrum 500 CCD result (inset) is more sensitive than film.

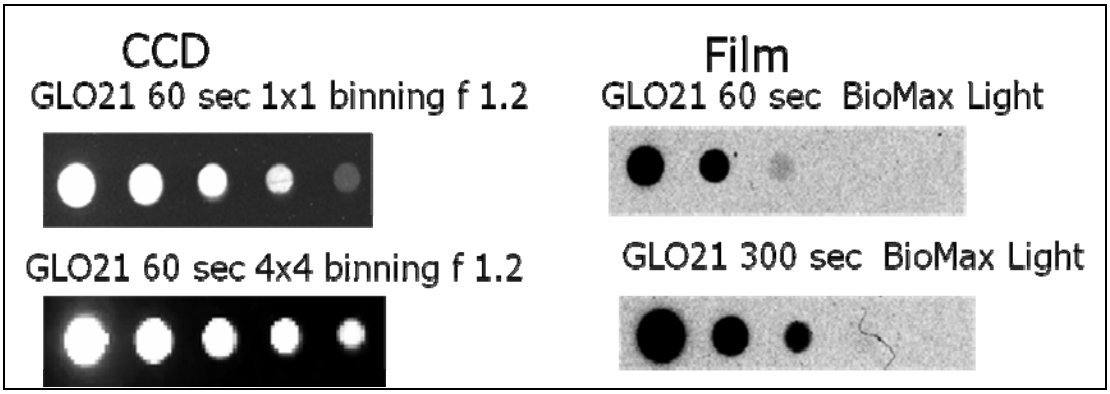

Figure 4. CCD and film exposure by a calibrated light source, GLO21, to compare sensitivity. A five-step GTLS calibrated light source (GLO21) was used to compare sensitivity between the BioSpectrum 500 system and typical chemiluminescence optimized film. The GTLS standard covers a 600 fold range from most intense (left) to least intense. The film cannot record the weak signals from the standard, while the CCD can quantitate all five intensities in 60 seconds or less.

Discussion

Chemiluminescent imaging of protein blots using the BioSpectrum 500 offers many advantages. CCD imaging greatly speeds and simplifies analysis, has a much higher dynamic range and is more sensitive to weak signals while accurately recording brighter signals without saturation.

Because the imaging cabinet acts as a benchtop darkroom, there is no need for a full traditional darkroom and all steps take place in room light in the laboratory. Once positioned on the imaging platform, the membrane is focused, door closed and image captured with a one button preset. Multiple captures are easily taken to get a full range of exposure, and the acquired image is available for immediate and accurate quantitation. The exposure typically takes from 10s to a few minutes.

In contrast, film requires a full darkroom with running water and has many more steps: while in the full darkroom, the film needs to be removed from the light tight package and placed in film cassette. The membrane, in plastic wrap, is then placed on top of the film and gentle pressure applied, usually by closing the film cassette, to ensure good contact (and sharp resolution) with the film. Any movement of the luminescent blot while situating on the film will result in spurious contact exposure creating a double image on the film.

Once exposed the film is removed and processed in the dark with developer and fixer and finally washed. Because the chemiluminescent signal rapidly drops with most peroxidase (luminol) based reagents, it is difficult to go back and reexpose the film without first processing the blot again by washing and adding fresh reagent.

Even then, the signal is less in the second round of processing. Once processed, the film must be scanned to quantitate the bands on the blot. Due to the very limited dynamic range of film, film is both easily saturated by bright signals while at the same time will not record the weak signals, giving poor data accuracy (figures 3 and 4).

Conclusion

Routine chemiluminescent imaging with the BioSpectrum 500 is fast and straightforward, yielding full 16 bit images for quantitation and publication. Chemiluminescent blots offer very high sensitivity, rivaling radiolabeled techniques, and can be easily reprobed without worrying about radioactivity disposal. Rapid, high-resolution image capture is possible through the use of cooled CCD 500 and 600 cameras and f/1.2 low light lenses. Unlike fluorescent blotting applications, no excitation light is needed. Typical chemiluminescent exposures for protein blots are from 10 seconds to 5 minutes. Northern and Southern blots generally require exposures ranging from 5 to 30 minutes.

References

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

7/9/10