



High Sensitivity Chemiluminescent 1D & 2D Immunoblotting (Westerns) Imaging

How To Tips for BioImaging Applications

Quantitative Immunoblotting: High Sensitivity with Day-to-Day Repeatability

Overview

Immunoblotting of SDS PAGE separated proteins is an ideal way to identify the presence and quantity of a specific protein in a complex mixture via antibody probes (Gallagher et al 2004). Briefly, proteins are solubilized in SDS sample buffer and separated by size through use of electrophoresis. Before the proteins can be exposed to immunoblotting reagents, the proteins must first be transferred out of the gel and attached to a membrane to make them accessible to detection reagents. This is accomplished via a second round of electrophoresis, perpendicular to the first, which moves the proteins onto a nitrocellulose or PVDF membrane. This forms an exact replica of the original gel, but with the protein attached to the reagent accessible surface of the membrane.



Fig. 1. Chemiluminescent blot with protein standards overlaid in the VisionWorksLS software.

Immunoblotting and subsequent development and visualization of the protein on the blot membrane are used to identify and quantitate specific proteins during purification or cell fractionation. In addition, prestained molecular weight standards are available, simplifying the identification, quantitation and sizing of the protein of interest (eg. Fig. 1).

Protocol

Typical chemiluminescent imaging protocol (Gallagher et al 2004):

1. Process membrane for immunodetection according to manufacturer's instructions. Typically either light emitting Luminol (HRP) or Dioxetane phosphate (AlkPhos) based reagents are used.
2. The membrane can be placed in a thin plastic bag or placed on a single sheet of plastic wrap; the visualization reagents are typically pooled in the small amount (1-2 ml) the plastic prior to adding the membrane. The membrane is gently laid on the plastic while evenly dispersing the reagents.



Fig. 2. UVP Heater: Temperature Stability for Western blots development.

3. Both chemiluminescent and chromogenic reactions are very temperature sensitive, and if possible the membrane should be placed on a temperature controlled surface (e.g. 30°C). The UVP Heater (Fig. 2) is ideal for immunoblot imaging and ensuring day-to-day consistency.



Fig. 3. BioSpectrum Imaging System

Chemiluminescent reaction will fade with time, typically over minutes, but the blot luminescence will still be visible after several hours of reaction, as long as the blot does not dry out. Once the exposure is complete, the membrane can be briefly rinsed in blocking solution and processed with chromogenic reagents such as DAB/NiCl₂ or TMP to visualize the bands by eye, simplifying molecular weight calculations and comparison to the more sensitive chemiluminescent image.

Conclusion

UVP's low light BioSpectrum Imaging Systems, in combination with Vision Works®LS Acquisition and Analysis Software, are ideal for day to day repeatable, chemiluminescent imaging.

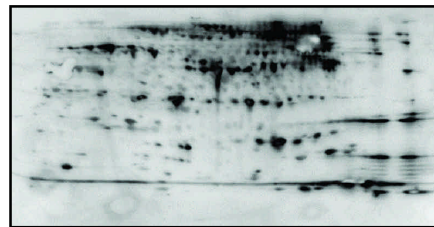


Fig. 5. Whole bacterial 2D gel. The primary antibody used was rabbit-anti-Escherichia coli antibody. The secondary antibody used was goat-anti-rabbit-HRP conjugate a total of 16.2 micrograms of E coli protein was separated by 2D SDS PAGE and immunoblotted. Image provided by Drs. Deb and Bulbul Chakravarti, Proteomics Center, Keck Graduate Institute of Applied Life Sciences

4. The membrane is sealed, quickly placed in the BioSpectrum® dark-room (Fig. 3), and the lens aperture set to 1.2 with typical chemiluminescent image capture settings as shown in Fig 4. A typical result is shown in Fig. 5.

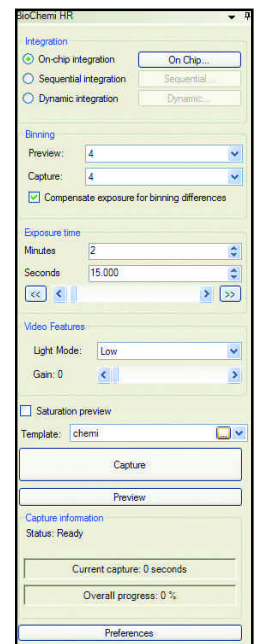
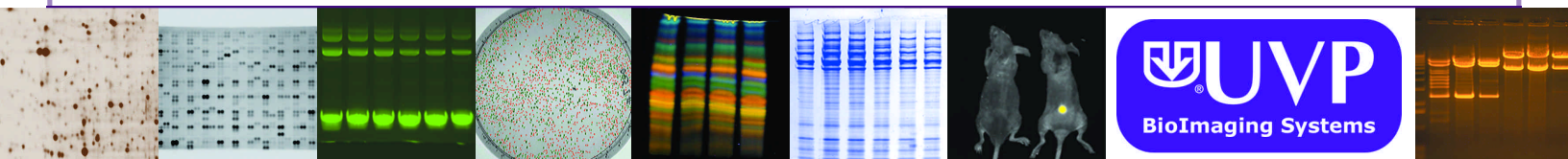


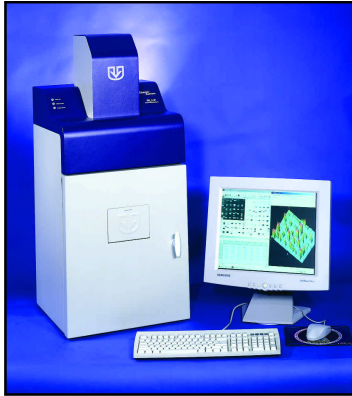
Fig. 4. Typical Chemiluminescent Capture Settings in the VisionWorksLS Software.

Reference: Gallagher, S.R., S.E. Winston, S.A. Fuller and J.G.R. Hurrell. 2004. Immunoblotting and immunodetection. In F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl (Eds.), Current Protocols in Molecular Biology. John Wiley & Sons, New York.



BioSpectrum Imaging System for Chemiluminescent Imaging

BioSpectrum Imaging System offers a multifunctional, automated design for a full spectrum of imaging applications.

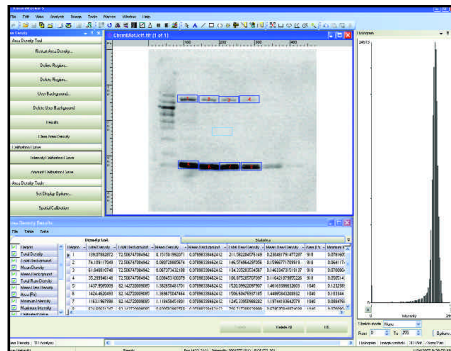


BioSpectrum 500 Imaging System connects to a computer installed with **VisionWorksLS Analysis Software**. Imaging applications include chemiluminescence, fluorescence and colorimetric. For plant/live animal studies, ask about the **BioSpectrum 600** which includes the **OptiChem HR Camera** for deeper cooling for higher sensitivity and extended integration times.

VisionWorksLS Acquisition and Analysis Software

Advanced, yet easy to use software enables researchers to quickly capture, analyze and generate reports

- Control the darkroom with a quick-link software interface
- Choose from an array of dynamic plug-ins to enhance software functionality
- Acquire images easily from a variety of capture and applications templates
- Enhance images with brightness/contrast, annotation, text, background correction options and more
- Create Digital Video Playback files of images
- Analyze images with 1D Analysis, Area Density and Colony Counting
- Write macros to simplify routine procedures
- Generate customizable reports



BioSpectrum 500 System Specifications

BioSpectrum Darkroom	Light tight with wide-access door Roll-out transilluminator tray Transilluminator timer Gel view window Epi UV, Visi-Blue and white light Drop-down white light plate Vertically adjustable chemi tray
Ultraviolet Transilluminator*	Select from FI-26X 302nm UV, 25x26cm FirstLight LM-26E, 254/302nm UV, 21x26cm LMS-26E, 254/302/365nm UV, 21x26cm Other models available
BioChem HR Camera	4 megapixel resolution/2048 x 2048 12-bit/16-bit digitized Cooled (regulated -28°C cooling) Quantum efficiency 55% at peak 500nm, 400nm 45% USB 2.0 Connectivity
Lens	Motorized 12.5 - 75mm f/1.2
Emission Filters	Ethidium Bromide, SYBR Gold/CY3 SYBR Green; additional filters available
Image Acquisition and Analysis*	VisionWorkLS Software provides comprehensive acquisition, analysis and documentation functionality
Optional Accessories	
Computer	Current high specification computer components available
Thermal Printer	Black and white digital thermal printer with 256 gray scale, archive quality prints
Converter Plates	UV to White Light: 25x26cm Visi-Blue™: 302nm to 460nm, 25x26cm UV to UV: 302 to 365nm UV, 25x26cm
Filters	Custom filters available
BioLite™ Illuminator	Filter selectable visible light transillumination (Custom filters available)
BioLite Multi-spectral Source	Directed visible light illumination of laboratory samples such as plants and animals
Gel Tools	Glowell Fluorescent Standards, Fluorescent Calibration Step Tablet, Gel Sentry, Gel Trays, Gel Cutter, Gel Ruler

* System components may require purchase separately in some areas. Contact your UVP dealer for details.

For a demonstration, quote or needs analysis, contact a UVP Biolmaging Specialist or Authorized Dealer.



UVP
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