Problem

Low-cost, High-resolution Colorimetric Western Blot Imaging

Solution

Imaging with the Analytik Jena UVP GelStudio 12MP Imager

Technique Creep – When Colorimetric Blots *Are* Enough

Introduction

Western blotting in the past decade has seen several developments, with a rise in attomolar-level detection using chemiluminescent substrates, fluorescent detection both in the visible and NIR wavelengths, improvements in quantitation using total protein stains, and even new modalities integrating capillary electrophoresis. There is no doubt, these exciting developments will open new doors for researchers, but importantly, it does not close the door to existing technologies. In fact, ongoing developments in this space only reflect the importance of Western blotting as a tool in the molecular researchers' toolbelt. And so, as there are many types of nails for the hammer, there are several chemistries for a Western blot. And colorimetric Western blotting remains a useful strategy that minimizes cost, while eliminating the need for highly-specialized equipment.

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There are several chemistries for colorimetric detection with varying ranges of sensitivity. In Table 1, we highlight some of the most common reagents used. While these chemistries have orders of magnitude less sensitivity and lower dynamic range than chemiluminescent or fluorescent Western blots, they are useful under certain circumstances, especially where source tissue is not a limiting factor. For example, a researcher may regularly express recombinant protein in bacteria, and Western

Table 1: Example Reagents for Colorimetric Detection (Source: Manufacturer website and technical documentation)

Enzyme Conjugated to 2° Ab	Substrate (catalog #)	Manufacturer	Catolog #	Color	Limit of Detection
AP	1-Step NBT/BCIP	ThermoScientific	34042	Black-purple	30 pg
AP	Immun-Blot Assay Kit (NBT/BCIP)	Bio-Rad	1706460	Black-purple	100 pg
HRP	Amplified Opti-4CN Substrate Kit	Bio-Rad	1708238	Blue-purple	5 pg
HRP	Metal Enhanced DAB Substrate Kit	ThermoScientific	34065	Brown / Black	17 pg
HRP	1-Step Ultra TMB Blotting	ThermoScientific	37574	Dark blue	20 pg
HRP	CN/DAB Substrate Kit	ThermoScientific	34000	Black	500 pg
HRP	DAB Substrate Kit	ThermoScientific	34002	Brown	1 ng
HRP	1-Step TMB-Blotting Solution	ThermoScientific	34018	Dark blue	1 ng
HRP	1-Step Chloronaphthol	ThermoScientific	34012	Blue-purple	5 ng

blotting is part of their QC pipeline to determine purity and quantity. In this pipeline, the user has the ability to readily augment the amount of protein loaded onto a gel and subsequently transferred to a membrane, thus helping increase the target antigen concentration above the limit of detection (LoD) of a colorimetric approach. Below we demonstrate colorimetric Western blotting using the UVP GelStudio Plus 12MP imager, an excellent entry-level gel and colorimetric blot instrument.

Methods

Source Material, SDS-PAGE, and Chromogenic Substrates

Myeloma protein IgG2a, was serially diluted from 1 mg/mL to 0.0004 mg/mL, boiled in NuPAGE 4x LDS Sample Buffer and

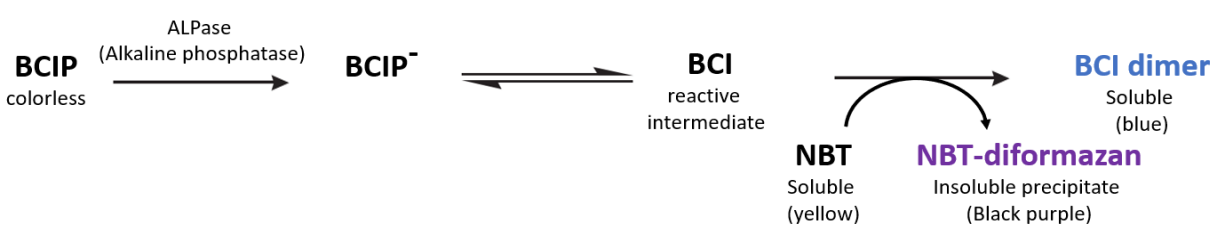

Figure 1: Colorimetric detection using nitroblue tetrazolium dye (NBT), 5-bromo-4-choloro-3-indoyl-phosphate (BCIP), and the enzyme Alkaline phosphatase (ALPase). After transferring proteins to a membrane, Western blotting proceeds with blocking, staining with primary antibody, and staining with the secondary antibody per usual. With colorimetric blots, the enzyme conjugated to the secondary antibody is reactive toward chromogenic substrates. As outlined above, NBT/BCIP chromogenic substrates, rely on ALPase being conjugated to the secondary antibody. After ALPase acts on BCIP, a highly reactive intermediate forms a stable dimer through tautomerization, which is soluble and blue in color. Two hydrogen ions released during dimerization, reduce the soluble yellow substrate NBT, forming a black-purple insoluble precipitate known as NBT-formazan. The black-purple precipitate is what is imaged by the researcher for protein detection/ quantitation.

10x Reducing Agent, are loaded on a mini NuPAGE Bis-Tris gel from ThermoFisher (Waltham, MA). After electrophoresis, the proteins were transferred to a PVDF membrane using an iBlot™ 2 from ThermoFisher. After transfer, we proceeded with colorimetric detection using alkaline phosphatase (ALPase), 5-bromo-4-chloro-3-indoyl-phosphate (BCIP), and nitroblue tetrazolium (NBT) (Figure 1), the principle components of the Immun-Blot™ Assay kit from Bio-Rad (Hercules, CA). Briefly, ALPase hydrolyzes BCIP, forming a reactive intermediate, which self- dimerizes. During dimerization, hydrogen ions are released which reduce NBT and form a visible black-purple precipitate on the membrane where the target antigen is located. Color development was stopped after 40 minutes followed by a 10-minute wash step.

Colorimetric Imaging with the UVP GelStudio 12MP

After drying, the membrane was placed directly on the imagining surface. Analytik Jena's VisionWorks 9.1 software was used for image capture and analysis. Both autoexposure and autofocusing were used. For quantitation, we used the area density tool in VisionWorks 9.1 and the values reported in Figure 2 are background subtracted values, converted to mass quantities.

Results and Conclusion

Using the UVP GelStudio 12MP imager, we were able to capture a high-resolution blot image in as little as 50 milliseconds (Figure 2). Importantly, the UVP GelStudio 12MP can detect signals near the reported limit of detection (LoD) of the Immun-Blot kit (inset, Figure 2) at ~350 pg without a prolonged color development procedure as recommended by the manufacturer. Indeed, extending the color development step to 4 hours per the manufacturer's recommendation, would likely produce more precipitate making the bands nearer the LoD more visible. In addition, users could opt for manual exposure settings in our software to optimize near-LoD detection.

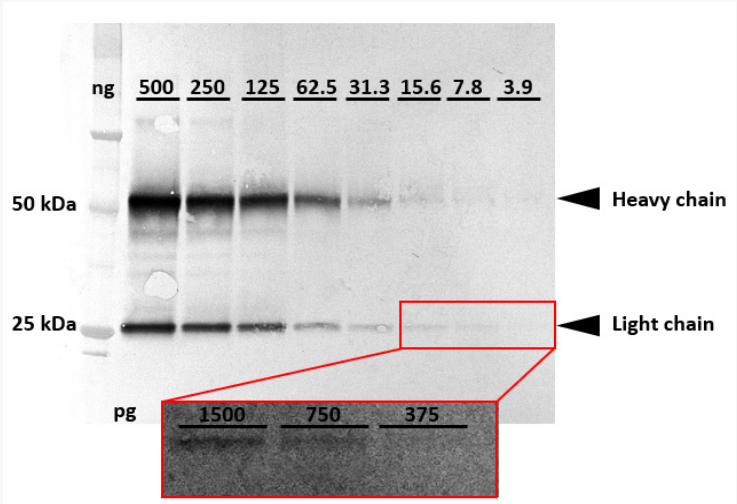

Figure 2: Colorimetric Imaging with the Analytik Jena UVP GelStudio 12 MP Imager. Purified IgG2a was serially diluted down to 3.9 ng for total protein and separated by SDS-PAGE. Proteins were transferred to a PVDF membrane and Western blotting was performed using the Immun-Blot™ assay kit from Bio-Rad per the manufacturer's instructions. The inset image contrast and brightness are adjusted to increase resolution of the bands present nearer the limit of detection of the kit.

For many laboratories, colorimetric blotting has a sufficient detection limit making the UVP GelStudio 12MP an excellent choice. If more sensitive technologies are needed, do not hesitate to contact our Applications Team to find out more about our Advanced Imager lineup.

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