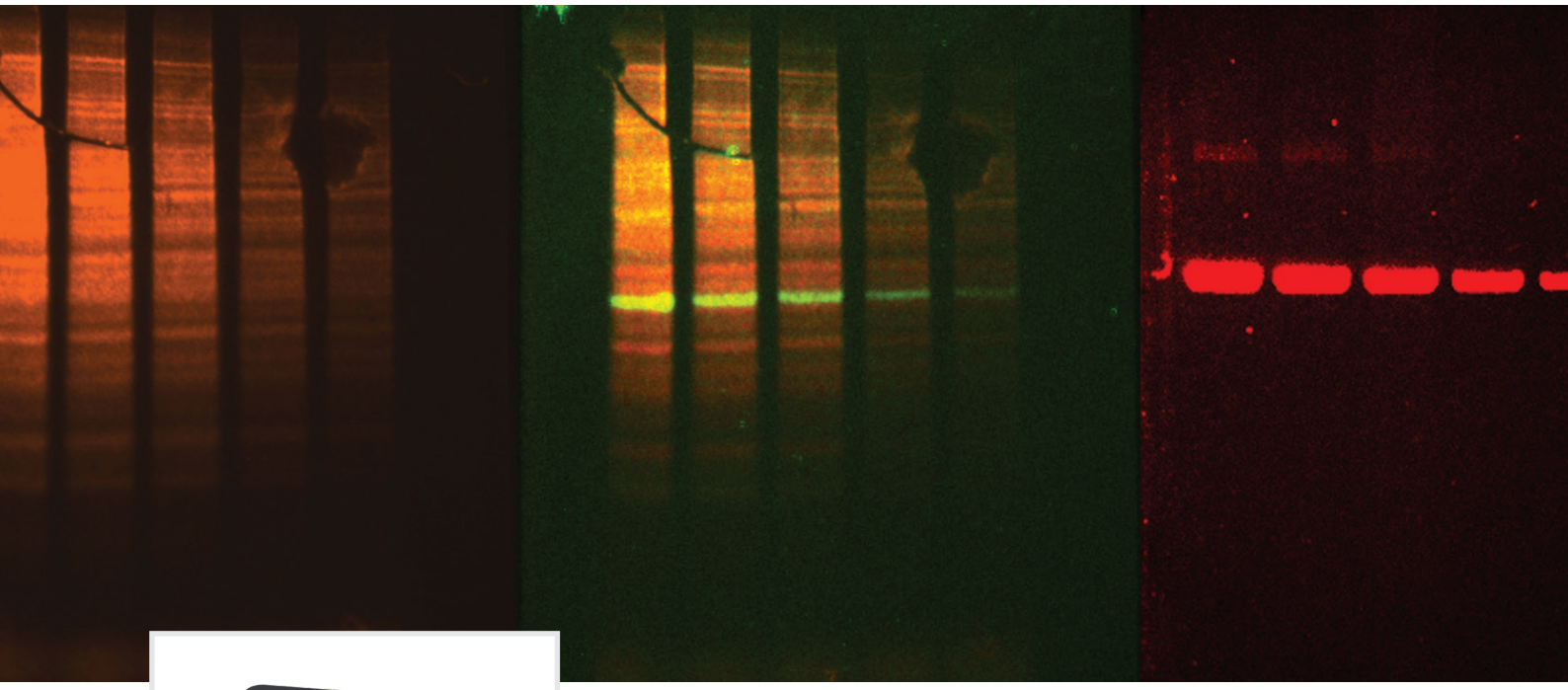

Multiplex Fluorescent Western Blot Detection Using the UVP ChemStudio Imaging System

Challenge

One touch imaging of multiple proteins on the same blot with NIR fluorescent tags

Solution

Automated imaging system with ultra sensitive cameras and lens, internal excitation LEDs and lasers all controlled by powerful and simple to use one touch software

Introduction

Western blotting is a commonly used analytical technique for the identification and quantification of specific proteins in a biological sample (Gallagher and Wiley, 2018). Traditionally, a target protein is interrogated by antigen-specific primary antibodies which are then probed by secondary antibodies conjugated to either Horseradish Peroxidase (HRP) or Alkaline Phosphatase (ALP) and followed by colorimetric or chemiluminescent detection.

Due to the improved signal-to-noise and multiplexing capabilities there continues to be growing interest in fluorescent protein (Western) blotting employing primary or secondary antibodies labeled with a fluorophore to perform non-enzymatic detection of protein expression.

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.

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Materials and Methods

- Imaging system: UVP ChemStudio
- Biotium's TrueBlack WB blocking kit, Cat#23013 (blocking buffer and antibody diluents)
- Wash buffer: TTBS (Tween (0.1% Tris Buffered Saline)
- HeLa lysate. The total protein concentration is determined by BCA assay. The lysis buffer contains 20mM Tris, 150mM NaCl, 1mM EDTA, 1% TritonX-100, pH 7.5
- Membrane: PVDF Millipore Immobilon-FL PVDF
- Primary antibodies (0.1µg/ml Primary Mouse or rabbit anti-Tubulin)
- Secondary antibodies goat-∝-rabbit IgG – CF 680R or goat-∝-mouse IgG – CF 770®

Sample Preparation

Using a serial dilution of 10 µg, 7.5µg, 5µg, 2.5µg, or 1µg HeLa cell lysate protein was added into micro sample tubes in total 10µl.
Lysate dilutions were pre-stained according to manufacturer's instructions (Biotium MnS Total Protein Prestain kit) lysate for 30 min with CF680T or CF770T for 30 min.
Loading buffer (4X LDS loading buffer)+ DTT was added to samples and heated at 95˚C using a dry temp lock for 3 min.
Samples were loaded on a 4-12% Bis-tris SDS-PAGE gel and separated at constant voltage at 200V for 30 min.
The separated proteins were then electro transferred to a PVDF membrane at 20V for 1 hour using an automated western blotting instrument according to manufacturer instructions.
Note there are a variety of alternatives include manual and semi-automated strategies (Gallagher and Wiley, 2018).

Blots were processed according to the following sequence:
Blocking, 4˚C 1h
Primary Ab binding, 4˚C 16h
Washing, 4˚C 5 x 10min
Secondary Ab binding, 4˚C 2h
Washing, 4˚C 5 x 10min

Imaging

The UVP ChemStudio Imaging System and the integrated 660/787 nm lasers were used for fluorescent imaging. Images were automatically acquired, composited with VisionWorks Touch software.

Briefly, the processed blot was positioned on the sample plate. One touch automated software set the optimal excitation and emission wavelengths, exposure and focus for maximal signal without saturation and automatically pseudo-colored to indicate the two different wavelengths and compositing as well as saving the original raw data images. Once acquired, the original unaltered image was archived, and a copy was used for image analysis.

Results and Discussion

Figure 1 illustrates the multiplex imaging capabilities of the UVP ChemStudio Imaging System, specifically separating out the signal of both Biotium CF680R and CF 770 fluorescence 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 UVP ChemStudio Imaging System with internal blue, green, red narrowband LEDs and 660nm and 787nm NIR excitation laser light sources provides a full range of excitation light wavelengths and also rapid high-resolution image capture through the use of deeply cooled 815 8.1 mpx with NIR and QE enhanced 615 3.2 mpx cameras and f/0.95 low light lenses.

Imaging Time

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

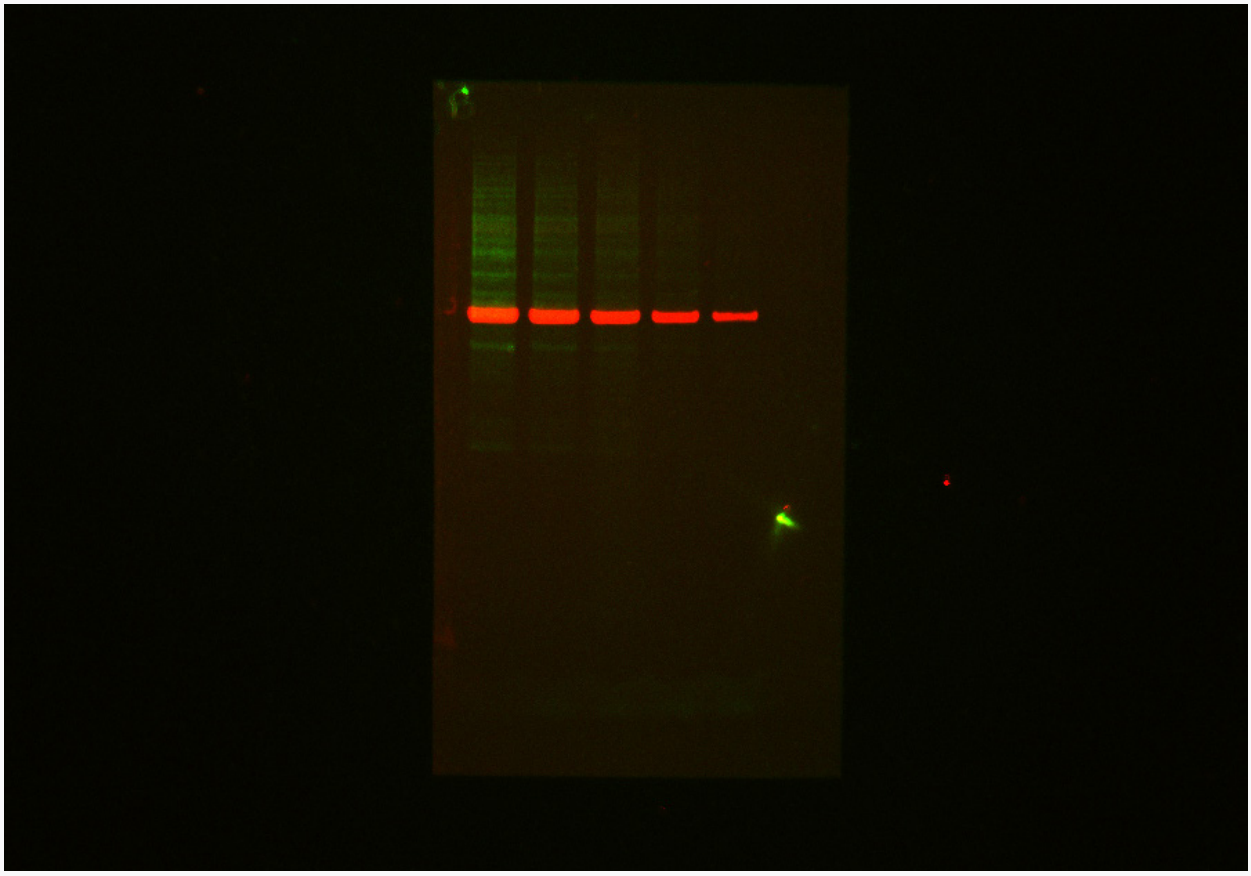

Figure 1: Protein blot stained with Biotium MnS Total Protein Prestain kit CF770T and Primary Rabbit anti-Tubulin/ goat-∝-rabbit IgG – CF 680R and visualized with the ChemStudio using the NIR 660/787 lasers set.

Conclusion

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

References

Gallagher, S.R. and Wiley, E.A. Current Protocols: Essential Laboratory Techniques. Wiley, 2018

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