Fluorescent Western Blotting

Application Overview

Western blotting is a commonly used analytical technique for identification and quantification of specific proteins in a biological sample. Traditionally target protein is interrogated by antigen specific antibodies which are then probed by secondary antibodies conjugated to either HRPO or ALP and followed by calorimetric or chemiluminescent detection.

Fluorescence western blotting employs secondary antibodies labeled with a fluorophore and performs non enzymatic direct detection of the protein expression. The two-color multiplexing abilities of BioSpectrum® Imaging System employing primary antibodies from different species and probing them with CyDyes™ tagged secondary antibodies allows simultaneous detection of multiple proteins on the same immunoblot.

Benefits over traditional chemiluminescent detection :

- Detection of multiple proteins on the same blot with help of multiplexing
- Eliminating the need to strip and re-probe
- Accurate quantitative analysis with broader dynamic range and highest linearity
- Longer stable signal
- Time and cost saving

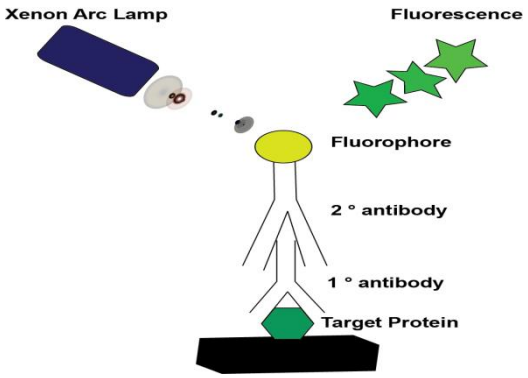

Figure.1 Schematic diagram of Fluorescent western blotting

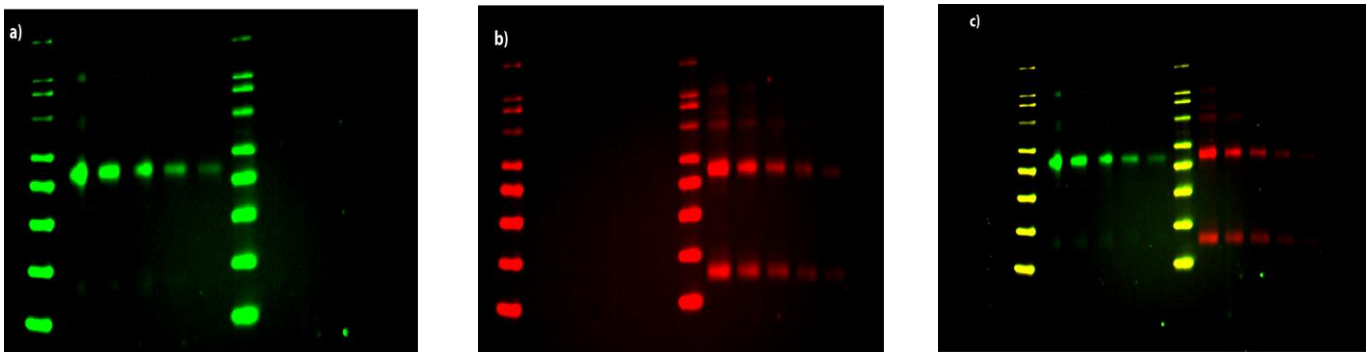

Figure.2 a) Rabbit IgG probed with Cy3–tagged goat anti-rabbit IgG b) Mouse IgG probed with Cy5-tagged goat anti-mouse IgG c) Multiplex fluorescence detection of two fold serial dilution of mouse and rabbit serum proteins probed with Cy3–tagged goat anti-rabbit IgG and Cy5-tagged goat anti-mouse IgG respectively on same immunoblot. CyDyes™ were imaged at 1 second of exposure with BioSpectrum® imaging system.