Low Background Membrane for Fluorescent Protein Detection in Western Blotting

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Overview

- **We demonstrate the use of novel, low-fluorescence PVDF membrane (Immobilon™ FL) for western blotting applications.**
- **The background fluorescence of the membrane is about 10 times lower than that of other commercially available PVDF membranes, and at least 2 times lower than the fluorescence of nitrocellulose membranes.**
- **The use of Immobilon FL membrane does not require any changes in standard western blotting protocol, and the membrane is shown to be compatible with variety of blocking reagents and transfer buffers, as well as total protein blot stains.**

Introduction

Polyvinylidene fluoride (PVDF) membranes are widely used in western blotting applications. The durability, low non-specific binding, high signal-to-noise ratio and re-probing characteristics of PVDF membranes make them ideal for protein blotting experiments where proteins are usually detected by chromogenic or chemiluminescent methods. Fluorescent detection on blotting membranes has thus far been limited by high background fluorescence resulting in low sensitivity. Nonetheless, fluorescent detection in western blotting offers several advantages, including practically unlimited signal life, digital image acquisition and much broader dynamic range compared to film. Moreover, imaging fluorescent blots eliminates needs for a dark room, film and X-ray processing equipment. Digital imaging allows accurate quantification of band intensities.

Table 1. Background Fluorescence of PVDF, Nitrocellulose and Immobilon FL Membranes at Different Excitation/Emission Wavelengths

Emission Color	Excitation wavelength	Emission wavelength	Fluorescence, RFU			Compatible fluorochromes
			Immobilon FL	PVDF	Nitrocellulose	
Blue	360	465	3364	34984	13000	Alexa Fluor 350, Marina Blue, Coumarin
Blue	405	465	1231	12470	3297	Pacific Blue
Green	430	535	1880	49749	9322	Alexa Fluor 430, Lucifer Yellow
Green	450	535	443	19123	2674	Alexa Fluor 430
Green	485	535	742	28806	2841	Fluorescein, Oregon Green 488, Cy2, Fluoro Emerald, Alexa Fluor 488
Green	492	535	1209	11688	1880	Fluorescein, Oregon Green 488, Oregon Green 514, Alexa Fluor 514, Alexa Fluor 488
Red-Orange	485	595	323	13367	1648	Phycoerythrin
Red-Orange	550	595	12300	15355	13311	Tetramethylrhodamine, Alexa Fluor 546, R-Phycoerythrin (R-PE), Rhodamine Red-X, Cy3, Cy3B
Red	485	635	102	3824	501	Alexa Fluor 610-R-PE
Red	550	635	68	1423	131	Alexa Fluor 610-R-PE, Nile Red
Red	590	635	272	1942	310	Alexa Fluor 594, Texas Red-X, Alexa Fluor 594

Fluorescence of blank membranes was measured using Tecan SPECTRAFluor Plus fluorometer, at 100 instrument gain. Immobilon P blotting membrane (Millipore, Billerica, MA,USA) and BA85 blotting membrane (Schleicher & Shuell BioScience, Keene, NH) were used as representative PVDF and nitrocellulose membranes.

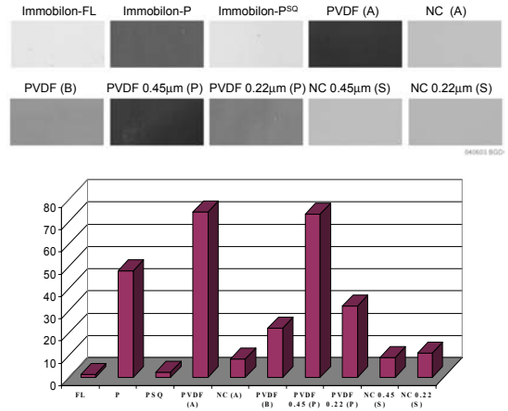

Figure 1. Auto-fluorescence background of commercially available blotting membranes. Each piece of blank membranes was scanned on Storm 840 Imaging system in blue fluorescence mode. Each background was determined using ImageQuant® 5.0 software and is shown in the graph.

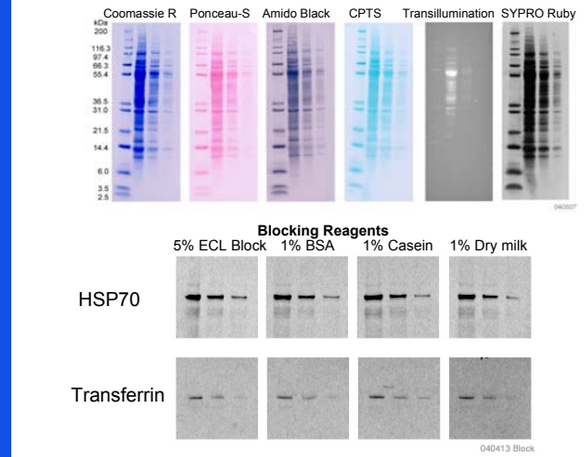

Figure 2. Compatibility of Immobilon FL blotting membrane with common blot stains and blocking reagents. Liver lysate protein load for blot staining was 20, 5 and 1.25 µg/lane. Dilutions of the lysate for HSP70 detection 1: 25, 50, 100 (L to R). Dilution of serum for transferrin detection 1: 128,000, 256,000, 512,000 (L to R).

Materials and Methods

Materials:
Common chemicals, human serum, casein, goat anti-transferrin antibody, mouse anti-HSP70 antibody, and anti-goat IgG FITC-conjugate were purchased from Sigma Aldrich (St. Louis MO, USA). Anti-mouse IgG Alexa Fluor® 430 conjugate was obtained from Molecular Probes (Eugene, OR, USA). ECL Block blocking reagent was from from Amersham Biosciences (Uppsala, Sweden). Storm® 840 Imaging System (Amersham) and Tecan SpectraFluor Plus (Zurich, Switzerland) were used to scan membranes and blots. NuPAGE 4-12% gel, running buffer and XCell SureLock™ Blot Module were from Invitrogen (Carlsbad, CA, USA).

Western blotting protocol:
Proteins were resolved in SDS PAGE gel at 200V for 35min and electrotransfered by tank method to membranes at 30V for 1hour. The blots were rinsed in Milli-Q® water for 5min and air dried. The blots were blocked in TBST buffer supplemented with blocking agent for 1hour, then rinsed twice with TBST. Blots were incubated with primary antibody diluted in TBST (1:1,000 and 1:2,500 for transferrin and HSP70 respectively) for 1hour, washed 4 times with TBST, and then incubated with secondary antibody (1:100 and 1:2,000 for transferrin and HSP70 respectively) for 1hour. Blots were washed 4 times in TBST and dried under vacuum for 1hour before visualization on Storm Imaging System.

Total protein blot stains:
Coomassie™ Blue: The blot was stained in 0.1% Coomassie brilliant blue R, 50% methanol, 7% acetic acid for 5 min and destained twice in 50% methanol, 7% acetic acid.

Ponceau-S: The blot was stained in 0.2% Ponceau-S red, 1% acetic acid for 5 min and destained for 2 min in Milli-Q water.

Amido Black: The blot was stained in 0.1% amido black, 25% isopropanol, 10% acetic acid for 10 min and destained twice in 25% isopropanol, 10% acetic acid.

CPTS: The blot was stained in 0.05% copper phthalocyanine tetrasulfonic acid, 12mM HCl for 5min and destained for 5min in 12mM HCl then for 2min in 20% methanol.

Transillumination: The dried blot was incubated in 20% methanol for 5 min and then imaged by an office scanner.

SYPRO® Ruby stain: The blot was incubated in 10% methanol, 7% acetic acid for 15min, then four times in Milli-Q water for 5 min, stained in SYPRO Ruby protein blot stain (Milecular Probes) for 15min and washed three times for 1 min in Milli-Q water. After air-drying for 2 hours, the blot was scanned on the Storm 840 Imaging System.

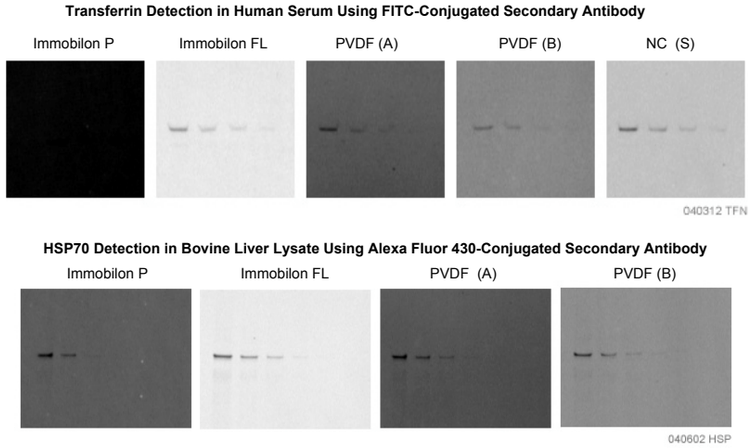

Figure 3. Detection of transferrin in serum and HSP70 in liver lysate was performed on Immobilon FL and other commercially available blotting membranes. Dilution of serum for transferrin detection; 1: 64,000, 128,000, 256,000, 512,000 (L to R), dilution of lysate for HSP70 detection; 1: 25, 50, 100, 200, 400, 800 (L to R). All membranes were scanned using Storm 840 imaging system (Amersham) in blue fluorescence mode.

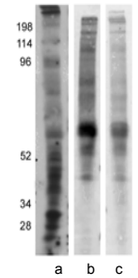

Figure 4. Detection of Connexin 43 (Cx43) in rat whole cell lysates. (a) - ECL detection using HRP-conjugated Protein A, (b) and (c) - fluorescent detection on Immobilon FL membrane using IRDye800-conjugated Protein A. Blocking reagents were 3% BSA (a), 1% dry milk (b), and 1:1 Odyssey Block (c). Fluorescent detection was done on Odyssey® Imaging System (Li-Cor Biotechnology, Lincoln, NE,USA). Data courtesy of Dr.J.Zimmerman and Dr. G. Yanochko, Salk Institute for Biological Studies, San Diego, California.

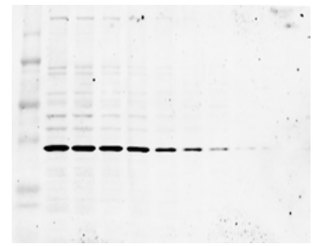

Figure 5. Detection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in HeLa cell lysates on Immobilon FL blotting membrane. 8ug, 6ug, 4ug, 3ug, 2ug, 1ug, 0.5ug, 0.2ug, 0.1ug.of cell lysate were loaded on the gel (left to right). Primary antibody was anti-G3PDH, secondary antibody was biotin conjugated anti-rabbit IgG. The proteins were detected with FITC-streptavidin. The membrane was scanned on BioRad FX imager. Data courtesy of Sysmex Corporation, Kobe, Hyogo, Japan.