Swift™ Membrane Stain 500X More Sensitive & >30X Faster than Ponceau-S Stain

Swift Membrane Stain™ is a unique, proprietary (patents pending), reversible, ready-to-use membrane stain for proteins on nitrocellulose or PVDF membranes. Swift Membrane Stain™ stains proteins faster and with 500X more sensitivity than the routinely used Ponceau-S stain. The lower detection limit of Swift Membrane Stain™ is ~0.5ng protein (BSA)/band on nitrocellulose membrane.

Swift Membrane Stain™ only stains proteins resulting in a clear background and no requirement for additional steps to remove background. The stronger staining allows for easier image capture due to the strong blue stain on a clear, white background.

Swift Membrane Stain™ can be complete removed from the membrane in <1 minute without affecting the biological or immunological properties of the immobilized proteins.

AIM

To evaluate the staining efficiency of Swift™ Membrane Stain and compare its staining and destaining to the routinely used Ponceau-S stain.

METHOD

A rat multiple-tissue blot (Cat. # TB39) was used in the analysis of the stains. The blot consists of 50µg protein lysate of the following tissues immobilized on a PVDF membrane:

Lane #	Tissue	Lane #	Tissue
1	Protein Marker	6	Rat Spleen
2	Rat Liver	7	Rat Testis
3	Rat Brain	8	Rat Ovary
4	Rat Lung	9	Rat Heart
5	Rat Kidney	10	Rat Pancreas

Swift Membrane Stain™

The PVDF membrane was transferred to a staining tray that was slightly larger than the membrane. 20ml methanol was added and the membrane rinsed for 5-10 seconds. (*Note: Nitrocellulose membranes do not require a methanol rinse*) 12ml Swift™ Membrane Stain was immediately added and the membrane was incubated on an orbital shaker at room temperature. Images were captured with a Microtek Scanmaker 5900 after 30 seconds and 5 minutes.

The Swift™ Membrane Stain was discarded and the membrane was rinsed in deionized water for 30 seconds and a scan was then taken.

The membrane was rapidly destained with the addition of 50ml Swift™ Destain [1X]. The membrane was incubated on an orbital shaker at room temperature and images captured after 1 and 5 minutes.

Ponceau-S Staining

A variety of methods and Ponceau-S compositions are recommended, however the method by Kruger[1] was used.

Basically, a 0.2% Ponceau-S solution was prepared in 10% acetic acid. The same membrane as above was rinsed extensively in DI water prior to Ponceau-S staining.

12ml Ponceau-S stain was added to the membrane and the membrane was then incubated and images captured as with the Swift™ Membrane Stain. The only difference was the membrane was destained in phosphate buffered saline (PBS) as per the protocol of Kruger and the destaining was allowed to proceed for >1 hour.

The above comparison was repeated, however Ponceau-S stain was performed first followed by Swift™ Membrane Stain. No difference in the results was seen (Data not shown).

Immunoblotting

Two kidney tissue blots (Cat. # TB31), containing whole kidney lysates from human, mouse and rat were used to compare the effects of Swift™ Membrane Stain on subsequent immunoblotting.

One membrane was treated with Swift™ Membrane Stain prior to antibody probing and a second membrane was simply washed in deionized water. Following destaining, both membranes were blocked with NAP-BLOCKER™, probed with an anti-actin antibody and then a secondary antibody tagged with HRP. The actin protein was detected with our femtoLUCENT™-PLUS chemiluminescene reagent.

RESULTS

Figure 1 clearly shows that the Swift™ Membrane Stain rapidly stains proteins (<30 seconds) and at a higher sensitivity than Ponceau-S. Although Ponceau-S stained proteins in 30 seconds it was difficult to see or capture detail due to the high red background, whereas the bands were clearly visibly with Swift™ Membrane Stain without the need for further washing/destaining.

In both cases, staining for 5 minutes allowed for stronger protein staining, but also an increase in background. The Swift™ Membrane Stain background was rapidly washed away with DI water, resulting in a membrane that had strongly stained protein bands on a white background and these were readily captured by scanning. Rinsing the Ponceau-S stain with DI water reduced the background, but also reduced the intensity of protein staining.

Swift™ Membrane Stain was rapidly removed (~1 minute) compared to the Ponceau-S stain that was still present after 5 minutes of destaining. In fact, the membrane was

left to destain for 1 hour and some bands were still present (Figure 1F).

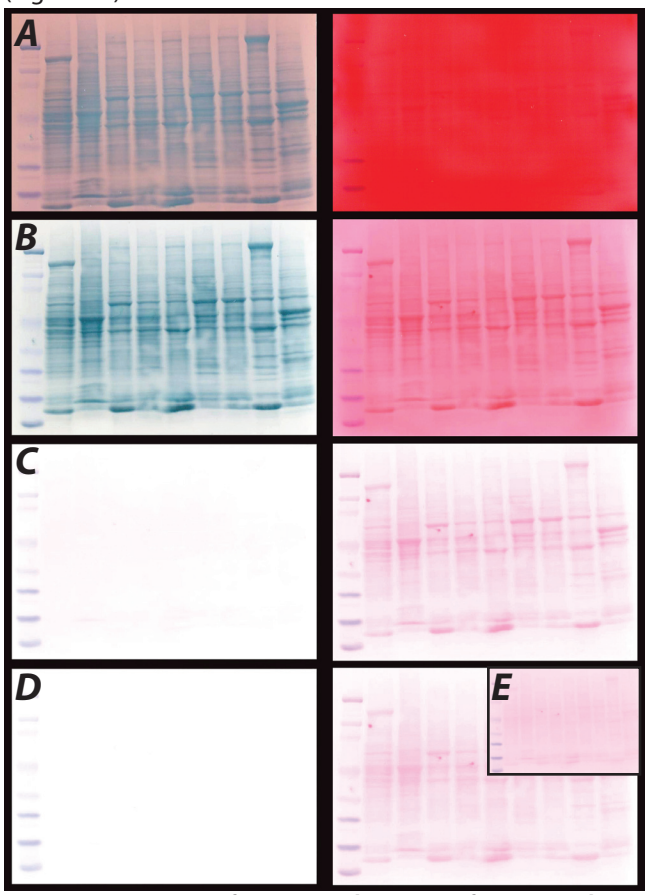

Figure 1: Comparison of staining & destaining of PVDF membrane between Swift™ Membrane Stain & Ponceau-S. Normal Rat Multiple Tissue Blot (Cat. # TB39) was probed with Swift™ Membrane Stain (left) or Ponceau-S (right), as described in the Method section. In both instances the membranes were incubate with the respective stain for 30 seconds (A), then rinsed in deionized water for 30 seconds (B) and destained as instructed for 1 (C) and 5 minutes (D). The Ponceau-S stained membrane was destained for a further 60 minutes (E).

Figure 2 shows a close up of two protein lanes stained with Swift™ Membrane Stain or Ponceau-S stain and clearly demonstrates the high level of protein staining of Swift™ Membrane Stain compared to Ponceau-S. The Swift™ Membrane Stain clearly stains far more "minor" proteins compared to the Ponceau-S stain.

Swift™ Membrane Stain had no deleterious effects on subsequent membrane probing and generate comparative chemiluminescent data compared to unstained membranes (Figure 3)

ORDERING INFORMATION

Cat. #	Description/ Size
786-677	Swift™ Membrane Stain/ 250ml
TB39	Rat Multiple Tissue Blot; *Liver, Brain, Lung, Kidney, Spleen, Testis, Ovary, Heart, Pancreas*/ 1 blot
TB31	Kidney Tissue Blot; *Human, Mouse, Rat*/ 1 blot
786-190	NAP-BLOCKER™/ 2 x 500ml
786-10	femtoLUCENT™ PLUS HRP/ For 1,500cm^2 membrane

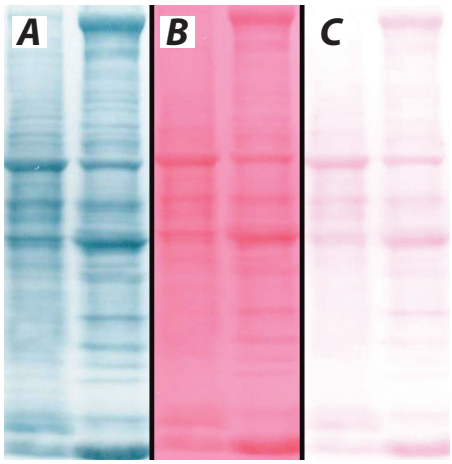

Figure 2: Comparison of staining & destaining of PVDF membrane between Swift™ Membrane Stain & Ponceau-S. An enlarged image comparing the number of protein bands stained by Swift™ Membrane Stain (A) or Ponceau-S (B,C). The image shows two lanes after 5 minutes of staining with the respective stain and a 30 second rinse in DI water (A, B) and a further 1 minute in DI water (C).

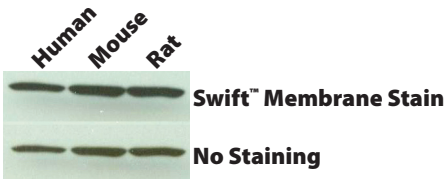

Figure 3: Swift™ Membrane Stain has no deleterious effects on downstream processing. A Kidney Tissue Blot (Cat. # TB31) was either stained with Swift™ Membrane Stain or left untreated prior to blocking and probing for actin according to the Method section.

DISCUSSION

Swift™ Membrane Stain clearly outperforms Ponceau-S stain in speed of staining and destaining, sensitivity and ease of image capture.

The entire Swift™ Membrane Stain procedure can be substantially reduced to approximately 2 minutes without loss of performance or sensitivity:

1. Stain for 30 seconds
2. Rinse for 30 seconds
3. Destain for 60 seconds

Another advantage of Swift™ Membrane Stain is that it stains only protein and no additional treatments are required to remove the background unlike numerous, commercially available membrane stains. The strong blue stain on a white background greatly simplifies image capture.

REFERENCES

1. Aebersold, R. et al (1987) Proc. Natl. Acad. Sci. USA, 84:6970
2. Kruger, N.J. (1996) Detection of Polypeptides on Blots Using Secondary Antibodies or Protein A. In J. M. Walker (Ed.), The Protein Protocols Handbook (pp. 313-321). New Jersey: Humana Press

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