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

Swift™ Membrane Stain is a unique, proprietary, 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.

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 completely 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:

<table>
<thead>
<tr>
<th>Lane #</th>
<th>Tissue</th>
<th>Lane #</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein Marker</td>
<td>6</td>
<td>Rat Spleen</td>
</tr>
<tr>
<td>2</td>
<td>Rat Liver</td>
<td>7</td>
<td>Rat Testis</td>
</tr>
<tr>
<td>3</td>
<td>Rat Brain</td>
<td>8</td>
<td>Rat Ovary</td>
</tr>
<tr>
<td>4</td>
<td>Rat Lung</td>
<td>9</td>
<td>Rat Heart</td>
</tr>
<tr>
<td>5</td>
<td>Rat Kidney</td>
<td>10</td>
<td>Rat Pancreas</td>
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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 30 seconds and 5 minutes.

Ponceau-S Staining
A variety of methods and Ponceau-S compositions are recommended; however, the method by Kruger1 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 visible with Swift™ Membrane Stain without the need for further washing/destaining.

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 (~30secs) 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 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).

**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, including destaining, 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**


**CITATIONS**


**ORDERING INFORMATION**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description/Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>786-677</td>
<td>Swift™ Membrane Stain / 250ml</td>
</tr>
<tr>
<td>TB39</td>
<td>Rat Multiple Tissue Blot: Liver, Brain, Lung, Kidney, Spleen, Testis, Ovary, Heart, Pancreas / 1 blot</td>
</tr>
<tr>
<td>TB31</td>
<td>Kidney Tissue Blot: Human, Mouse, Rat / 1 blot</td>
</tr>
<tr>
<td>786-190</td>
<td>NAP-BLOCKER™ / 2 x 500ml</td>
</tr>
<tr>
<td>786-10</td>
<td>femtoLUCENT™ PLUS HRP / For 1,500cm² membrane</td>
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