Visualization of proteins electro-transferred on Hybond ECL and Hybond-P using Deep Purple Total Protein Stain

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Deep Purple™ Total Protein Stain® can be used to stain blots as well as gels. Results show that the blot staining protocol delivers rapid, robust, and highly sensitive results when using either nitrocellulose or polyvinylidene difluoride (PVDF) membranes. Detection sensitivity was shown to be at least 16 times greater than Sypro™ Ruby Blot Stain.

Introduction
Deep Purple Total Protein Stain is one of the most sensitive stains currently used for detecting and quantitating protein in 1-D and 2-D electrophoresis studies. As a gel stain, Deep Purple Total Protein Stain is characterized by its brightness, low background and artifact levels, and quantitative linearity over more than four orders of magnitude (1, 2).

Here we demonstrate the use of Deep Purple Total Protein Stain as a simple, quick, and highly sensitive stain for detecting and quantitating proteins transferred to Hybond™ ECL™ nitrocellulose and Hybond-P PVDF membranes.

Methods
Membranes were stained according to manufacturer’s instructions. Please see the application note Visualization of proteins electro-blotted on Hybond ECL and Hybond-P using Deep Purple Total Protein Stain [11-0025-43], available at www.amershambiosciences.com, for detailed methods.

Results and discussion
Deep Purple staining of protein blotted to Hybond-P
The Deep Purple blot staining protocol is simple and quick, requiring 30–40 min. In addition, blots can be stained in either a wet or dry format.

Deep Purple Total Protein Stain sensitively and quantitatively stained protein on Hybond-P while exhibiting only low levels of background interference, even though the protein concentration was very low. The detection sensitivity was approximately 1 ng of protein (Fig 1A). This compares with a detection sensitivity of 16 ng for identical blots stained with Sypro Ruby Blot Stain (Fig 1B). However, this underestimates the actual sensitivity of the Deep Purple blot stain as the nominal protein quantities (Table 1) are the actual amounts loaded onto gels. Electrotransfer was not 100% efficient. Post-transfer staining of the gels revealed that enough protein remained in the gel to be detected with Deep Purple Total Protein Stain.

In a direct dot blot comparison, Deep Purple Total Protein Stain showed a detection sensitivity at least 16 times greater than Sypro Ruby Blot Stain (Fig 2).

Deep Purple Total Protein Stain performed equally well for both 1-D and 2-D separated proteins blotted to Hybond-P. The chemical stability of the Hybond-P in a range of solvents makes it especially suited for use in subsequent studies such as N-terminal sequencing and mass spectrometry.
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References

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Table 1. GE Healthcare low molecular weight markers were prepared at the following concentrations (ng) per 5 µl and loaded into precast 4–20% gradient mini gels.

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<th>Protein</th>
<th>107.3</th>
<th>53.6</th>
<th>26.8</th>
<th>13.4</th>
<th>6.7</th>
<th>3.4</th>
<th>1.7</th>
<th>0.8</th>
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<td>phosphorylase b</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>human serum albumin</td>
<td>128.8</td>
<td>66.4</td>
<td>33.2</td>
<td>16.6</td>
<td>8.3</td>
<td>4.1</td>
<td>2.1</td>
<td>1.0</td>
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<td>ovalbumin</td>
<td>235.5</td>
<td>117.6</td>
<td>58.8</td>
<td>29.4</td>
<td>14.7</td>
<td>7.4</td>
<td>3.7</td>
<td>1.8</td>
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<tr>
<td>carbonic anhydrase</td>
<td>128.8</td>
<td>66.4</td>
<td>33.2</td>
<td>16.6</td>
<td>8.3</td>
<td>4.1</td>
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<td>1.0</td>
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<td>soybean trypsin inhibitor</td>
<td>128.0</td>
<td>64.0</td>
<td>32.0</td>
<td>16.0</td>
<td>8.0</td>
<td>4.0</td>
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<td>α-lactalbumin</td>
<td>186.0</td>
<td>93.0</td>
<td>46.5</td>
<td>23.2</td>
<td>11.6</td>
<td>5.8</td>
<td>2.9</td>
<td>1.5</td>
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</tbody>
</table>

Deep Purple staining of protein blotted to Hybond ECL
Deep Purple Total Protein Stain also delivered sensitive results with low background when used with Hybond ECL. Figure 3 shows a 2-D gel separation of a complex tissue sample blotted to Hybond ECL and then stained with Deep Purple Total Protein Stain.

Hybond ECL electrotransfers stained with Deep Purple exhibited a detection sensitivity of 1 ng per band (data not shown).

Conclusions
Deep Purple Total Protein Stain is a widely used protein gel stain that exhibits excellent sensitivity and quantitative linearity. It is compatible with subsequent analyses such as mass spectrometry and N-terminal sequencing chemistry.

These characteristics are transferable to the quantitation of proteins electrotransferred to Hybond-P (PVDF) and Hybond ECL (nitrocellulose) membranes. The blot staining procedure is rapid and robust, producing results at least 16 times more sensitive than Sypro Ruby, with low background interference. Deep Purple Total Protein Stain can be used with nitrocellulose or PVDF membranes, and blots can be stained wet or dry.

Fig 2. Dot blots stained with Deep Purple Total Protein Stain (upper image) and Sypro Ruby Blot Stain (lower image). Replicate dot blots of BSA in a four-fold dilution series on Hybond-P starting at (from left to right) 3125 ng, 781 ng, 195 ng, 49 ng, 12 ng, and 3 ng BSA per spot.

Fig 3. Blot of ras-transformed fibroblast protein extract stained with Deep Purple Total Protein Stain on Hybond ECL.

References

Acknowledgements
The authors thank Anna Lucantoni from GE Healthcare and Melissa Mico from Fluorotechnics for their technical support.

Ordering Information
Deep Purple Total Protein Stain (5 ml) RPN6305
Deep Purple Total Protein Stain (25 ml) RPN6306
Hybond-P (30 cm x 3 m, 1 roll)* RPN303F
Hybond ECL (30 cm x 3 m, 1 roll)* RPN303D

* Other sizes are available.

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