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.

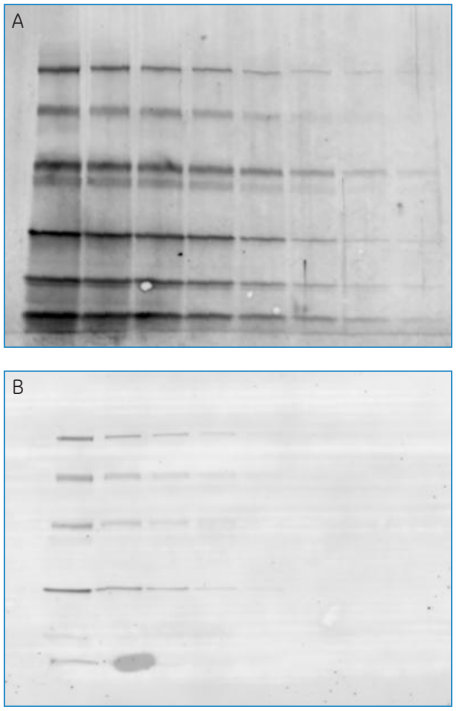

Fig 1. Comparison of Deep Purple Total Protein Stain with Sypro Ruby Blot Stain for use with Hybond-P. Low molecular weight markers were two-fold serially diluted from approximately 128–1 ng (Table 1), and separated by electrophoresis on Tris-Glycine 4–20% gradient mini gels. Gels were blotted to Hybond-P using a TE 22 Mini Tank Transfer Unit. (A) Blot stained with Deep Purple Total Protein Stain. (B) Blot stained with Sypro Ruby Blot 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.

✧ See licensing information on page 16.

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.

phosphorylase b	107.3	53.6	26.8	13.4	6.7	3.4	1.7	0.8
human serum albumin	132.8	66.4	33.2	16.6	8.3	4.1	2.1	1.0
ovalbumin	235.5	117.6	58.8	29.4	14.7	7.4	3.7	1.8
carbonic anhydrase	132.8	66.4	33.2	16.6	8.3	4.1	2.1	1.0
soybean trypsin inhibitor	128.0	64.0	32.0	16.0	8.0	4.0	2.0	1.0
α-lactalbumin	186.0	93.0	46.5	23.2	11.6	5.8	2.9	1.5

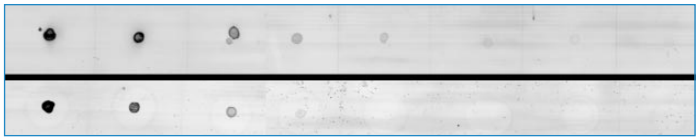

Fig 2. Dot blots stained with Deep Purple Total Protein Stain (upper image) and Sypro Ruby Blot Stain (lower image). Replicate dot blots of BSA in a four-fold dilution series on Hybond-P starting at (from left to right) 3125 ng, 781 ng, 195 ng, 49 ng, 12 ng, and 3 ng BSA per spot.

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

References

1. Bell, P. J. L. and Karuso, P. Epicocconone: A novel fluorescent compound from the fungus *Epicoccum nigrum*. *J. Am. Chem. Soc* **125**, 9304–9305 (2003).

2. Mackintosh, J. A. *et al.* A fluorescent natural product for ultra sensitive detection of proteins in 1-D and 2-D gel electrophoresis. *Proteomics* **3**, 2273–2288 (2003).

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

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