Comparison of Deep Purple Total Protein Stain and Sypro Ruby in 1-D and 2-D gel electrophoresis

Key words:

Deep Purple Total Protein Stain • protein staining • 1-D/2-D electrophoresis • fluorescence detection

Introduction

Protein separation by 1-D or 2-D gel electrophoresis is one of the most widely used forms of protein analysis. Methods are available for the detection of proteins within gels using colorimetric or fluorescent endpoints. Of the colorimetric methods available, Coomassie™ Brilliant Blue is relatively insensitive and silver staining, whilst sensitive, exhibits a poor dynamic range and is less user friendly. Fluorescent stains tend to be sensitive and have a wide dynamic range.

In this application note, we compare the fluorescent stain Deep Purple™ Total Protein Stain (1,2) with Sypro™ Ruby.

Products used

Amersham Biosciences Products used:

Hoefer miniVE Vertical Electrophoresis System	80-6418-77
Ettan IPGPhor IEF System	80-6414-02
Ettan DALT*twelve* Large Vertical System	80-6466-27
Typhoon 9410 Variable Mode Imager & ImageQuant Image Analysis Software	63-0038-55
Personal Densitometer SI	63-0016-46
Low Molecular Weight-SDS Marker Kit	17-0446-01
PlusOne Silver Staining Kit, Protein	17-1150-01
Deep Purple Total Protein Stain	RPN6305
Pharmalyte™, broad range pH 3–10	17-0456-01
PlusOne Bind-Silane	17-1330-01
Immobiline DryStrip pH 3–10 NL, 24 cm	17-6002-45
Immobiline DryStrip pH 4–7, 24 cm	17-6002-46

Other materials required

Sypro Ruby (Molecular Probes Inc.)	S-21900
BioChemi imaging system (UVP Ltd.)	

Methods

Electrophoresis

For 1-D SDS polyacrylamide gels, low-molecular weight protein standards were separated on 15% polyacrylamide gels according to standard protocols. Electrophoresis was performed on Hoefer™ miniVE electrophoresis apparatus. Total loadings used were 512 ng down to 125 pg in doubling dilutions. The proteins contained in these standards were phosphorylase b (molecular weight 97 000), bovine serum albumin (66 000), ovalbumin (45 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100) and α-lactalbumin (14 400).

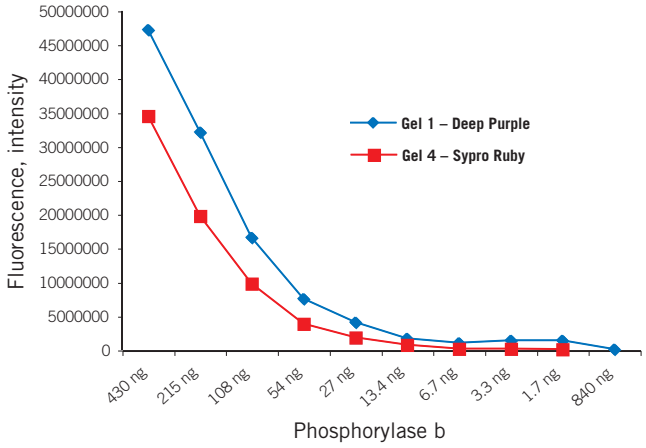
Two-dimensional gel electrophoresis was performed according to recommended protocols (3) on Ettan IPGphor™ IEF System for the first dimension and Ettan™ DALT*twelve* electrophoresis system for the second dimension. Immobiline™ DryStrip gels pH 3–10 NL, 24 cm or pH 4–7 were used for the first dimension and 12.5% polyacrylamide gels in the second dimension. The sample consisted of a mixture of 250 µg protein from the human mammary gland cell line, HBL100 and 250 µg protein from the human ductal breast cell carcinoma, BT474.

Protein staining and detection

Deep Purple staining, for both 1-D and 2-D SDS gels, was performed according to the manufacturers' protocols. Gels were first placed in fix solution (7.5% acetic acid, 10% methanol) overnight. Following 4 × 10 min washes in water, gels were incubated with gentle agitation for 1 h in stain (made up as a 1:200 dilution in water). Development of the stain was performed with 3 × 10 min incubations in a 1:1000 dilution of concentrated ammonia (sp. gr. 0.88–0.89). Gels were then left in signal stabilization solution (0.75% glacial acetic acid) for a minimum of 10 min prior to imaging on a glass plate. Typhoon™ 9410 Variable Mode Imager was used with a green (532 nm) laser and 560LP filter. Quantitation of fluorescence intensity was carried out using ImageQuant™ Image Analysis Software. For UV excitation and detection, a BioChemi™ imaging system was used containing a 365 nm UV transilluminator and a cooled CCD camera (–60 °C).

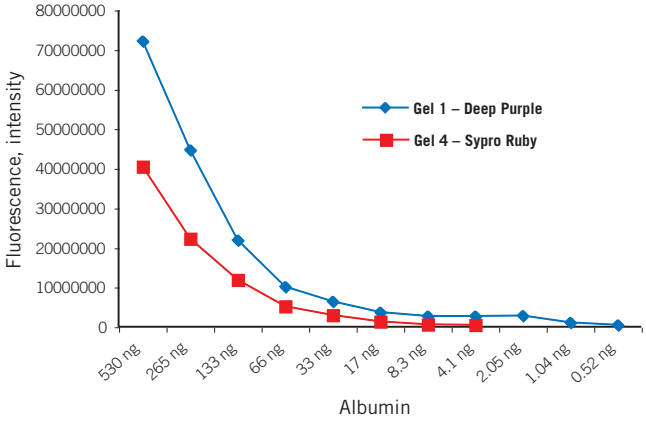
Gel 1 – Deep Purple – Phosphorylase b

Gel 4 – Sypro Ruby – Phosphorylase b



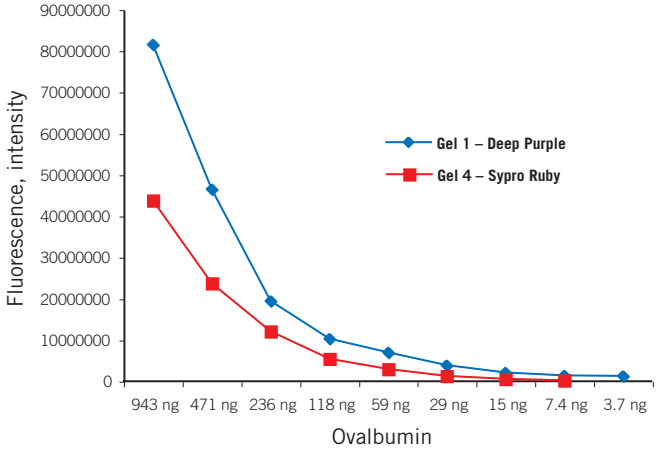
Gel 1 – Deep Purple – Albumin

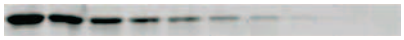
Gel 4 – Sypro Ruby – Albumin



Gel 1 – Deep Purple – Ovalbumin

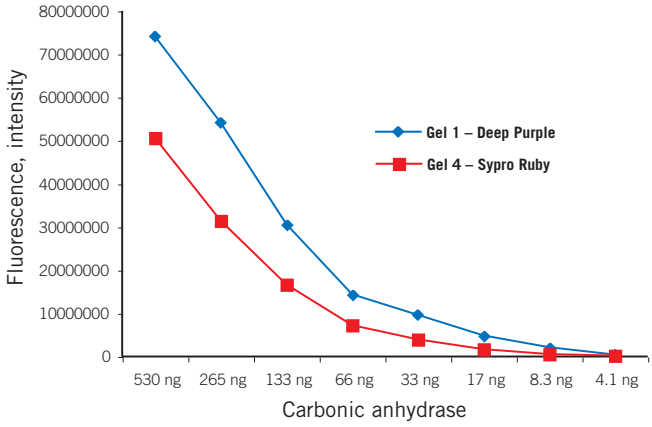
Gel 4 – Sypro Ruby – Ovalbumin





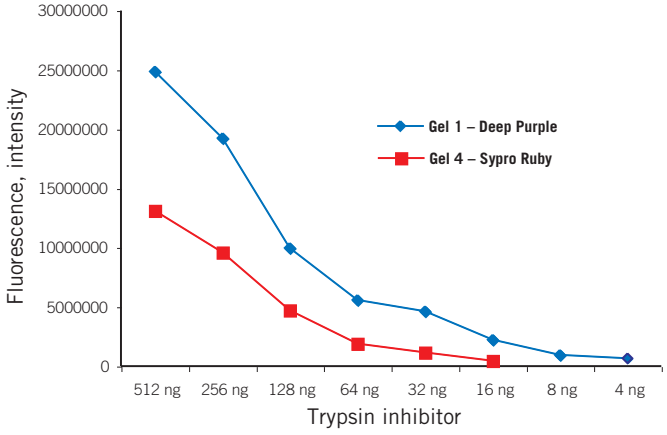
Gel 1 – Deep Purple – Carbonic anhydrase

Gel 4 – Sypro Ruby – Carbonic anhydrase



Gel 1 – Deep Purple – Trypsin inhibitor

Gel 4 – Sypro Ruby – Trypsin inhibitor



Gel 1 – Deep Purple – Alpha lactalbumin

Gel 4 – Sypro Ruby – Alpha lactalbumin

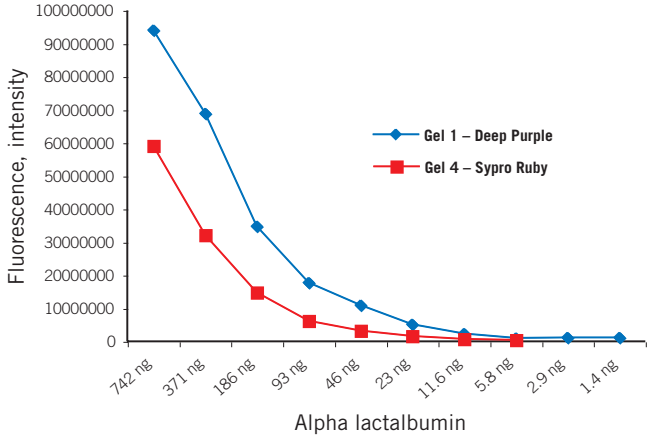


Fig 1. Comparison of individual proteins stained with either Deep Purple or Sypro Ruby. The left-hand column shows the fluorescent images of the loading dilution series for six separate proteins stained with either one of the two dyes. The right-hand column shows the relative signal intensity and relative sensitivity for the images' protein dilution series.

Staining with Sypro™ Ruby was performed as follows: Gels for 1-D analysis were fixed in 7.5% acetic acid, 30% methanol for 1 h, stained overnight, washed in water for 4 × 30 min and destained in 7.5% acetic acid, 10% methanol for at least 30 min. Gels for 2-D analysis were fixed in 7.5% acetic acid, 10% methanol for 30 min, incubated in stain overnight and destained in 7.5% acetic acid, 10% methanol for at least 30 min. For all gels, imaging was on the Typhoon 9410 imager with the green laser and 610BP30 filter.

Silver staining was carried out according to the protocol for PlusOne™ Silver Staining Kit, Protein. Gels were initially fixed in 10% acetic acid, 40% methanol for a minimum of 30 min. They were then incubated in sensitization solution (30% ethanol, 0.5% glutaraldehyde, 0.2% sodium thiosulphate, 6.8% sodium acetate) for 30 min, washed 3 × 5 min in water, then stained for 20 min in 0.25% silver nitrate, 0.04% formaldehyde. After washing 2 × 1 min in water, the gels were developed for up to 2 × 5 min in developer (2.5% sodium carbonate, 0.02% formaldehyde), the precise time depending on the level of staining achieved. Gels were then placed in stop solution containing 1.5% EDTA for a minimum of 10 min. Imaging of gels was performed using Personal Densitometer™ SI.

For this comparison study, the gels were run and stained with each of the protein stains in triplicate (gels 1–3 stained with Deep Purple; gels 4–6 stained with Sypro Ruby; gels 7–9 stained with silver).

Results and discussion

Stain comparison on 1-D gels

Deep Purple exhibits excellent proportionality of signal to protein level as shown on 1-D SDS polyacryalmide gels, with a clear and even background. As silver stain easily becomes saturated at higher protein levels due to the relatively poor dynamic range, accurate quantitation is much more difficult and the data is not shown due to the significant level of variability observed.

Quantitation of the areas under the peaks was performed in triplicate for each of the gel stains on each of the proteins on the gel. Analysis of the quantitation data across the dilution series (Fig 1) showed that in general Deep Purple is between two- and four-fold more sensitive than Sypro Ruby and for some proteins such as albumin, the level of this increase can be up to eight-fold.

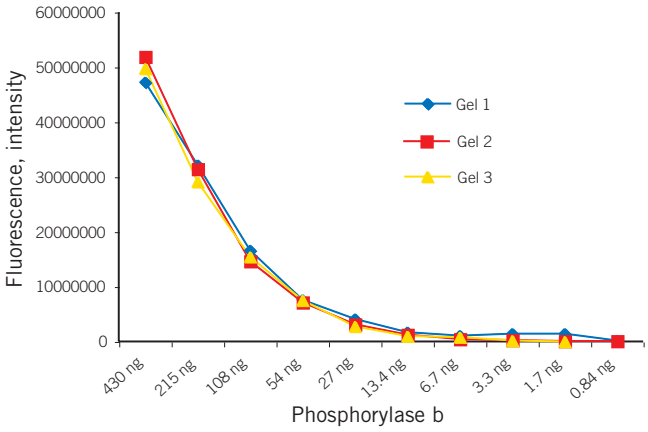


Fig 2. Reproducibility of Deep Purple staining of proteins illustrated by the quantitation of phosphorylase b on triplicate gels (gel 1 data is shown in Fig 1).

Fluorescence intensity was analyzed for phosphorylase b across all three gels processed with Deep Purple (Fig 2). This clearly demonstrates the high intensity of signal and the excellent reproducibility of staining; similar results were obtained for all the other proteins analyzed (data not shown).

Stain comparison on 2-D gels

Deep Purple staining of 2-D gels is characterized by strong, positive fluorescent signals with low level, clear backgrounds. Figure 3 shows fluorescent images of 2-D gels on which a lysate containing a mix of normal and cancerous cell types was run. The gels were stained with either Deep Purple or Sypro Ruby and processed using DeCyder™ Differential Analysis Software. The same small area has been expanded on each gel, centered on a matched spot. It clearly demonstrates the good signal intensity and clarity of background associated with Deep Purple while the speckling often associated with Sypro Ruby staining is clearly visible. The use of Deep Purple is therefore highly compatible with automated spot-detection programs.

A silver-stained gel was also compared to Deep Purple (data not shown), but, as discussed earlier for 1-D gels, silver staining suffers from a poor dynamic range and "volcano-like" effects and is therefore very much less amenable to quantitation than gels stained with Deep Purple.

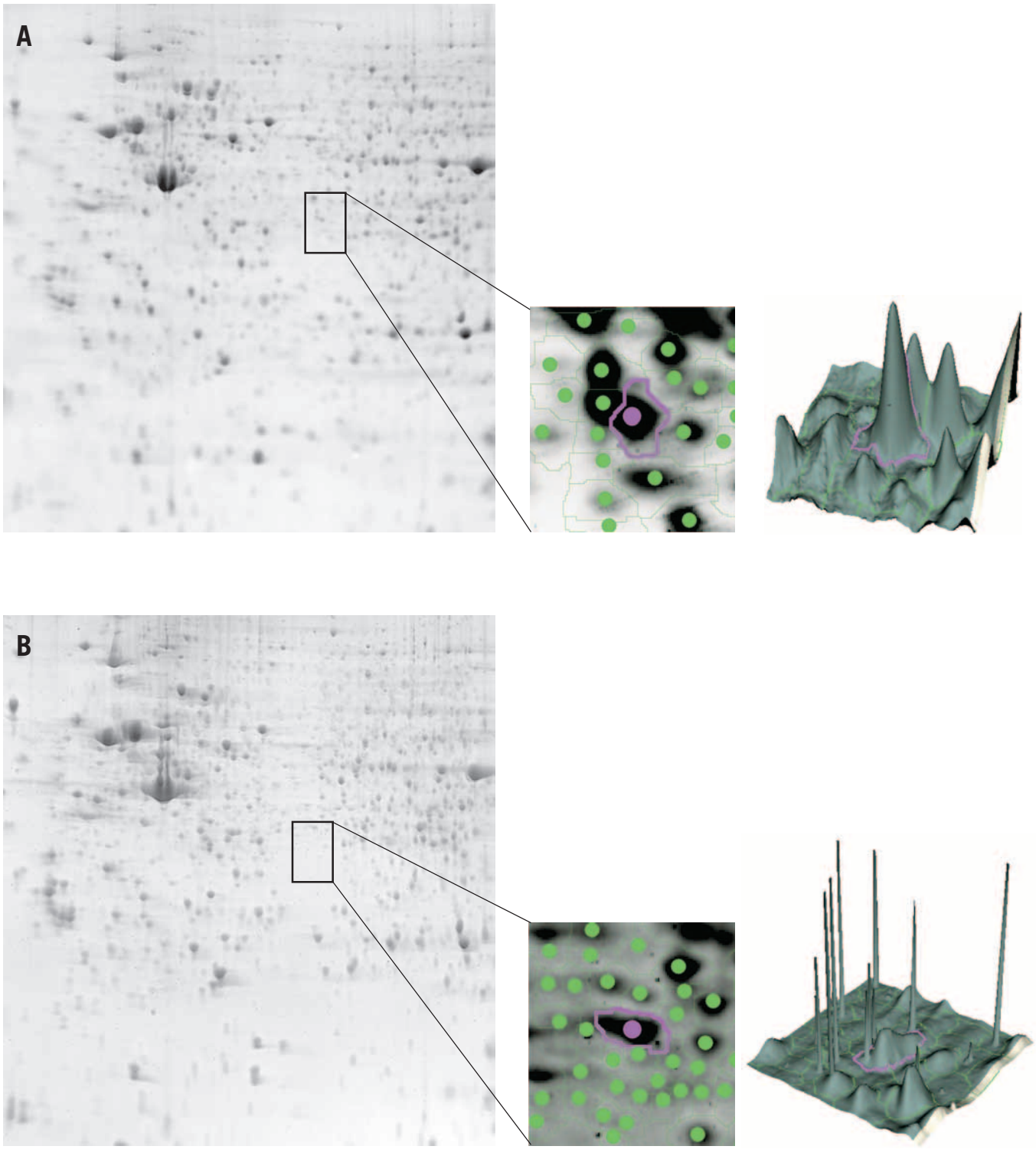


Fig 3. Comparison of Deep Purple and Sypro Ruby for the fluorescent staining of 2-D gels. Images of a preparative gel loaded with a cell lysate containing a mixture of normal and cancerous breast cell lines (A) stained with Deep Purple and (B) stained with Sypro Ruby. Area indicated in gel, centered on the same matched spot for both stains, was analyzed in DeCyder software and is presented as a 3-D surface plot rotated through 90° relative to the indicated area, for viewing clarity.

Alternative imaging devices

Imaging of Deep Purple stained gels can be carried out with a variety of instrumentation. Whilst our recommendation is for a laser scanner with 532 nm laser and 560LP filter, other settings can be used such as 488 nm laser or even a 457 nm laser. Signal intensities will be dependent on laser power as well as optical extinction at the particular wavelength. Other fluorescent imagers with suitable light sources may be used, either exciting in the visible region of the spectrum or the ultraviolet region of the spectrum. Figure 4 shows a fluorescent image of a gel taken on a laser imager compared with the same gel imaged on a 365 nm transilluminator with CCD detection. Similar sensitivity levels are observed. UV excitation may be useful for manual band/spot picking from 1-D or 2-D gels in proteomics applications.

Fig 4. Images of an SDS-PAGE gel containing protein markers stained with Deep Purple and imaged (A) on a Typhoon 9410 imager with 532 nm laser and 560LP filter and (B) on the BioChemi CCD-based imager with 365 nm UV transilluminator.

Conclusions

Deep Purple Total Protein Stain is a new, ultrasensitive fluorescent protein stain that can be successfully applied to both 1-D and 2-D electrophoresis gels. Imaging of the gels can be performed with a variety of instrumentation. Deep Purple has significant benefits in comparison with other traditional protein stains such as Sypro Ruby and silver staining, including a potential increase in sensitivity with clear and even background. It is particularly suitable for quantitation and amenable for use with 2-D image analysis software programs.

References

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