Enabling manual spot picking through colorimetric post-staining of Deep Purple Total Protein Stain treated 2-D gels

key words: Deep Purple Total Protein Stain • Coomassie Blue • silver • 2-D gels • manual spot picking • MALDI ToF-Pro mass spectrometer • dual stain

Introduction

This application note demonstrates the compatibility of combining Deep Purple™ Total Protein Stain with Coomassie™ Brilliant Blue or silver stains to enable manual protein spot picking from 2-D electrophoresis gels.

Proteins in gels stained with colorimetric Coomassie Blue or silver stain can be picked manually. Yet both stains have drawbacks. Coomassie Blue is relatively insensitive (1), and silver stain, while providing adequate sensitivity, offers poor dynamic range and can be time consuming to use (2).

Although fluorescent stains are sensitive with a wide dynamic range, they are unsuitable for manual spot picking as they are invisible to the naked eye and require the use of laser scanning instruments. Manual spot picking can be performed using an ultraviolet (UV) transilluminator, however stain signal fades with time and UV is hazardous. Deep Purple Total Protein Stain is a highly sensitive fluorescent stain, linear over four orders of magnitude (3, 4), and enables good quantitation of both abundant and scarce proteins.

When manual spot picking is required, it is therefore attractive to consider combining the methods: to treat 2-D gels initially with Deep Purple Total Protein Stain and then post-stain the gels with either Coomasssie Blue or silver stain.

Our results confirm that this combination approach provides good, reliable protein quantitation without sacrificing quality—no interference in mass spectrometry analysis was observed with the dual-stained gels.

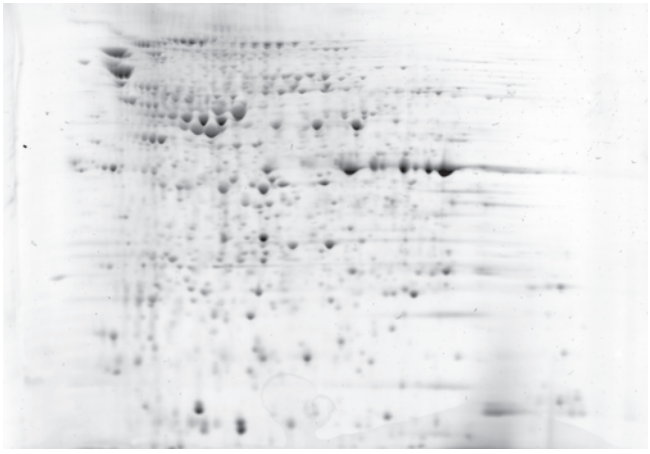

Fig 1. A preparative gel image containing 500 µg total protein from *E. coli* stained with Deep Purple Total Protein Stain. Proteins were focused on Immobiline DryStrips and were separated in the second dimension on Ettan DALT gels.

Products used

Amersham Biosciences products used:

Electrophoresis systems and consumables

Immobline™ DryStrip Reswelling Tray	80-6465-32
Ettan™ IPGphor™ II IEF System	80-6505-03
Ettan IPGphor Manifold	80-6498-38
DALT*twelve* Gel Caster	80-6467-22
Ettan DALT*twelve* Large Vertical System	80-6466-27
Immobline DryStrip pH 3–10 NL, 24 cm	17-6002-45
PlusOne™ Bind-Silane	17-1330-01
PlusOne ReadySol™ IEF 40% T, 3% C	17-1310-01
PlusOne TEMED	17-1312-01
PlusOne Ammonium Persulfate	17-1311-01
PlusOne Sodium Dodecylsulfate (SDS)	17-1313-01
PlusOne Tris	17-1321-01
PlusOne Glycine	17-1323-01
DeStreak™ Rehydration Solution	17-6003-19
Pharmalyte™, broad range pH 3–10	17-0456-01
Immobiline DryStrip Cover Fluid	17-1335-01
Trifluoroacetic Acid	17-6002-76

Amersham Biosciences

Imaging

Typhoon™ 9410 Variable Mode Imager & ImageQuant™ Image Analysis Software	63-0038-55
ImageScanner™ II	18-1170-84

Staining

Deep Purple™ Total Protein Stain	RPN6305
PlusOne Coomassie Tablets, PhastGel™ Blue R350	17-0518-01
PlusOne Silver Staining Kit, Protein	17-1150-01

Digestion and mass spectrometry

Ettan Trypsin, sequencing grade	17-6002-75
Ettan MALDI-Tof Pro	18-1156-53

Other materials required

Decon™ 90 (Decon Laboratories Ltd)
Hydrochloric acid
Methanol
Glacial acetic acid
Ammonia solution, puris, 25–30%
Acetonitrile
Ammonium bicarbonate
Potassium ferricyanide
Sodium thiosulphate
Recrystallized αCHCA (alpha-cyano-4-hydroxy-cinnamic acid) (LaserBioLabs)
Crew™ wipers (Aldrich)

Methods

1-D and 2-D electrophoresis

Using the Immobiline DryStrip Reswelling Tray, 24 cm Immobiline DryStrips pH 3–10 NL were rehydrated overnight in DeStreak Rehydration Solution including 500 µg *E. coli* protein. Isoelectric focusing (IEF) was carried out the next day using an Ettan IPGphor II IEF System with an Ettan IPGphor Manifold. The run included three phases; 500 V for 8 h; a gradient from 500 to 4000 V for 2 h; and 8000 V for 7 h. After equilibration (5) the strips were loaded on four large format 12.5% SDS-PAGE (Laemmli) gels. Gel solutions were prepared using standard procedures with PlusOne electrophoresis reagents and cast on DALT*twelve* Gel Caster with PlusOne Bind-Silane-coated low-fluorescence glass plates (26 x 20 cm) and 1 mm spacers. An overlay of 0.5% (w/v) agarose in running buffer containing 0.001% bromophenol blue was applied. Second-dimension electrophoresis was performed using the Ettan DALT*twelve* Large Vertical System. Initially, gels were run at 2.5 W/gel for 30 min and then at 15 W/gel until the bromophenol dye front reached the bottom of the gel.

Deep Purple total protein staining and detection

After electrophoresis, the glass plates were disassembled and the gels placed overnight in fixation solution (7.5% [v/v] acetic acid, 10% [v/v] methanol). Staining was performed the next day following the standard protocol (6). After staining, the glass-backed gels were imaged using a Typhoon 9410 Variable Mode Imager. Imaging was performed using a 532 nm laser for excitation with a 560LP emission filter and a photo multiplier tube set to 460 V.

Post-staining 2-D gels with Coomassie Blue and silver

After imaging, two of the gels stained with Deep Purple Total Protein Stain were prepared for Coomassie Blue staining by fixing them overnight in 7.5% [v/v] acetic acid and 10% [v/v] methanol. The other two gels stained with Deep Purple Total Protein Stain were also prepared for silver staining through overnight fixation in 40% [v/v] ethanol and 10% [v/v] acetic acid. Coomassie Blue staining was performed the next day for 1.5 h according to standard protocol; gels were then placed in a destaining solution (20% [v/v] methanol, 5% [v/v] acetic acid), with one or two solution changes until the background was clear. Scanning was performed using the ImageScanner flatbed scanner at a setting of 300 dpi. Silver staining was also performed following the overnight fixation using the PlusOne Silver Staining Kit for proteins. The silver staining protocol was modified to make the staining compatible with mass spectrometry (7), which requires the omission of both glutaraldehyde in the sensitizer solution and formaldehyde from the silver solution, while using double the amount of formaldehyde in the developer. The silver stain was developed for 10 min for all gels and scanning was performed as with the Coomassie stained gels.

Mass spectrometry and protein identification

Before manual spot picking was performed, the gels were placed in highly pure water for at least 2 h. Eight spots from all four gels were chosen randomly and were manually picked into microplates, and then digested with Ettan Trypsin enzyme according to protocol (8) using the same volumes but with modifications for the manual method. Following digestion and extraction, the peptides were spotted onto MALDI targets. Peptide-mass fingerprinting was performed using Ettan MALDI-ToF Pro following the manufacturer's recommended protocol (9). MALDI-generated mass spectra were internally calibrated using trypsin peaks. The ProFound search engine (10) was used to search peptide masses against the National Center for Biotechnology Information non-redundant protein sequence database (11).

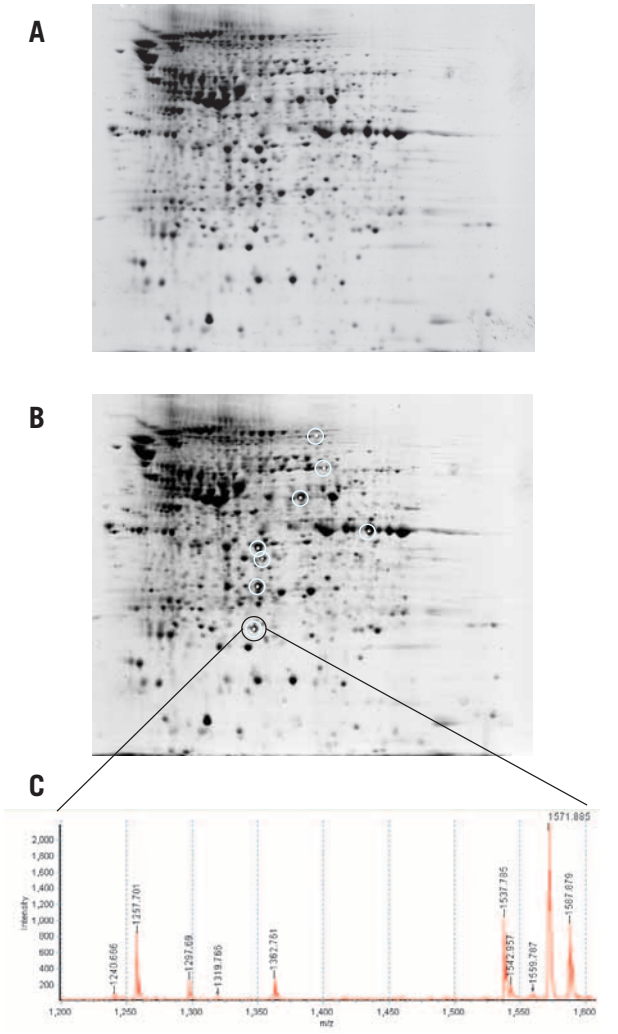

Fig 2. Gel dual stained with Deep Purple Total Protein Stain and Coomassie Blue. **A.** Image of a gel treated first with Deep Purple Total Protein Stain and then with Coomassie Blue. **B.** Image of the same gel as in Figure 2A, but with manually picked spots indicated. **C.** Mass spectrum of the spot highlighted in Figure 2B.

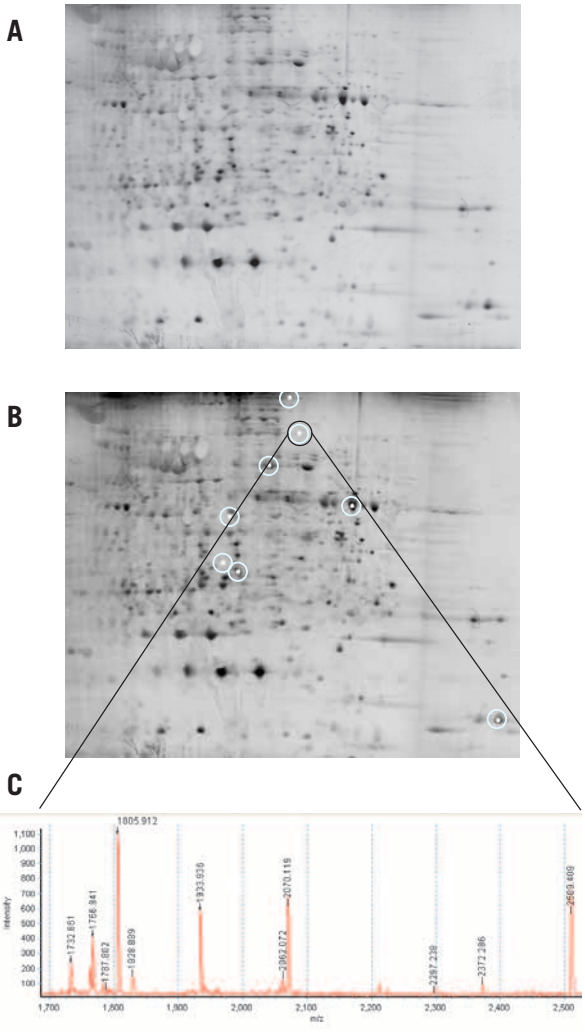

Fig 3. Gel dual stained with Deep Purple Total Protein Stain and silver. **A.** Image of a gel treated first with Deep Purple Total Protein Stain and then with silver stain. **B.** Image of the same gel as in Figure 3A, but with manually picked spots indicated. **C.** Mass spectrum of the spot highlighted in Figure 3B.

Results and discussion

Colorimetric post-staining of 2-D gels

Deep Purple Total Protein Stain provides a clear, even background that produces a high signal-to-noise ratio and good proportionality between the fluorescent signal and protein level. As a result, the stain is highly sensitive with reliable quantitation. To be able to further investigate any spots of interest using mass spectrometry when spot picking needs to be performed manually, poststaining with Coomassie Blue or silver makes the gel spots visible to the naked eye. Figure 1 shows a 2-D gel run with *E. coli* total protein with Deep Purple Total Protein Stain. Figure 2 shows gels successfully stained first with Deep Purple Total Protein Stain and subsequently with Coomassie Blue. Eight spots per gel were selected for manual picking (Fig 2B). Similarly, gels initially treated with Deep Purple Total Protein Stain were post-stained with silver, as shown in Figure 3. Eight spots per gel were also selected for manual picking from this collection of gels.

Digestion and protein identification

To verify that protein identification is not affected when gels stained with Deep Purple Total Protein Stain are post-stained with Coomassie Blue or silver, mass spectrometry was performed. Figures 2C and 3C show peptide-mass fingerprinting results for one Coomassie Blue-stained spot and one silver-stained spot. Ninety three percent of the proteins in the Coomassie Blue-stained plugs and 88% in the silver-treated plugs were positively identified. In addition, our results show that dual staining does not interfere with mass spectrometry determination. The spectra in Figures 2C and 3C have a low background signal, making the spectra easy to interpret and resulting in good scores for expectation and sequence coverage.

Conclusion

Dual staining, first with Deep Purple Total Protein Stain and then with Coomassie Blue or silver, takes advantages of the strengths of each stain. Deep Purple Total Protein Stain provides high sensitivity and enables quantitation of both abundant and scarce proteins. Post-staining gels with Coomassie Blue or silver allows the protein spots to be clearly seen, enabling manual spot picking. Dual staining does not interfere with analysis by mass spectrometry and the resulting spectra exhibit low background, facilitating easy interpretation.

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