Detection and Quantitation of Proteins Using a Novel Fluorescent Dye

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Introduction

Protein detection and quantitation commonly utilize the ability of certain dyes to bind proteins nonspecifically with an accompanying change in the spectral properties of the bound dye. Fluorescent dyes with this ability are particularly useful for protein analysis due to the high detection sensitivity and dynamic range achievable using fluorescence. Such dyes are now widely applied in proteomic analyses, particularly as fluorescent gel stains (for example, SYPRO Ruby protein gel stain).

A novel dye, Flamingo Pink™, undergoes dramatic fluorescence enhancement in the presence of denatured protein. A gel-staining protocol using Flamingo Pink was developed, and the optimized formulation generated for staining has been termed Flamingo™ fluorescent gel stain. To investigate the utility of this product, 1-D and 2-D gels were stained and imaged. Limits of sensitivity under different imaging conditions and linearity with respect to protein load were evaluated. Detection sensitivity and compatibility with subsequent mass spectrometric analysis were compared between Flamingo and SYPRO Ruby.

Results

Fluorescence of Flamingo Pink was almost undetectable in the absence of protein and markedly enhanced in the presence of 100 µg/ml protein (Figure 1).

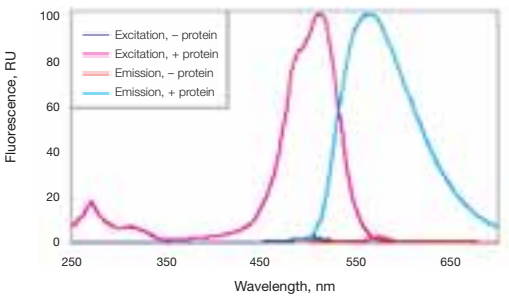


Fig. 1. Fluorescence spectra of Flamingo Pink in the presence and absence of protein. Fluorescence spectra generated by 165 nM Flamingo Pink in 50 mM sodium formate, pH 4.0, with and without 100 µg/ml chicken trypsin inhibitor (added as a 1:100 dilution of 10 mg/ml protein in 8 M urea). Excitation and emission wavelengths were 480 nm and 610 nm, respectively.

Fluorescence of Flamingo Pink increased linearly across the range of protein concentrations tested (4–80 µg/ml) (Figure 2).

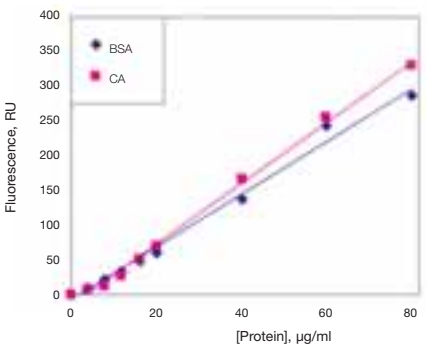


Fig. 2. Protein quantitation in solution. Fluorescence measurements (excitation 510 nm, emission 540 nm) were taken of 165 nM Flamingo Pink in 50 mM sodium formate, pH 4.0, containing various concentrations of urea-denatured bovine serum albumin (BSA) or bovine carbonic anhydrase (CA). Averages of two measurements for each protein are shown.

Table 1. Flamingo fluorescent gel stain protocol.

Step	Reagent	Volume*	Time
Fix	40% ethanol, 10% acetic acid	200 ml	>2 hr
Stain	Flamingo fluorescent gel stain	100 ml	>3 hr**
Background reduction***	0.1% (w/v) Tween 20	200 ml	10 min

* Volumes given are those needed to stain a 13 x 9 cm gel.

** Optimal staining sensitivity is reached within 3 hr. Longer staining (8 hr to overnight) is required for optimal linearity.

*** Destaining is not normally required for gels stained with Flamingo, but this step can result in some background reduction.

Flamingo fluorescent gel stain allowed imaging and detection of as little as 0.25 ng protein using 532 nm laser excitation, and 0.5 ng protein with 300 nm UV transillumination (Figure 3).

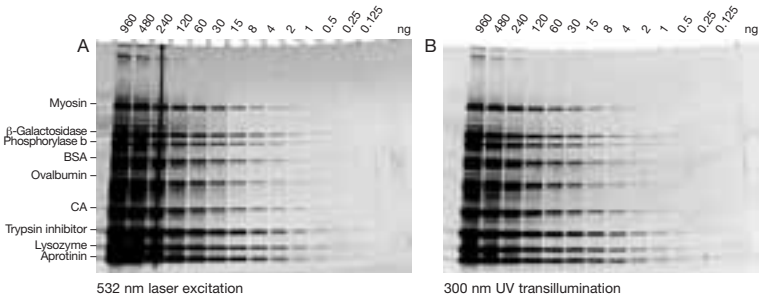


Fig. 3. Sensitivity of protein detection using Flamingo with two different imaging systems. A dilution series of Bio-Rad SDS-PAGE standards was run on 4–20% Criterion™ Tris-HCl gels. Each protein was loaded in the amount (in ng) indicated, and gels were stained with Flamingo. **A**, Gel imaged using 532 nm laser excitation and the Molecular Imager FX™ system (555 nm longpass emission). **B**, Gel imaged with 300 nm UV transillumination using the VersaDoc™ imaging system (5 min exposure). Gel images were transformed to optimize visual limit of sensitivity.

Fluorescence measured after staining with Flamingo increased linearly with increasing protein loads, from the limit of detection (0.25 ng) to close to 1 µg protein (Figure 4).

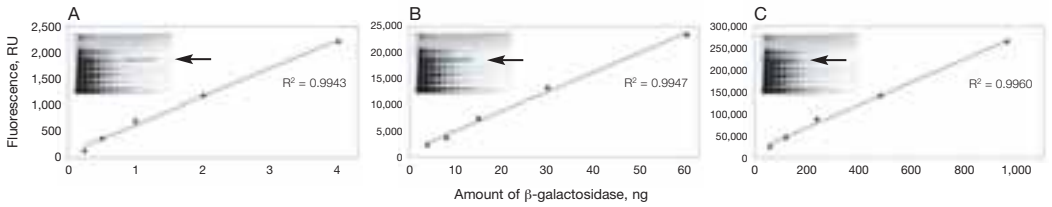


Fig. 4. Determination of linear dynamic range of Flamingo. A dilution series of Bio-Rad SDS-PAGE standards was run on a 4–20% Criterion Tris-HCl gel and stained with Flamingo. The gel was imaged using the Molecular Imager FX system. Fluorescence from each β-galactosidase band was quantitated using Quantity One® software following background subtraction. Shown is β-galactosidase band fluorescence in the range **A**, 0.25–4 ng; **B**, 4–60 ng; **C**, 60–960 ng. Inset gel images show the bands selected for quantitation.

Following 2-D electrophoresis, Flamingo allowed visualization of as many or more proteins from a total *E. coli* lysate as did SYPRO Ruby (Figure 5).

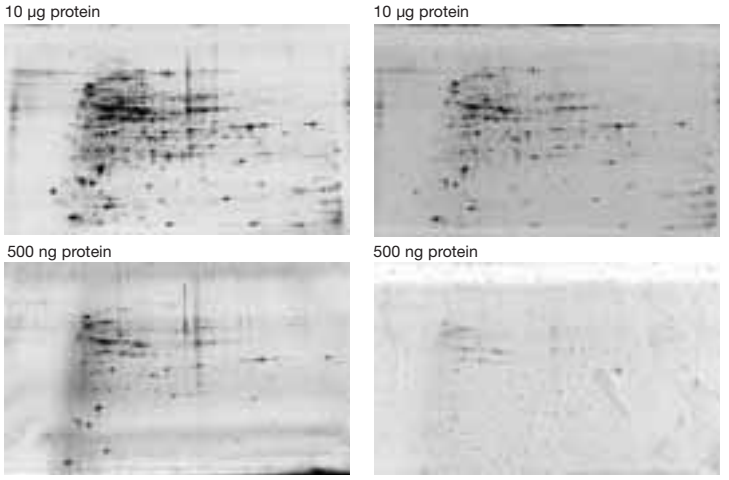
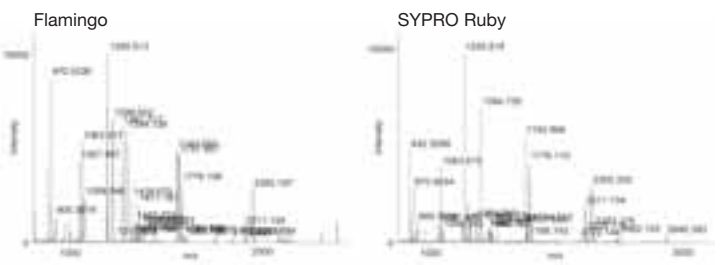


Fig. 5. Comparison of 2-D gel staining with Flamingo versus SYPRO Ruby. Total *E. coli* protein samples in the amounts indicated were separated by 2-D electrophoresis. Separation in the first dimension was performed using 11 cm pH 3–10 NL ReadyStrip™ IPG strips and in the second dimension using 8–16% Criterion Tris-HCl gels. Gels were stained with either Flamingo, as described in Table 1, or SYPRO Ruby, according to the manfacturer's instructions. All gel images were generated with the Molecular Imager FX system using default instrument settings found to be optimal for SYPRO Ruby (532 nm excitation, 555 nm longpass emission). Gel images were transformed manually to maximize the number of visible spots.

MALDI tryptic peptide analysis of a protein recovered from a 2-D gel stained with Flamingo yielded more identifiable peptides than a similar analysis of protein from a gel stained with SYPRO Ruby (Figure 6).



33 peaks (out of a possible 55) correspond with theoretical *E. coli* β-galactosidase tryptic peptides.

25 peaks (out of a possible 55) correspond with theoretical *E. coli* β-galactosidase tryptic peptides.

Fig. 6. MALDI tryptic peptide mass fingerprinting from stained gels. Gel plugs containing approximately 24 ng *E. coli* β-galactosidase were excised from SDS-PAGE gels that had been stained either with Flamingo or SYPRO Ruby, and the recovered protein was subjected to MALDI tryptic peptide mass analysis (Proteome Research Services, Ann Arbor, MI).

Conclusions

- Flamingo Pink undergoes pronounced fluorescence enhancement in the presence of protein.
- Fluorescence enhancement of Flamingo Pink is linearly related to protein concentration and can be used to quantitate protein in solution.
- Flamingo fluorescent gel stain can be used to stain SDS-PAGE gels using a simple protocol requiring as little as two steps and 5 hr.
- Flamingo allows protein detection down to 0.25 ng in gels imaged using 532 nm laser excitation.
- Flamingo allows protein detection down to 0.5 ng in gels imaged using 300 nm UV transillumination.
- Flamingo allows linear quantitation of gel-separated proteins in a wide mass range covering 3 orders of magnitude (0.25–960 ng).
- Flamingo is compatible with 2-D gel analysis and allows visualization of separated proteins with greater sensitivity than SYPRO Ruby.
- Flamingo is compatible with MALDI tryptic peptide mass fingerprinting and compares favorably to SYPRO Ruby.