Comparative Performance of Fluorescent Total Protein Stains on One- and Two-Dimensional PAGE Gels

Courtenay Hart and Steve Harwood
Invitrogen Corporation • 1600 Faraday Avenue • Carlsbad, California 92008 • USA

Introduction

In this study, the staining performance of four fluorescent stains was compared using Bis-Tris (NuPAGE® Novex® gels) and Tris-Glycine (Novex® gels) buffered polyacrylamide gels in 1-D and 2-D formats.

Four stains were evaluated: SYPRO® Ruby Protein gel stain (Invitrogen), Lucy 506 gel stain (Sigma), Flamingo™ gel stain (Bio-Rad), and Deep Purple™ gel stain (Amersham). Each stain was used in accordance with the manufacturer's recommended protocol (see Table 1). Gels were imaged with several instruments at various excitation wavelengths and emission filters to determine the optimal imaging conditions for each stain. Imagers used were the Lumi-Imager™ (Roche), UV transillumination with a cooled CCD camera and 520/20 bandpass (bp) (Lucy) or 600/20 bp emission filter (all others); the SafeImager™ blue-light transilluminator (Invitrogen) in a GeneGenius Bioimaging system (Syngene), blue transillumination with an orange plastic filter; the FLA-3000 laser scanner (Fuji Instruments) with 473 nm excitation with 520 nm longpass (LP) emission or 532 nm excitation with 580 nm LP; and the FX Pro Plus (Bio-Rad), 488 nm excitation with 555 nm LP emission filter. Fluorescent images were analyzed using Progenesis™ PG240, Totallab™ TL120, and TT900S2S software packages (Nonlinear Dynamics).

The mechanism of SYPRO® Ruby gel stain binding is through electrostatic interaction of the disulfonates with basic amino acid residues and through coordination of the ruthenium atom with the polypeptide backbone (1). Fixation to remove SDS reduces background and improves sensitivity. Lucy is a nonfixative stain that binds protein via hydrophobic interaction with the lypophylic tail of SDS bound to protein (Sigma product insert). Flamingo™ is a fixative stain that is nonfluorescent in solution but becomes fluorescent when bound to protein (Bio-Rad product insert). Deep Purple™ is a nonfluorescent dye that becomes fluorescent upon conversion with base or reaction with lysine amines (3,4). The primary mechanism of dye binding is through hydrophobic interaction with the lypophilic tail of SDS bound to protein. The stain employs a weak (low % alcohol) fixation to remove SDS from the gel background and the dye binds both to lysines and residual SDS present in the gel and bound to the protein (3,4).

Stains that bind proteins via SDS may exhibit differential staining of proteins, as not all proteins bind SDS to saturation. Examples of proteins that bind significantly less SDS are glycoproteins and proteins at their isoelectric point. Also, SDS derivatization is not performed at elevated temperature for 2-D GE as it is for 1-D PAGE, such that less SDS is bound to proteins on 2-D gels (3,4).

Table 1 – Summary of Staining Protocols

Protocol Step	SYPRO® Ruby Stain	Deep Purple™ Stain	Lucy 506 Stain	Flamingo™ Stain
Fixation	2X 15 min 50% methanol/ 10% acetic acid	1 hour 10% methanol/ 7.5% acetic acid	None	120 min 40% ethanol/ 10% acetic acid
Wash	None	30 min sodium bicarbonate	None	None
Stain	No dilution 30 min heated	200X dilution 60 min	5000X dilution 60 min	10X dilution 180 min
Destain	30 min methanol/ acetic acid, water rinse	2X 15 min methanol/ acetic acid	Brief acetic acid, water rinse	(optional) 10 min 0.1% Tween 20, water rinse

Figure 1 – Estimated Lower Limit of Detection for Gel Stains

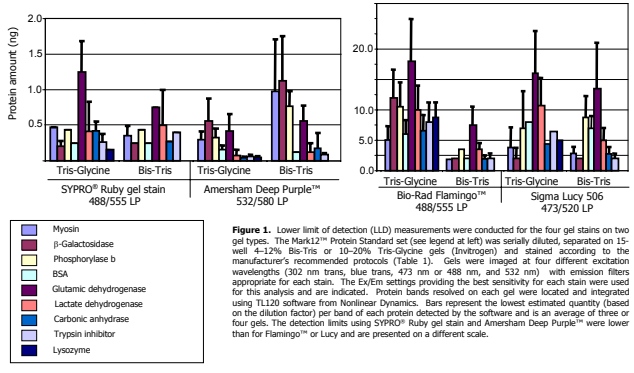

Figure 1. Lower limit of detection (LLD) measurements were conducted for the four gel stains on two gel types. The Mark12™ Protein Standard set (see legend at left) was serially diluted, separated on 15-well 4–12% Bis-Tris or 10–20% Tris-Glycine gels (Invitrogen) and stained according to the manufacturer's recommended protocols (Table 1). Gels were imaged at four different excitation wavelengths (302 nm trans, blue trans, 473 nm or 488 nm, and 532 nm) with emission filters appropriate for each stain. The Ex/Em settings providing the best sensitivity for each stain were used for this analysis and are indicated. Protein bands resolved on each gel were located and integrated using TL120 software from Nonlinear Dynamics. Bars represent the lowest estimated quantity (based on the dilution factor) per band of each protein detected by the software and is an average of three or four gels. The detection limits using SYPRO® Ruby gel stain and Amersham Deep Purple™ were lower than for Flamingo™ or Lucy and are presented on a different scale.

Figure 2 – Response of Fluorescent Gel Stains as a Function of Protein Quantity

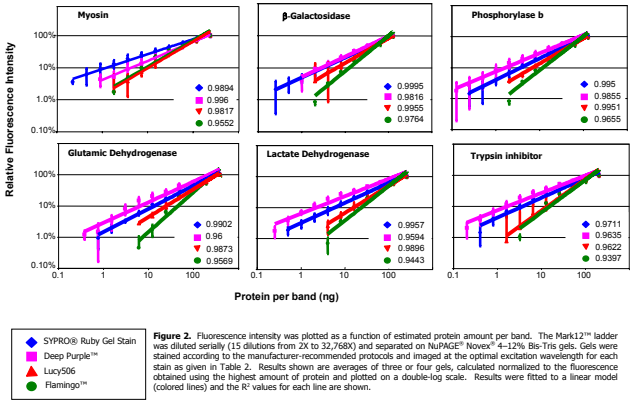

Figure 2. Fluorescence intensity was plotted as a function of estimated protein amount per band. The Mark12™ ladder was diluted serially (15 dilutions from 2X to 32,768X) and separated on NuPAGE® Novex® 4–12% Bis-Tris gels. Gels were stained according to the manufacturer-recommended protocols and imaged at the optimal excitation wavelength for each stain as given in Table 2. Results shown are averages of three or four gels, calculated normalized to the fluorescence obtained using the highest amount of protein and plotted on a double-log scale. Results were fitted to a linear model (colored lines) and the R^2 values for each line are shown.

Figure 3 – Differential Protein Detection in 1-D Gels

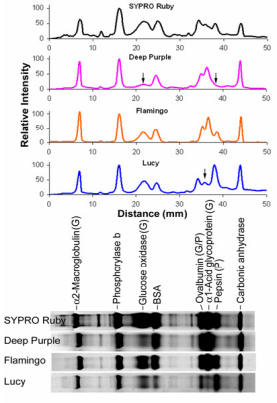

Figure 3. A mixture of glycosylated (G), phosphorylated (P), and nonglycosylated/phosphorylated proteins, all at 125 ng/band were separated on 4–12% Bis-Tris gels and stained according to the manufacturer's recommended protocols (Table 1). Gels were imaged at the excitation/emission settings providing the best sensitivity for each stain, as indicated in Table 2. The fluorescence intensity of each stain was measured and normalized to the phosphorylase b peak. Gel bands in the lower image directly correlate with peaks in the upper image. Arrows indicate weakly stained proteins.

Figure 4 – Staining of 2-D Gels

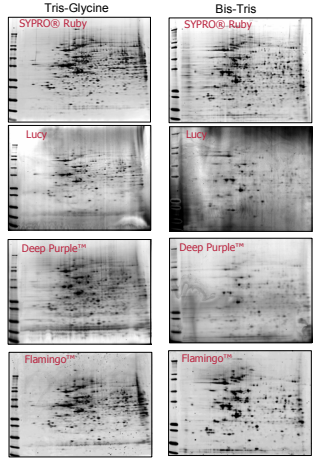

Figure 4. Jurkat extracts (10 µg protein) were separated using the Zoom® benchtop proteomics platform using pH 4-7 IPG strips for isoelectric focusing and second dimension SDS-PAGE on 4-12% Bis-Tris gels or 10-20% Tris-Glycine gels and stained according to the manufacturer's recommended protocols (Table 1). Gels were imaged at the excitation/ emission settings providing the best sensitivity for each stain, as indicated in Table 2. All gels of each gel type for all stains (12 gels) were first warped using TT900S2S software. Triplicate gels of each stain were grouped and the same area of each gel was selected for spot detection using Progenesis PG240 software. Spots were counted if they were detected in at least two of three gels.

Figure 5 – Comparison of Relative Fluorescent Intensities

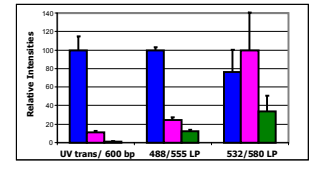

Figure 5. Direct comparison of the fluorescent intensities of an 8X dilution of Mark12™ markers in 10–20% Tris-Glycine gels (averaged for 9 proteins) at three excitations, background subtracted, and normalized to the intensity of the brightest stain at each excitation. Lucy uses a green emission filter, so its signal could not be directly compared.

Table 2 – Summary of Results

Attribute	SYPRO® Ruby Stain	Deep Purple™ Stain	Lucy 506 Stain	Flamingo™ Stain
Optimal Wavelength excitation/emission nm	488/555 LP	532/580 LP	473/520 LP	488/555 LP
Sensitivity 1-D gels 2-D gels (number spots detected)	Tris-Glycine Bis-Tris 0.43 ng 0.4 ng 806 838	Tris-Glycine Bis-Tris 0.2 ng 0.49 ng 617 436	Tris-Glycine Bis-Tris 7.0 ng 5.9 ng 461 240	Tris-Glycine Bis-Tris 9.1 ng 3.0 ng 436 234
Minimum protocol time	90 minutes	180 minutes	60 minutes	310 minutes
Solution changes	5	5	3	2 or 4
Hands-on time	15 min	25 min	5 min	10 min
Storage/Stability	RT 9 months	-20°C 3 months	4°C	4°C 6 months
Multiplexing compatible	Yes	No	Not determined	Not determined

Conclusions

- Sensitivity of Ruby® gel stain and Deep Purple™ stain were roughly equivalent on 1-D gels with both stains detecting on average less than 0.5 ng protein for either gel type. The sensitivity of SYPRO® Ruby gel stain was consistent between gel types and showed little size bias, whereas Deep Purple™ stain tended to have lower sensitivity on Bis-Tris gels and with larger proteins (Fig. 1). Lucy and Flamingo™ gel stains were approximately one order of magnitude less sensitive than SYPRO® Ruby gel stain on 1-D gels and tended to have lower sensitivity on Bis-Tris gels (Fig. 1).
- Of all stains tested, SYPRO® Ruby gel stain gave the most sensitive results on 2-D gels; Deep Purple™, Lucy, and Flamingo™ stains detected 23%, 42% and 46% fewer spots, respectively on TG gels and 48%, 71%, and 72% fewer spots respectively on BT gels.
- Differential Staining of protein types: Deep Purple™ stain showed poor staining of the glycoprotein glucose oxidase as well as the phosphoprotein pepsin, while Lucy showed poor staining of the glycoprotein α_1 acid glycoprotein as well as reduced staining of glucose oxidase. This may be explained by the SDS-meditated mechanism by which these two stains bind protein.
- All stains provided a near linear response with varying amounts of protein. R^2 values ranged from 0.94 to 0.9995 (Fig. 2).
- When the stains were directly compared using the same protein sample and the same emission filter set (Fig. 5):
  - With UV excitation, SYPRO® Ruby gel stain was nine times brighter than Deep Purple™ stain and 83 times brighter than Flamingo™ stain.
  - With 488 excitation, SYPRO® Ruby gel stain was 4 times brighter than Deep Purple™ stain and 8 times brighter than Flamingo™ stain.
  - With 532 nm excitation, Deep Purple™ stain was 1.3 times brighter than SYPRO® Ruby gel stain and 3 times brighter than Flamingo™ stain.

References

1. Berggren, K. N. (2002). Proteomics 2(5): 486-98.
2. Chevalier, F. (2004). Phytochemistry 65(11): 1499-506.
3. Smejkal, G. (2004). Expert Rev. Proteomics 1(4): 381-387.
4. Smejkal, G. B., (2004). Electrophoresis 25(15): 2511-9.