Oriole™ Fluorescent Gel Stain: Characterization and Comparison with SYPRO Ruby Gel Stain

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Introduction

Fluorescent stains are widely used to visualize and quantitate proteins following SDS-PAGE due to their high sensitivity and wide dynamic range. Oriole fluorescent gel stain is a novel reagent for staining SDS-PAGE gels. It is applied in a simple one-step staining protocol, and is optimally imaged using UV transillumination. The ability to be imaged using UV light is a desirable feature because UV-based imaging equipment is relatively simple and inexpensive and tends to be more generally available to laboratory researchers.

SYPRO Ruby protein gel stain is another commercial reagent for fluorescent staining of SDS-PAGE gels. It shares with Oriole stain the property of bright fluorescence when excited with UV light, but requires a more complicated and time consuming staining protocol. In this study, the performance of Oriole gel stain is characterized with respect to its spectral properties, detection sensitivity, protein-to-protein variability, and compatibility with mass spectrometry. These properties are evaluated relative to SYPRO Ruby stain.

Methods

Fluorescence spectra were generated with a Cary Eclipse fluorescence spectrophotometer (Varian, Inc.). Criterion™ Tris-HCl, 18-well, 4–20% linear gradient gels (Bio-Rad Laboratories, Inc.) were used for SDS-PAGE. Gels were loaded with a dilution series of broad range SDS-PAGE standards (Bio-Rad). Dilutions were prepared to give the following sample load (for each individual protein) in a loading volume of 5 µl: 960 ng, 480 ng, 240 ng, 120 ng, 60 ng, 30 ng, 15 ng, 8 ng, 4 ng, 2 ng, 1 ng, 0.5 ng, 0.25 ng, 125 pg, and 60 pg. 2-D gels were run using 40 µg of *E. coli* total protein. First dimension was run on 11 cm pH 5–8 ReadyStrip™ IPG strips (Bio-Rad). Second dimension was run on Criterion Tris-HCl 8–16% linear gradient SDS-PAGE gels. Following electrophoresis, gels to be stained with Oriole stain were stained for 1.5 hr (unless otherwise indicated) in 100 ml of staining solution. No fixing or destaining treatments were employed. Gels to be stained with SYPRO Ruby were stained according to manufacturer's instructions.

Following staining, gels were transferred to water and photographed with the Molecular Imager® VersaDoc™ MP 4000 imaging system (Bio-Rad) using UV transillumination, a 520 nm LP filter, and a 10 sec exposure. Images were analyzed with Quantity One® software (Bio-Rad).

Gels used for evaluation of mass spectrometric protein identification were loaded with dilutions of broad range SDS-PAGE standards (960, 240, and 60 ng) and stained with either Oriole or SYPRO Ruby gel stains. Gel plugs were excised from each dilution of β-galactosidase, ovalbumin, and lysozyme using the EXQuest™ spot cutter (Bio-Rad). Following excision, the gel plugs were treated with 20 µl of 5 µg/ml trypsin in 50 mM ammonium carbonate buffer, pH 8.0. Eluted peptides were cleaned up with ZipTip C18 pipette tips (Millipore) and spotted on a MALDI target prespotted with 2,5 dihydroxybenzoic acid matrix. Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) was performed using an autoflex II instrument and the data were analyzed using flex Analysis software (both from Bruker Daltonics).

Staining Protocol Comparison

The basic staining protocol for SYPRO Ruby stain consists of four steps and lasts overnight plus 90 min. Oriole stain uses a single step protocol that lasts 90 min (Table 1).

Table 1. Basic staining protocols for SYPRO Ruby and Oriole gel stains.

Step*	SYPRO Ruby Gel Stain	Oriole Gel Stain
Fixing	Fix 2 x 30 min (Total time: 1 hr)	(No fix step)
Staining	Stain overnight	Stain 90 min
Destaining or washing	Wash 30 min	(No destain or wash step)

* All steps were carried out at room temperature.

Results and Discussion

Fluorescence Spectra

Oriole fluorescent stain has broad UV excitation with a maximum around 270 nm (Figure 1). It is thus optimally imaged using UV transillumination. UV transilluminator-based imaging systems typically use lamps that emit broad spectrum UV light centered around 300 nm, which is very compatible with the excitation properties of Oriole stain. Oriole stain is poorly excited by visible light (above 400 nm), so compatibility with imaging systems that use visible light for excitation is not expected.

The fluorescence emission of Oriole stain is broad as well, covering most of the visible range with a maximum around 600 nm (Figure 1). The emission is visibly red-orange in color. There is practically no overlap between excitation and emission, so low-background imaging is possible simply using a longpass filter that excludes UV light. Most of the emitted light is transmitted, allowing short exposures with high sensitivity.

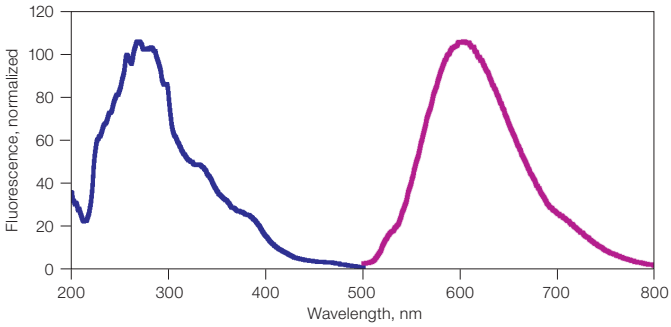


Fig. 1. Fluorescence excitation and emission spectra of Oriole stain. Oriole stain has its excitation maximum at 270 nm and emission maximum at 604 nm, making it compatible with UV-based imagers. —, Excitation spectrum; —, Emission spectrum.

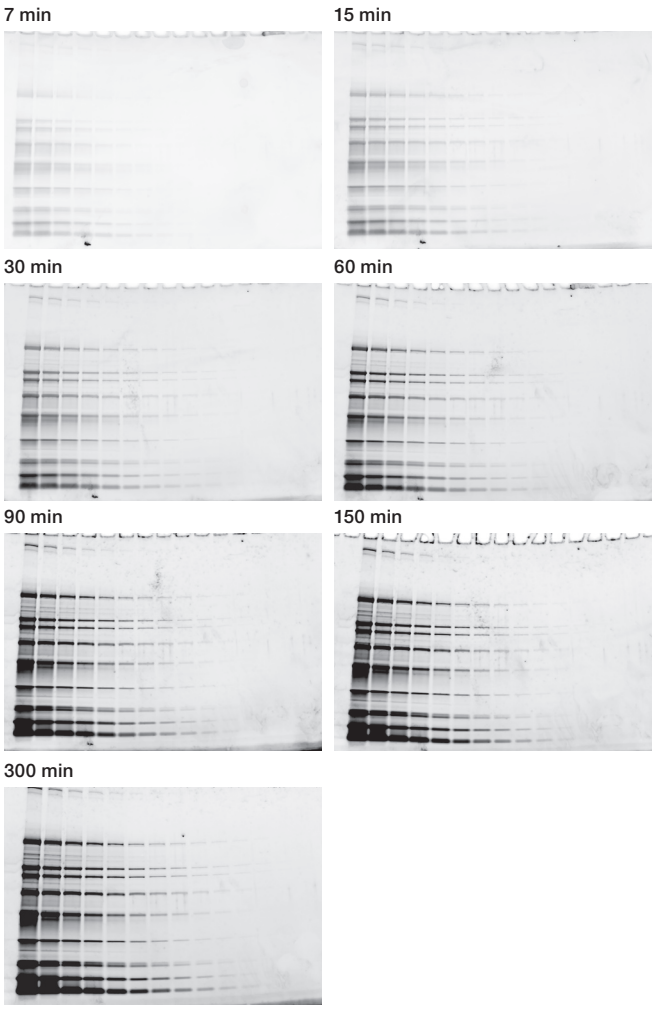


Fig. 2. Time course of staining. A single gel was stained with 100 ml of Oriole stain. The gel was imaged and returned to the staining solution at the indicated intervals from the commencement of staining.

Time Required for Optimal Staining

Staining with Oriole stain is complete within 90 minutes. Overstaining did not occur within 5 hours of staining (Figure 2).

Comparison to SYPRO Ruby Stain

Appearance of Stained Gels

The relative appearance, background, and sensitivity of gels stained with Oriole stain and SYPRO Ruby stain were evaluated using gels that were loaded, run, and imaged identically, and presented without adjusting or transforming the resulting images. A gel stained with Oriole stain and a gel stained with SYPRO Ruby stain gave images of comparable brightness (Figure 3). Standards bands are visible on both images out to the tenth or eleventh dilution (2 ng and 1 ng per band, respectively, on these gels). The gel stained with SYPRO Ruby stain showed visibly higher background staining than the gel stained with Oriole stain, but otherwise, the appearance of the two gel images is similar.

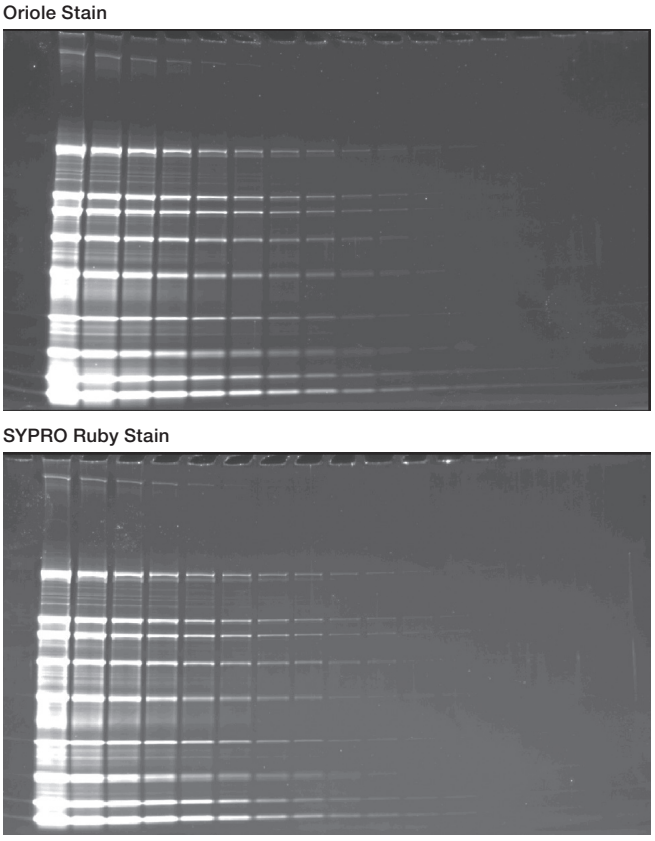
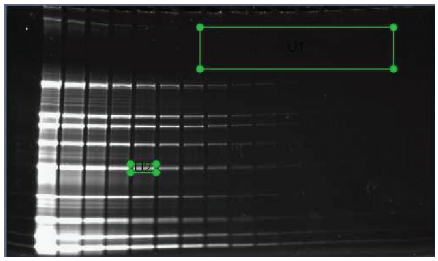


Fig. 3. Comparison of gels stained with Oriole and SYPRO Ruby stains. Unadjusted images of two identically loaded gels, stained with Oriole and SYPRO Ruby gel stains respectively. Both gels were imaged on a VersaDoc MP 4000 imaging system using UV transillumination, a 520 nm LP filter, and a 10 sec exposure.

Staining Intensity and Background

Background and staining intensity were evaluated from the gels shown in Figure 3 using Quantity One software. Background intensity was measured in a rectangular area containing no protein bands. Staining intensity of a representative protein band (60 ng ovalbumin) was determined following background subtraction. Figure 4 shows the position of the areas used for evaluation. As shown in Table 2, the staining intensity of a selected protein band is similar between Oriole stain and SYPRO Ruby stain, but the background intensity is significantly higher with SYPRO Ruby stain.

Oriole Stain



SYPRO Ruby Stain

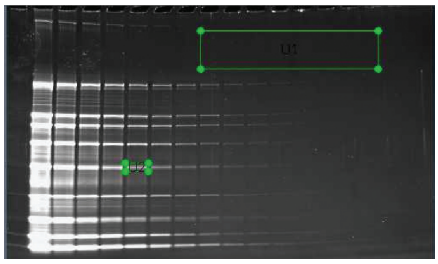


Fig. 4. Comparison of band intensity in gels stained with Oriole and SYPRO Ruby stains. The images shown in Figure 3 were analyzed with Quantity One software. Areas evaluated for background and staining intensity are indicated by the green boxes.

Table 2. Background and staining intensity of gels stained with Oriole and SYPRO Ruby stains.

Stain	Protein Band Intensity* (60 ng ovalbumin)	Background Intensity*
Oriole	1068	348
SYPRO Ruby	1154	578

* Arbitrary pixel intensity units per mm^2 of gel.

Limit of Sensitivity

Effective visualization of proteins loaded in low amounts generally requires some image transformation. Altering the pixel intensity scale can often allow faint protein bands to become more visible and give a lower apparent limit of sensitivity. The images shown in Figure 3 were independently adjusted to make the faintest bands more visible. Following this operation, the Oriole stain appears to be more sensitive than SYPRO Ruby stain as judged by the visual limit of detection (Figure 5).

Fig. 5. Limit of visual detection of protein bands. The images from Figure 2 were inverted, cropped to show protein loads ≤4 ng, and adjusted to show the limit of sensitivity. All of the standards are detectable on the Oriole-stained gel at a sample load of 0.5 ng/band whereas the limit of sensitivity on the SYPRO Ruby-stained gel is 1 ng/band.

Protein-to-Protein Variability

Ideally, a protein stain should exhibit little protein-to-protein staining variability. Staining intensity of a particular spot or band should depend only on the amount of protein present, not the individual properties of the protein(s) that result from amino acid composition or posttranslational modifications. In this regard, Oriole stain is comparable to SYPRO Ruby stain (Figure 6). Although some differences in protein-to-protein staining intensity are apparent with both stains, they exhibit a similar degree of protein-to-protein staining variability among different proteins.

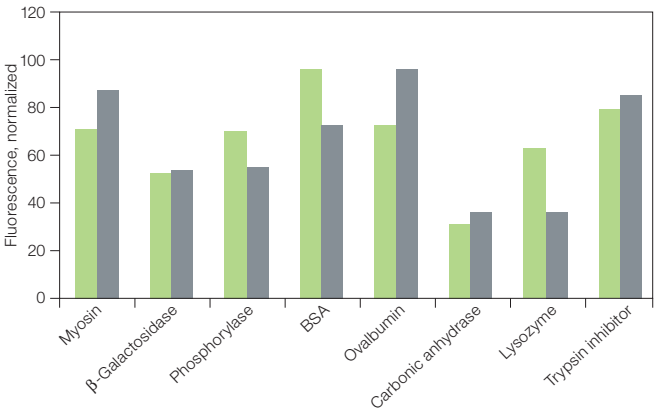


Fig. 6. Protein-to-protein variability in staining intensity. Fluorescence intensity of various proteins in gels stained with Oriole and SYPRO Ruby stains was measured. Fluorescence associated with protein bands containing 60 ng of protein was determined following image background subtraction. The data for each stain were normalized to the band exhibiting the most fluorescence (100). ■, SYPRO Ruby stain; ■, Oriole stain.

Table 3. Mass spectrometic analysis of proteins from gels stained with Oriole and SYPRO Ruby stains. Summary of data comparing MS-based protein identification between Oriole and SYPRO Ruby stain. The data were evaluated with respect to three different parameters — the number of peptides matched to known tryptic peptides, the percentage of the full sequence represented by the identified peptides, and the MASCOT score.

Protein	Approximate Quantity in Gel Plug, ng	# of Peptides Matched		% Coverage		MASCOT Score	
		Oriole	SYPRO Ruby	Oriole	SYPRO Ruby	Oriole	SYPRO Ruby
β-Galactosidase	160	52	40	62	45	212	82
	40	42	31	48	34	107	67
	10	18	19	16	18	79	33
Ovalbumin	160	25	23	48	71	32	107
	40	10	8	20	29	28	45
	10	15	11	48	32	50	26
Lysozyme	160	12	11	68	68	72	48
	40	9	15	53	58	53	56
	10	13	9	68	53	53	24

Compatibility with Mass Spectrometric Protein Identification

Fluorescent protein stains are widely used in conjunction with proteolytic digestion of excised protein followed by identification of the protein by mass spectrometric analysis of the resultant peptide mixture. Relative compatibility of the Oriole stain and the SYPRO Ruby stain with mass spectometric analysis was therefore evaluated. In the majority of cases, the performance of Oriole stain exceeded that of SYPRO Ruby stain in terms of number of peptides matched, percent coverage, and MASCOT score (Table 3).

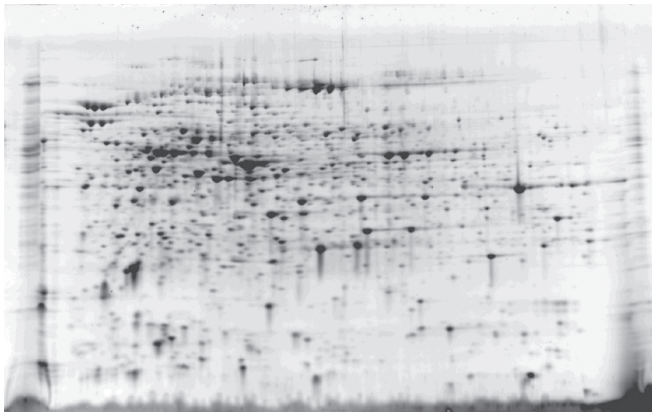


Fig. 7. 2-D gel stained with Oriole stain. *E. coli* protein (40 µg) was run on an 11 cm pH 5–8 ReadyStrip IPG strip for the first dimension and Criterion Tris-HCl 8–16% gel for the second dimension. The gel was stained with Oriole stain and was imaged on the Molecular Imager VersaDoc 4000 imaging system.

2-D Electrophoresis

Fluorescent stains are also widely applied to 2-D gel analysis. The high sensitivity and wide dynamic range afforded by fluorescence imaging is particularly useful in comparative proteomic studies of complex samples. A 2-D gel stained with Oriole stain exhibited a clear, sharp 2-D image without apparent interference by carrier ampholyte, CHAPS, or any other component of the 2-D sample (Figure 7).

Conclusions

A fluorescent stain must fulfill a number of criteria in order to be useful in proteomics applications. It should be sensitive enough to visualize minor sample components present at quantities down to 1 ng or less. It should exhibit minimal protein-to-protein variability in staining intensity. It should be fully compatible with the standard workflow consisting of separating proteins by 2-D electrophoresis and identifying them by peptide mass spectrometric fingerprinting. Oriole fluorescent stain fulfills all of these criteria and was found to equal or exceed the performance of SYPRO Ruby stain in all cases tested. Oriole stain shares with SYPRO Ruby stain the property of being very effectively excited by UV light. It is thus compatible with relatively simple and inexpensive UV light-based imaging systems. Additionally, Oriole stain is remarkably rapid and simple to use. It is unique among commonly used fluorescent protein stains in that complete staining is accomplished in a single 90-minute step without the need for fixing or destaining.

Information in this tech note was current as of the date of writing (2009) and not necessarily the date this version (Rev A, 2010) was published.

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