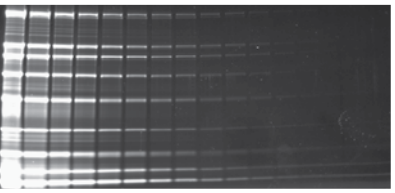

ELECTROPHORESIS

ORIOLE™ Fluorescent Gel Stain

- Rapid fluorescent gel staining. Complete procedure including imaging takes less than two hours
- Fully compatible with downstream proteolysis and mass spectrometric analysis
- Nanogram sensitivity and low background
- Wide dynamic range (3 orders of magnitude)

High Sensitivity Fluorescent Protein Staining in 90 Minutes

Introduction

Oriole fluorescent gel stain is an easy-to-use, rapid and sensitive fluorescent stain for the visualization and quantitation of proteins in SDS-PAGE gels. It is intended for use with imaging instruments that use UV light excitation.

The product is available in three configurations — 200 ml, 1 L, and 5 L.

- **The 200 ml size** — fully diluted and ready to use; provides enough stain for four gels run on a Mini-PROTEAN® system or two gels run on a Criterion™ system, and is a good size for evaluation or occasional use
- **The 1 L size** — also fully diluted and ready to use; provides enough stain for 20 gels run on a Mini-PROTEAN system, ten on a Criterion system, four on a PROTEAN® II system, or two on a PROTEAN Plus unit, and is the ideal size for routine use
- **The 5 L size** — contains concentrated components to prepare 5 L of staining solution and can be diluted to 1x according to demand. This is an ideal configuration for laboratories with high staining throughput and is economical to use

Rapid and Convenient Staining

Oriole fluorescent gel stain uses a one-step, room temperature staining protocol without prior fixing or subsequent destaining steps. The complete procedure, including imaging, can be completed in less than two hours (Table 1). This contrasts with SYPRO Ruby fluorescent gel stain, which requires fixing and destaining steps and for which overnight staining is recommended.

Table 1. Basic staining protocols for SYPRO Ruby and Oriole stains. All steps were carried out at room temperature.

Processing Step	SYPRO Ruby Stain	Oriole Stain
Fixing 1	30 min	None
Fixing 2	30 min	None
Staining	Overnight	90 min
Wash	30 min	None
Total time	~18 hr	90 min

BIO-RAD

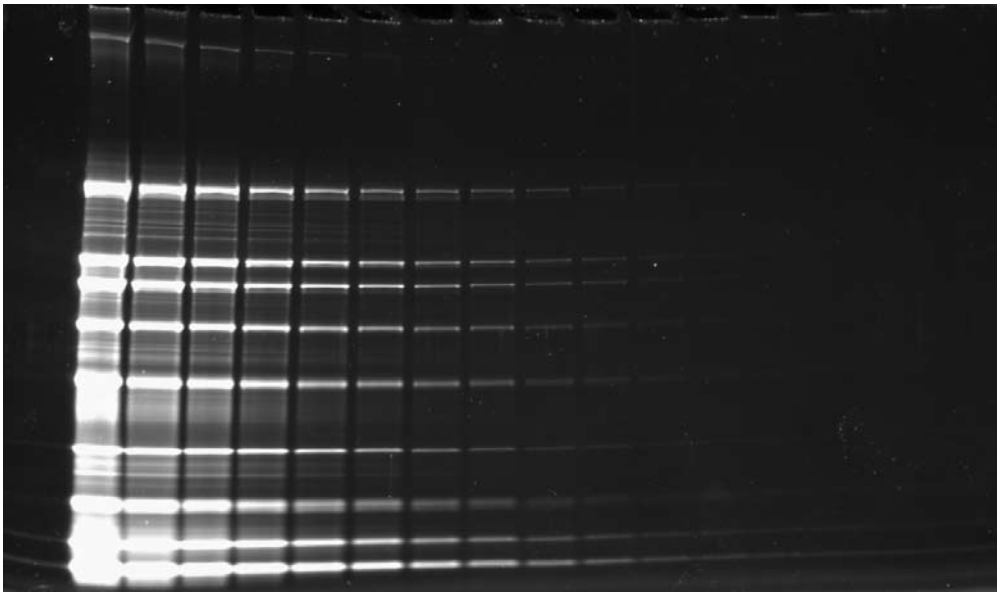

Fig. 1. Unadjusted image of a gel stained with Oriole stain. A dilution series of Bio-Rad SDS-PAGE standards was run on a 4–20% Criterion Tris-HCl linear gradient gel, stained with Oriole stain, and imaged using a Molecular Imager® VersaDoc™ 4000 imaging system with image settings for SYPRO Ruby stain. The resulting image file was not adjusted. Dilutions were prepared in a loading volume of 5 µl to give the following sample load (for each individual protein): 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, 60 pg.

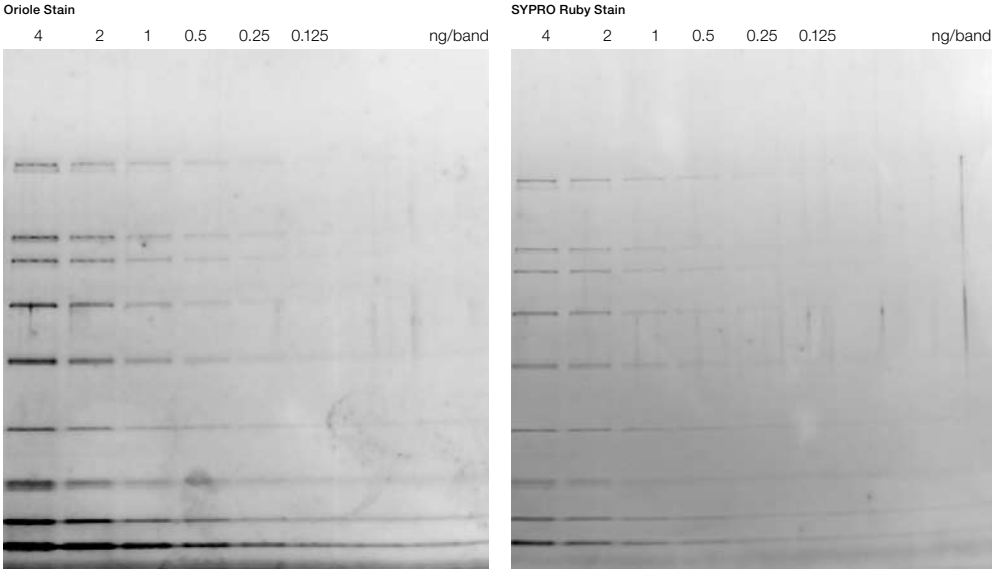

Fig. 2. Oriole stain versus SYPRO Ruby stain, images adjusted to show the limit of sensitivity. The image from Figure 1 was inverted, cropped to show protein loads ≤ 4 ng, and adjusted to show the limit of sensitivity. An identical gel was stained with SYPRO Ruby stain and imaged and adjusted similarly for comparison.

Nanogram Sensitivity and Low Background

The dye in Oriole stain is highly fluorescent and binds tightly to proteins. Background staining is low and the limit of sensitivity is below 1 ng. The sensitivity of Oriole stain is better than the sensitivity of SYPRO Ruby stain even with imager settings optimal for the SYPRO Ruby stain (Figures 1 and 2).

Compatibility With Mass Spectrometry

Oriole fluorescent protein gel stain is fully compatible with downstream mass spectrometric (MS) analysis. In the majority of cases, the performance of Oriole stain exceeds that of SYPRO Ruby stain in terms of the number of peptides matched and percent sequence coverage).

Wide Dynamic Range

Oriole stain has a linear range for quantitation that spans three orders of magnitude (Figure 3). This allows protein quantitation in samples of varying concentration over a wide range of relative abundance.

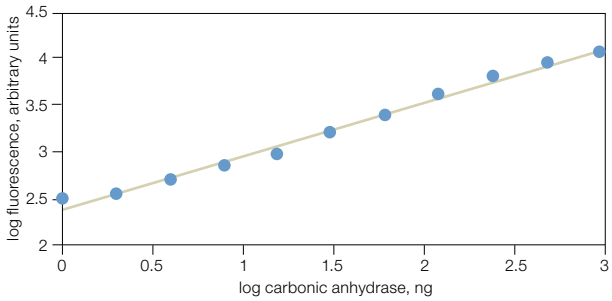

Fig. 3. Linearity of Oriole stain. A dilution series of carbonic anhydrase was run on a Criterion gel, stained with Oriole stain, and imaged on the Molecular Imager VersaDoc 4000 imaging system. Fluorescence associated with the carbonic anhydrase band was plotted following background subtraction.

Compatibility With Multiple Imaging Systems

Oriole fluorescent protein gel stain has broad UV excitation with a maximum at 270 nm and broad visible emission with a maximum at 604 nm (Figure 4). This allows excellent compatibility with a wide range of UV-based imaging equipment including all UV-based Bio-Rad imagers (Molecular Imager® GelDoc™ XR+, ChemiDoc™ XRS+, and VersaDoc imaging systems) and the UV epi-illumination-based EXQuest™ spot cutter.

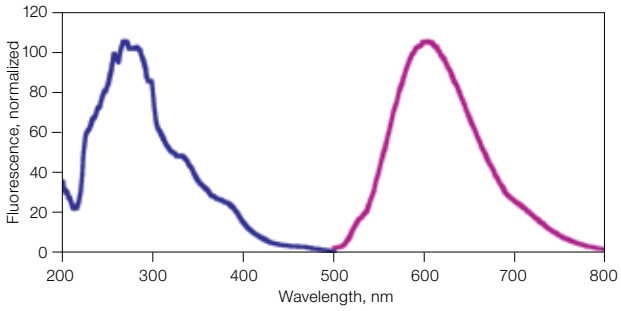

Fig. 4. Fluorescence excitation and emission spectra of Oriole stain. Oriole stain has its excitation maximum at 270 nm and emission maximum at 604 nm, making it compatible with UV-based imagers. —, Excitation spectrum; —, Emission spectrum.

Oriole stain is fully compatible with both 1-D and 2-D SDS-PAGE gels and gives excellent images of complex samples using the Molecular Imager GelDoc, ChemiDoc, and VersaDoc imagers (Figures 5, 6, and 7).

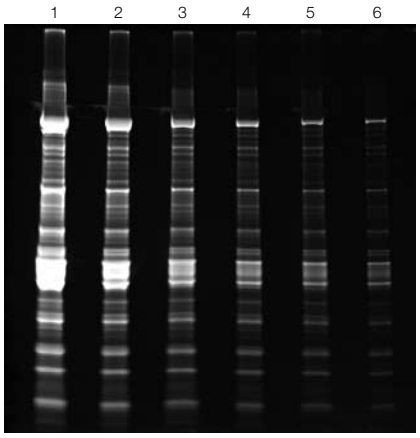

Fig. 5. 1-D gel stained with Oriole stain and imaged on a Molecular Imager GelDoc XR+ system. Dilutions of salmon muscle extract were run on a 4–20% Criterion gel, stained with Oriole stain, and imaged on the Molecular Imager GelDoc XR+ imaging system. Amount of protein loaded from lanes 1 through 6 was 20, 10, 5, 2.5, 1.25, 0.625 µg/lane.

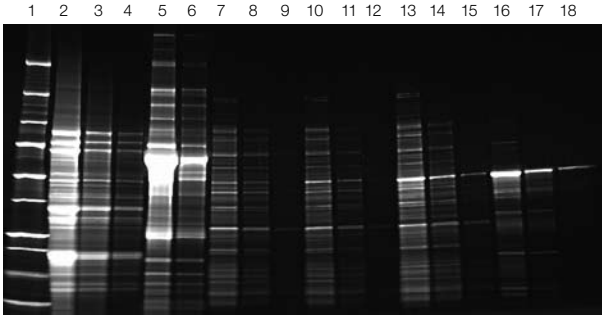

Fig. 6. 1-D gel stained with Oriole stain and imaged on a Molecular Imager ChemiDoc XRS+ system. Various samples were run on a 4–20% Criterion gel, stained with Oriole stain, and imaged on the Molecular Imager ChemiDoc XRS+ system. Lane 1, Precision Plus Protein™ prestained standards; lanes 2, 3, and 4, soy extract (20, 4 and 0.8 µg respectively); lanes 5 and 6, human serum (25 and 5 µg respectively); lanes 7, 8, and 9, flowthrough fractions of *E. coli* extract run on the Profinia™ protein purification system (2, 0.4, and 0.08 µl respectively); lanes 10, 11, and 12, wash fractions of *E. coli* extract (2, 0.4, and 0.08 µl respectively); lanes 13, 14, and 15, Profinia control lysate (2, 0.4, and 0.08 µl respectively); lanes 16, 17, and 18, elution fractions of *E. coli* extract (3.2, 0.64, 0.128 µg respectively).

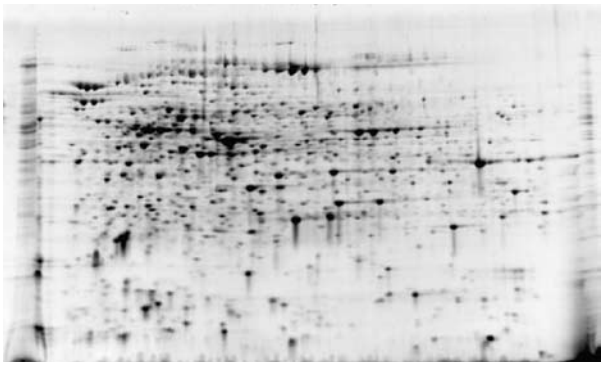

Fig. 7. 2-D gel stained with Oriole stain. *E. coli* protein (40 µg) was run on an 11 cm pH 5–8 ReadyStrip™ IPG strip for the first dimension and Tris-HCl 8–16% Criterion gel for the second dimension. The gel was stained with Oriole stain and was imaged on the Molecular Imager VersaDoc imaging system.

Ordering Information

Catalog #	Description
161-0495	**Oriole Fluorescent Gel Stain**, 1x, 200 ml
161-0496	**Oriole Fluorescent Gel Stain**, 1x, 1 L
161-0497	**Oriole Fluorescent Gel Stain**, kit for 5 L

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