



Oriole™ Fluorescent Gel Stain

Instruction Manual

Catalog #

161-0495, 1x solution, 200 ml

161-0496, 1x solution, 1 L

161-0497, kit for 5 L

For technical support call your local Bio-Rad office.

In the U.S. call 1-800-424-6723.



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Section 1

Introduction and General Information

1.1. Introduction

Oriole™ fluorescent gel stain is an easy to use, rapid, and sensitive stain for visualization and quantitation of proteins separated by SDS-PAGE. The product is available in three configurations. The 200 ml and 1 L sizes are provided ready to use. The product is also available as a kit containing components to make 5 L of ready to use staining solution.

The staining procedure is a simple one-step protocol that can be completed in as little as 90 minutes. Gels stained with Oriole fluorescent gel stain may be visualized with a variety of different UV-based fluorescence imaging systems.

Oriole fluorescent gel stain gives exceptional sensitivity and dynamic range (see pages 14–16) and is compatible with subsequent analysis by enzymatic digestion and mass spectrometry. It is thus particularly well suited to proteomics applications.

1.2. Product Description

Oriole fluorescent gel stain comes in three package configurations.

The 200 ml size — fully diluted and ready to use; provides enough stain for four mini format Mini-PROTEAN® gels (~8.6 x 6.8 cm), or two midi format Criterion™ gels (13.3 x 8.7 cm).

The 1 L size — fully diluted and ready to use; provides enough stain for 20 Mini-PROTEAN gels (~8.6 x 6.8 cm), ten Criterion gels (13.3 x 8.7 cm), four large format PROTEAN® II gels (16 x 16 cm or 16 x 20 cm), or two large format PROTEAN Plus gels (25 x 20.5 cm).

The 5 L kit — contains concentrated components to prepare 5 L of staining solution and can be diluted to 1x according to demand.

1.3. Storage

The product is stable for at least 18 months from the date of manufacture or until the expiration date on the label when stored at 24°C or below. Consult the expiration date

before using. Avoid prolonged exposure to temperatures greater than 37°C and protect from light.

1.4. Materials and Equipment Required but Not Supplied

- Staining containers — Any glass or plastic tray capable of holding the recommended volume of solution may be used
- Imaging equipment — Gels are best imaged using a UV-based fluorescence imager capable of excitation near 270 nm and detection near 604 nm such as the Molecular Imager® Gel Doc™ XR+, Molecular Imager® ChemiDoc™ XRS+, VersaDoc™ MP 4000, ExQuest™ spot cutter, and VersaDoc MP 5000 systems. For a more complete list of compatible imaging systems, see pages 12–13
- Laboratory shaker or rocker
- Powder-free latex, vinyl, or nitrile gloves

1.5. Reagents Required but Not Supplied

Methanol, reagent grade (for 5 L kit only)

1.6. Safety Considerations

Oriole fluorescent gel stain is a dilute solution of a fluorescent dye. The working solution is flammable and should be handled in a manner that prevents exposure to open flame or sparks. The complete properties of the dye component have not been investigated. Eye protection and gloves should be worn and general laboratory safety precautions followed while handling both the diluted and undiluted product.

1.7. Disposal Considerations

Laws governing the disposal of laboratory chemicals vary by region. Consult the MSDS (available online at www.bio-rad.com) and check local laws for proper disposal guidelines.

1.8. Fluorescence Characteristics

Oriole fluorescent gel stain has a fluorescence excitation maximum of 270 nm and a fluorescence emission maximum of 604 nm.

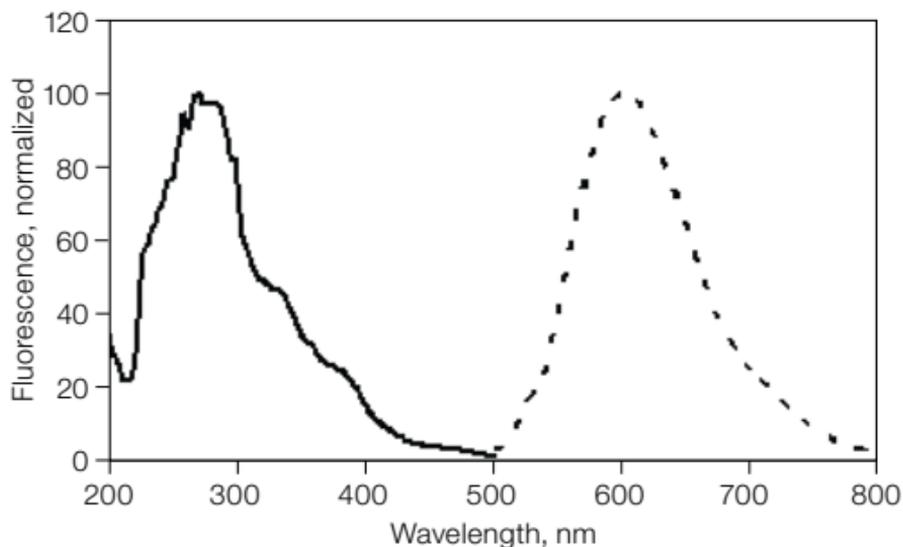


Fig. 1. Fluorescence excitation and emission spectra of Oriole stain. Oriole stain has its excitation maximum at 270 nm and emission maximum at 604 nm, making it compatible with UV-based imagers. —, Excitation spectrum; ----, emission spectrum.

Section 2

Instructions

2.1. General Considerations

Best results are obtained by using clean technique. Any dust or dirt transferred to the surface of the gel may appear in the fluorescence image as smudges or speckles. Oriole™ fluorescent gel stain is exceptionally sensitive. Contaminant proteins such as keratin will appear in the gel image if care is not taken to minimize such contamination.

All glassware used should be cleaned with laboratory glassware cleaner and rinsed with distilled or deionized water. Use dust-free gloves and limit dust exposure by keeping reagent vessels and gel trays covered as much as possible. If gels are cast in the laboratory, the glass plates used should be thoroughly cleaned with lint-free laboratory wipes.

Oriole fluorescent gel stain is very sensitive, and less protein can be visualized than what is possible using a visible stain like Coomassie Blue. Sensitivity is of the

same general order as silver stain or other fluorescent protein stains. Oriole fluorescent gel stain has a wide dynamic range, and variability in the amount of protein to be visualized can be accommodated simply by varying the exposure settings during imaging. As a general rule, the maximum quantity of protein recommended for visualization with Oriole fluorescent gel stain is 1–2 μg for individual proteins and 10–20 μg for complex mixtures on 1-D gels. The limit of sensitivity for individual proteins is 1 ng or less.

Oriole fluorescent gel stain is moderately light sensitive. If gels are left in stain for more than 90 min, the gel tray should be covered with aluminum foil or an opaque lid.

Oriole fluorescent gel stain is intended only for staining 1-D and 2-D SDS-PAGE gels. Native gels and IEF gels cannot be stained with Oriole stain. Oriole stain is not recommended for staining protein blots.

Instructions given are for standard 1 mm thick SDS-PAGE gels. Thicker gels may benefit from longer stain times and larger volumes of solution.

Molecular weight standards that have been prestained with a visible dye such as Precision Plus Protein™ All Blue, Dual Color or Kaleidoscope™ prestained standards do not stain with Oriole fluorescent gel stain and cannot be imaged by fluorescence in gels stained with Oriole stain. We recommend the use of unstained protein standards on gels to be stained with Oriole stain.

2.2. Stain Solution Preparation

The 200 ml and 1 L configurations are provided ready to use.

The 5 L kit comprises 5 individual 1 L bottles, each containing 590 ml of Oriole fluorescent gel stain diluent, and a single bottle containing 50 ml of Oriole gel stain concentrate. Staining solution (1x) is prepared as follows.

- To a 1 L bottle holding 590 ml of diluent, add (in sequence):
 - 400 ml methanol (reagent grade)
 - 10 ml of Oriole fluorescent gel stain concentrate
- Mix well by shaking
- Stain is now ready to be used

NOTE: Use only methanol in preparing staining solution from the 5 L kit. The use of water, ethanol, or other solvents will result in poor staining performance.

Recommended Stain Volume

Gel Size	Volume of Stain Solution per Gel
Ready Gel® or Mini-PROTEAN gel (8.6 cm x 6.8 cm)	50 ml
Criterion gel (13.3 cm x 8.7 cm)	100 ml
PROTEAN II gel (16 cm x 16 cm or 16 cm x 20 cm)	250 ml
PROTEAN Plus gel (25.6 cm x 23 cm)	500 ml

2.3. Gel Staining

NOTE: Do not fix or wash gel prior to staining. This will make staining less sensitive.

1. Place gel directly into a clean tray containing the recommended volume of Oriole fluorescent gel stain. Cover the tray, place on a rocker or shaker, and agitate as vigorously as possible without splashing liquid or damaging the gel.

2. Stain for 90 min for maximum sensitivity.
3. Cover the tray to exclude light if the gel is stained longer than 90 min.

NOTE: Best results are obtained if the gels are left in staining solution no longer than 2 hr.

4. Transfer gel to water prior to imaging. This step prevents exposure of the imaging equipment to moderately corrosive staining solution.

NOTE: Destaining is not necessary. Staining intensity persists when the gel is stored in water.

Stained gels can be stored in water for up to 6 months and imaged without significant loss of sensitivity if protected from light and stored at 2–8°C.

2.4. Gel Imaging

Gels stained with Oriole stain are visualized using UV light excitation. Bio-Rad Molecular Imager Gel Doc XR+, Molecular Imager ChemiDoc XRS+, EXQuest spot cutter, VersaDoc MP 4000, or VersaDoc MP 5000 systems are recommended for imaging gels stained with Oriole stain. If the imaging equipment has no preprogrammed imaging function for Oriole fluorescent gel stain, the imaging setting for SYPRO Ruby stain or ethidium bromide that uses UV transillumination is recommended.

Any imaging system using UV light excitation may be used to image Oriole fluorescent gel stain. Such imaging systems almost always have midrange (300 nm, 306 nm, or 312 nm) UV excitation and red or orange emission filters as standard options for imaging ethidium bromide-stained gels. The excitation and emission properties of Oriole stain are very compatible with ethidium bromide, therefore imager settings for ethidium bromide can be used when imaging gels stained with Oriole stain.

Imaging systems capable of imaging gels stained with Oriole fluorescent gel stain include the following:

Manufacturer	Imaging system	Recommended settings
Bio-Rad Laboratories	Gel Doc, ChemiDoc, VersaDoc, EXQuest spot cutter systems	For Gel Doc and ChemiDoc systems, use Standard (302 nm) UV lamp with Standard Filter (580 nm bandpass for ethidium bromide). For VersaDoc systems use Standard (302 nm) UV lamp and either the 520 nm longpass filter or the 605 nm bandpass filter (either one or the other is included with the instrument)
GE Healthcare	ImageQuant	UV 302 illumination with ethidium bromide (orange) emission filter
Fuji	LAS 3000, LAS 4000	312 nm illumination with ethidium bromide emission filter
Alpha Innotech	Alphamager, FluorChem	UV 302 with standard (orange) emission filter

Manufacturer	Imaging system	Recommended settings
UVP	BioDoc-It, VisiDoc-It, DigiDoc-It, MultiDoc-It, Photo Doc-It, BioSpectrum, EC3	Midrange (302 nm) UV excitation with ethidium bromide red emission filter
Carestream	Gel Logic	UV trans excitation with 590 nm bandpass (ethidium bromide) emission filter
Kodak	2200, 4000MM, 4000MM Pro, 4000R, 4000R Pro	306 nm UV excitation with ethidium bromide standard orange (600 nm) emission filter
Syngene	G:Box, InGenius, U:Genius	Midrange UV excitation with ethidium bromide emission filter
Biometra	BioDocAnalyze	Midrange (312 nm) UV excitation with ethidium bromide emission filter

Imaging systems using laser light excitation or other visible light source excitation are not recommended for imaging gels stained with Oriole fluorescent gel stain. These include PharosFX™ system (Bio-Rad), Typhoon, Storm, and Ettan DIGE Imager (GE Healthcare), Odyssey (Li-Cor), FLA (Fuji), and FM Bio (Hitachi) systems.

Section 3

Technical Information

3.1. Sensitivity of Staining – 1-D Gels

The dye in Oriole™ stain is highly fluorescent and binds tightly to proteins. Background staining is low, and the limit of sensitivity is generally below 1 ng.

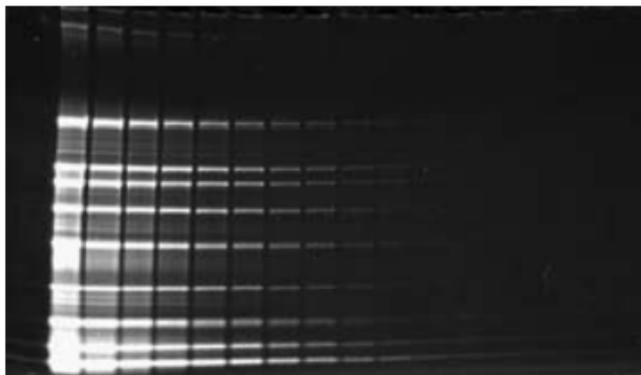


Fig. 2. Unadjusted image of a gel stained with Oriole stain.

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 MP 4000 imaging system with image settings for SYPRO Ruby stain. The resulting image file was not adjusted.

4 2 1 0.5 0.25 0.125 ng/band

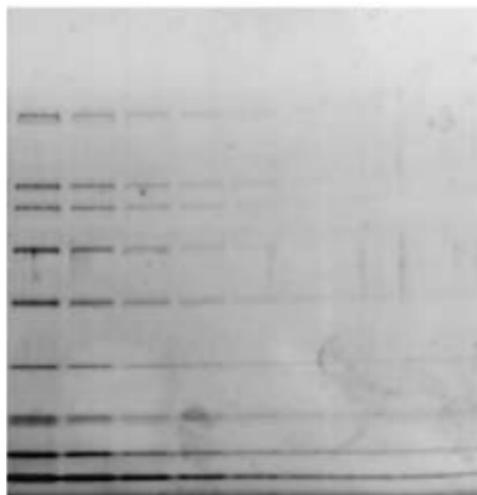


Fig. 3. Image of the gel stained with Oriole stain, adjusted to show the limit of sensitivity. The image from the previous figure was inverted, cropped to show protein loads ≤ 4 ng, and adjusted to show the limit of sensitivity.

3.2. Compatibility With Mass Spectrometry

Oriole fluorescent protein gel stain is fully compatible with downstream proteolysis and mass spectrometric analysis.

3.3. Protein-to-Protein Variability

Oriole fluorescent gel stain will stain most proteins and exhibits little protein-to-protein variability in staining intensity.

3.4. Dynamic Range

Oriole fluorescent gel stain has a dynamic range of up to three orders of magnitude. This allows protein quantitation in samples of varying concentration over a wide range of relative abundance.

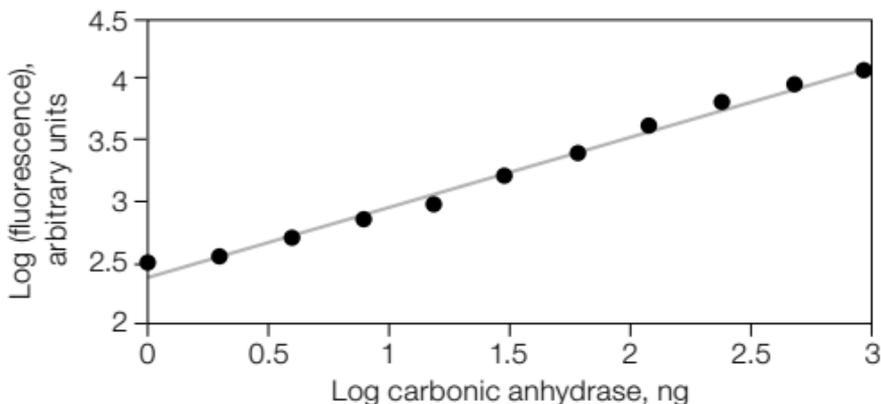


Fig. 4. Linearity of Oriole stain. A dilution series of carbonic anhydrase was run on a Criterion gel, stained with Oriole stain, and imaged on the Molecular Imager VersaDoc MP 4000 imaging system. Fluorescence associated with the carbonic anhydrase band was plotted following background subtraction.

3.5. 2-D Gel Staining

Oriole fluorescent gel stain is ideal for staining 2-D polyacrylamide gels. Clear background-free images are obtained without interference from CHAPS, carrier ampholytes, or other components of the first-dimension separation.

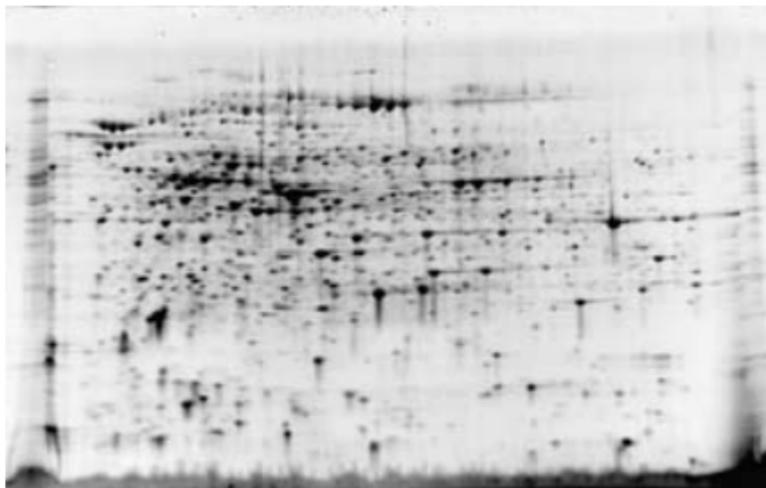


Fig. 5. 2-D gel stained with Oriole stain. *E. coli* protein (40 μ g) was run on an 11 cm pH 5–8 ReadyStrip™ IPG strip for the first dimension and Criterion Tris-HCl 8–16% gel for the second dimension. The gel was stained with Oriole stain and was imaged on the Molecular Imager VersaDoc MP 4000 imaging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

Section 4

Troubleshooting

Problem	Possible Cause	Remedy
Bands or spots not visible	No protein on gel	Verify that there is actually protein on the gel by staining with another method such as Bio-Safe™ Coomassie stain.
	Malfunctioning imaging system	Check instrument manual for troubleshooting, or contact the imaging instrument manufacturer.
Poor staining sensitivity	Insufficient staining time	Stain sensitivity maximizes after 90 min.
	Dirty staining trays	Make sure that the staining trays and other equipment have been thoroughly cleaned with laboratory glassware cleaner.
	Insufficient stain volume	Follow the recommendations for stain volume appropriate to the gel size (Section 2.2).

continued

Problem	Possible Cause	Remedy
Poor staining sensitivity	Reuse of the stain	Reuse of Oriole stain is not recommended.
	Use of nonrecommended solvent for the 5 L kit	Use only methanol and provided diluent.
	Fixing or washing the gel prior to staining	Do not fix or wash gel prior to staining.
High or uneven background staining	Dirty equipment or staining trays	Make sure that the staining trays and other equipment have been thoroughly cleaned with laboratory glassware cleaner.
	Too much time in staining solution	Restrict time of staining solution treatment to 90–120 min. Background resulting from overstaining can be reduced by washing the gel in water for 30 min or more.

continued

Problem	Possible Cause	Remedy
High or uneven background staining	Reagent impurities when preparing stain solution from the 5 L kit	Use high quality reagents.
Speckles or blotches in the gel image	Particulate material from reagents, staining tray, dust, or gloves	<p>Make sure that the staining trays are thoroughly cleaned.</p> <p>Limit the time that the gels and staining solution are exposed to open air.</p> <p>Use dust-free gloves and handle gels only by the edges.</p>
Uneven staining	Insufficient shaking during staining	Ensure that the gel is well agitated during staining.
Gel shrinkage	Some gel shrinkage occurs during staining	The gel will reswell following transfer to water.

Section 5

Product Information

5.1. Oriole Fluorescent Gel Stain

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

5.2. Related Products

Catalog #	Description
163-2091	ReadyPrep™ Proteomics Grade Water, 500 ml
170-8640	Molecular Imager VersaDoc MP 4000 System
170-8650	Molecular Imager VersaDoc MP 5000 System
170-8251	Molecular Imager ChemiDoc XRS+ System
170-8170	Molecular Imager Gel Doc XR+ System
345-9920	Criterion Gel/Blotting Trays, pack of 12
161-0786	Bio-Safe Coomassie stain, 1 L
161-0787	Bio-Safe Coomassie stain, 5 L
161-0363	Precision Plus Protein Unstained Standards
161-0378	Precision Plus Protein Standards Plugs
161-0303	SDS-PAGE Standards, high range
161-0304	SDS-PAGE Standards, low range
161-0317	SDS-PAGE Standards, broad range

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