Flamingo™ Fluorescent Gel Stain

Instruction Manual

Catalog Numbers
161-0490, 20 ml
161-0491, 100 ml
161-0492, 500 ml

For technical support call your local Bio-Rad office, or in the US call
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Table of Contents

Section 1
Introduction and General Information

1.1 Introduction

Flamingo fluorescent gel stain allows sensitive fluorescence visualization of proteins that have been separated by 1-D or 2-D SDS-PAGE. It comes as a 10x stock solution that is diluted with water by the user to its working concentration. It is supplied in three product sizes: 20 ml, 100 ml, and 500 ml.

Flamingo fluorescent gel stain contains a dye which binds to denatured protein. This dye is normally non-fluorescent in solution, but becomes strongly fluorescent when protein-bound. The staining procedure is a simple two-step protocol that can be completed in as little as 5 hours. Gels to be stained are fixed with ethanol/acetic acid solution prior to staining with the gel stain solution. A destaining step is not normally required, but may be employed to reduce background.

Gels stained with Flamingo fluorescent gel stain may be visualized with a variety of different fluorescence imaging systems. The most optimal imaging systems are laser-based fluorescence scanners capable of exciting and detecting at 510 nm and 535 nm respectively, such as the Molecular Imager FX™ system. Gels may also be imaged using systems that use 300 nm UV transillumination such as the VersaDoc™ imaging system.

Flamingo fluorescent gel stain gives exceptional sensitivity and dynamic range and is compatible with subsequent analysis by enzymatic digestion and mass spectrometry. It is thus particularly well-suited to proteomics applications. Stained gels may be stored in the dark at 2–8°C for up to six months without significant loss of imaging sensitivity.

1.2 Product Description

Flamingo fluorescent gel stain is a 10× stock solution that comes in three package sizes. Instructions are included.

- The 20 ml size stains 4 mini gels (Ready Gel®) or 2 Criterion™ gels.
- The 100 ml size stains 20 mini gels (Ready Gel), 10 Criterion gels, 4 PROTEAN® II gels, or 2 PROTEAN® Plus gels.
- The 500 ml size stains 100 mini gels (Ready Gel), 50 Criterion gels, 20 PROTEAN II gels, or 10 PROTEAN Plus gels.

1.3 Storage

The product is stable for at least 6 months when stored at 2–8°C. Avoid exposure to temperatures greater than 37°C and protect from light.

1.4 Materials and Equipment Required but not Supplied

- Staining containers—Glass trays are recommended, plastic trays may be used if cleaned and rinsed thoroughly
- Imaging equipment—Gels are best imaged using a laser-based fluorescence scanner capable of exciting and detecting near 510 nm and 540 nm respectively, such as the Molecular Imager FX system. Gels may also be imaged using systems based on the UV transilluminator and CCD camera such as the VersaDoc imaging system
- Laboratory shaker or rocker
- Powder-free latex, vinyl or nitrile gloves

1.5 Reagents Required but not Supplied

- Acetic Acid, reagent grade
- Ethanol, reagent grade
- Filtered, distilled or deionized water.

1.6 Safety Considerations

Flamingo fluorescent gel stain is a dilute alcoholic solution of an organic dye. It is flammable in its 10x concentrated form and should be handled in a manner that prevents exposure to open flame or sparks. The diluted working solution is minimally hazardous and non-flammable. However, 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.

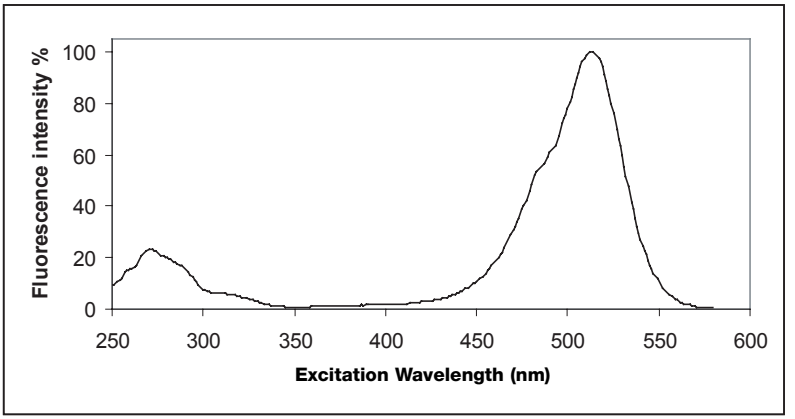
1.7. Disposal Considerations

Laws governing the disposal of laboratory chemicals vary by region. Consult the MSDS and check local laws for the proper disposal guidelines.

1.8. Fluorescence Characteristics

In the presence of protein, Flamingo fluorescent gel stain has a fluorescence excitation maximum of 512 nm and a fluorescence emission maximum of 535 nm. Flamingo fluorescent gel stain has a secondary excitation at 271 nm and can also be excited with UV light (Fig. 1).

A



B

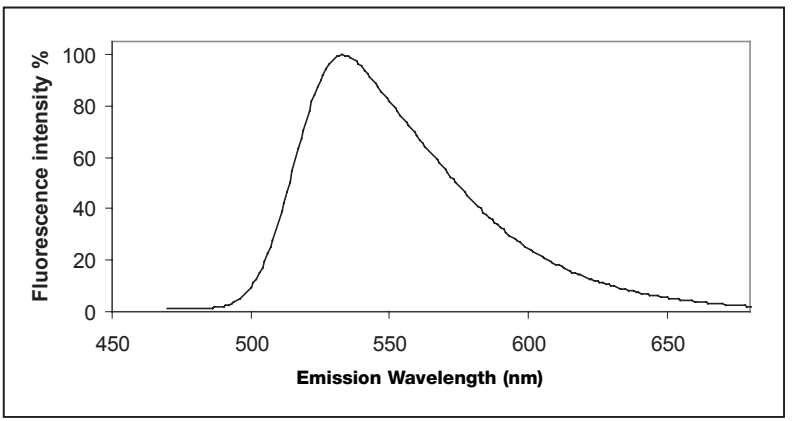


Fig. 1. Excitation (A) and Emission (B) spectra for Flamingo fluorescent gel stain in the presence of protein (denatured carbonic anhydrase).

Section 2
Instructions

2.1 General Considerations

Best results are obtained using clean technique. Any dust or dirt transferred to the surface of the gel may appear in the fluorescence image as smudges or speckles. Flamingo 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.

Flamingo fluorescent gel stain may be exposed to typical room light for up to 8 hours without any observable effect on the fluorescence intensity of the stained protein bands or spots. However, if gels are ok as is left in stain solution for more extended periods, photobleaching may occur. This can be prevented by covering the gel trays with aluminum foil or an opaque lid.

Instructions given are for standard 1 mm thick SDS-PAGE gels. Thicker gels may benefit from longer fix and stain times and larger volumes of solution. Native PAGE gels may also be stained with this procedure.

2.2 Prepare Fix Solution

The fix solution consists of 40% (v/v) ethanol and 10% (v/v) acetic acid. Prepare the solution with distilled or deionized water that has been filtered. The quantity of fix solution required depends on the number of gels to be stained and the size of the gel as indicated in the table below.

Gel Size	Volume of Fix Solution per Gel
Ready Gel (8.6 cm × 6.8 cm)	100 ml
Criterion (13.3 cm × 8.7 cm)	200 ml
PROTEAN II (16 cm × 16 cm)	500 ml
PROTEAN Plus (25.6 cm × 23 cm)	1,000 ml

2.3 Fix Gels

Remove the gel(s) from the gel cassette or plates. Place gel in a clean glass tray with the volume of fix solution indicated above. Cover the tray, place on a rocker or shaker and agitate gently. Fix for at least 2 hours.

Note: Gels may be left in fix solution for up to 24 hours. Shortened fix time or insufficient fix solution may reduce sensitivity.

2.4 Prepare Working (1x) Stain Solution

Working (1x) stain solution is prepared by diluting one volume of Flamingo fluorescent gel stain with 9 volumes of distilled or deionized water that has been filtered. The quantity of stain solution required depends on the number of gels to be stained and the size of the gel as indicated in the table below.

Gel Size	Volume of Stain Solution per Gel
Ready Gel (8.6 cm × 6.8 cm)	50 ml
Criterion (13.3 cm × 8.7 cm)	100 ml
PROTEAN II (16 cm × 16 cm)	250 ml
PROTEAN Plus (25.6 cm × 23 cm)	500 ml

Working (1x) stain solution should be prepared within one day of its intended use.

2.5 Stain Gels

Carefully pour off the fix solution and add Flamingo fluorescent gel stain to the staining tray. Cover the tray, place on a rocker or shaker and agitate gently. Stain for at least 3 hours.

Gels may be left in the stain solution for extended periods. If the gel is to be left in the stain solution for longer than 8 hours, cover the gel tray with aluminum foil or other opaque material to limit light exposure.

Stained gels may be stored for up to six months and imaged without significant loss of sensitivity. Gels held for long-term storage should be placed in sealable plastic bags with 5–10 ml of stain solution and stored in the dark at 2–8°C.

2.6 Background Reduction (Optional)

Note: The dye in Flamingo fluorescent gel stain has very low fluorescence when not protein-bound. A background reduction or destaining step is normally not required but the following step will slightly lower background staining and may allow some proteins to be more sensitively detected.

Carefully pour off the stain solution and replace with an equal volume of 0.1% (w/v) Tween 20. Cover the tray, place on a rocker or shaker and agitate gently for 10 minutes. Rinse gels with distilled or deionized water prior to imaging.

2.7 Gel Imaging

The primary fluorescence excitation maximum of Flamingo fluorescent gel stain is at 512 nm. There is a minor excitation maximum at 271 nm. The fluorescence emission maximum is at 535 nm (see Section 1.8 Fluorescence Characteristics). Flamingo fluorescent gel stain therefore gives the most sensitive results when used with visible laser light-based imaging systems equipped with 473, 488 or 532 nm laser light sources. The Molecular Imager FX system is particularly well suited for imaging gels stained with Flamingo fluorescent gel stain, and provides high-resolution images suitable for proteomics applications. If the accompanying software does not have a have a preprogrammed imaging function for Flamingo fluorescent gel stain, the image settings for SYPRO® Ruby stain may be used. If an alternative laser-based scanner is used, choose imaging settings that match the fluorescence characteristics of Flamingo fluorescent gel stain. Examples of compatible excitation sources and emission filter combinations include the following:

- Green laser (532 nm) with 555 nm longpass emission filter
- Blue laser (473 nm) with 530 nm emission filter
- Blue laser (488 nm) with 560 nm longpass emission filter

Flamingo fluorescent gel stain may also be used with UV transilluminator-based systems such as the VersaDoc imaging system. However, the limit of sensitivity with UV transillumination will not be as low as that achievable with a visible laser light-based imaging system.

If UV transillumination is used, make sure that the transilluminator has 300 nm bulbs installed.

Section 3
Technical Information

3.1. Sensitivity with Laser Scanning

When scanned on the Molecular Imager FX system, an SDS-PAGE gel stained with Flamingo fluorescent gel stain should exhibit a limit of sensitivity in the range of 0.25–0.5 ng (Fig. 2).

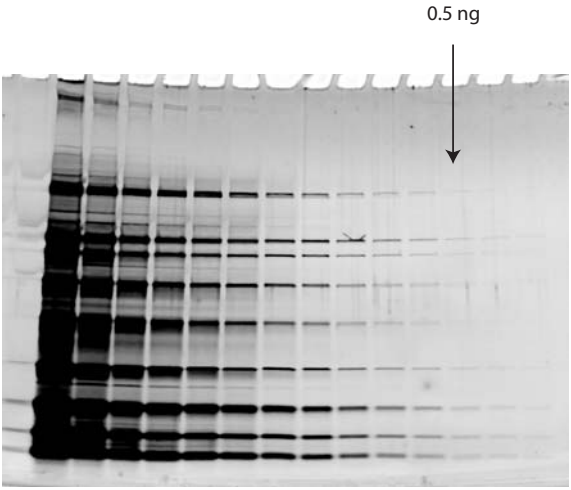


Fig. 2. Serial 2-fold dilutions of SDS-PAGE molecular weight standards, broad range, on a Criterion Tris-HCl 4–20% gel. The gel was stained with Flamingo fluorescent gel stain and imaged with the Molecular Imager FX system. The arrow indicates the lane containing 0.5 ng of each standard.

3.2. Sensitivity with UV Transillumination

When scanned on the VersaDoc 4000 imaging system, an SDS-PAGE gel stained with Flamingo fluorescent gel stain should exhibit a limit of sensitivity in the range of 0.5–2 ng (see Fig. 3).

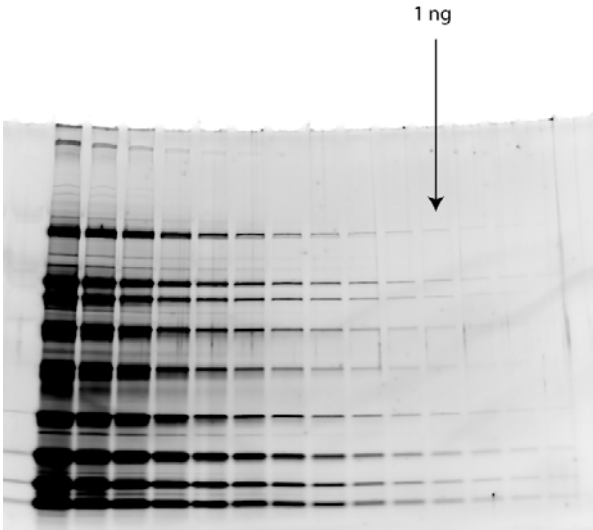


Fig. 3. Serial 2-fold dilutions of SDS-PAGE molecular weight standards, broad range, on a Criterion Tris-HCl 4–20% gel. The gel was stained with Flamingo fluorescent gel stain and imaged with the VersaDoc 4000 imaging system using an exposure time of 5 min. The arrow indicates the lane containing 0.5 ng of each standard.

3.3. Protein-to-Protein Variability

Flamingo fluorescent gel stain will stain most proteins and exhibits less protein-to-protein variability than other staining methods.

3.4. Linearity of Staining

Flamingo fluorescent gel stain allows linear quantitation over a wide dynamic range. Staining with the standard 5 hour protocol (2 hour fix, 3 hour stain) will allow linear quantitation from 1 to 100 ng. If the staining step is extended to overnight (at least 12 hours), the linear range for quantitation can be extended to over three orders of magnitude (0.5 to 1 µg).

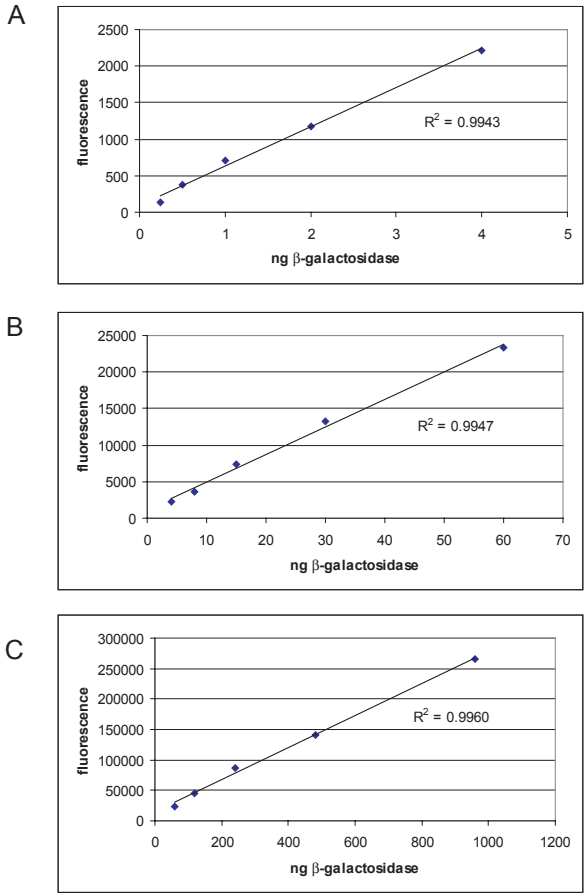


Fig. 4. Serial 2-fold dilutions of β-galactosidase, run on a Criterion™ Tris-HCl 4–20% gel. The gel was stained with Flamingo Fluorescent Gel Stain and imaged with the Molecular Imager FX system. Fluorescence associated with the β-galactosidase band was determined using Quantity One® 1-D Analysis Software. **A**: Fluorescence associated with protein band in the range 0.25–4 ng. **B**: Fluorescence associated with protein band in the range 4–60 ng. **C**: Fluorescnece associated with protein band in the range 60–960 ng.

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 (e.g. Bio-Safe™ Coomassie stain).
	Malfunctioning imaging system	Check instrument manual for troubleshooting.
Poor staining sensitivity	Residual SDS in gel	Confirm the correct amount of fix solution is used. (See page 5)
High or uneven background staining	Dirty equipment or staining trays	Make sure that the staining trays and other equipment used have been thoroughly cleaned with laboratory glassware cleaner. Use glass staining trays, as these are more likely to be thoroughly clean.
	Reagent impurities	Make sure that the water and reagents used for preparing and fixing the gel are of the highest possible quality. Proteomics grade water may be used to prepare the stain solution.

Problem	Possible Cause	Remedy
Speckles or blotches in the gel image	Particulate material from water, reagents, staining tray, ambient dust and gloves	Filter water used to prepare fix solution and stain solution if laboratory water system does not have a filter. Make sure that the staining trays are thoroughly clean. Limit the time that the gels and staining solution are exposed to open air. Use dust-free gloves and handle gels only by the edges. Particulate material may be washed from the surface of the gel prior to imaging by washing with 0.1% (v/w) Tween 20 prior to imaging (See Section 2.6).

Section 5
Product Information

4.1. Flamingo Fluorescent Gel Stain

Catalog #	Description
161-0490	Flamingo Fluorescent Gel Stain, 10x Solution, 20 ml
161-0491	Flamingo Fluorescent Gel Stain, 10x Solution, 100 ml
161-0492	Flamingo Fluorescent Gel Stain, 10x Solution, 500 ml

4.2. Related Products

Catalog #	Description
170-6531	Tween 20, EIA grade, 200 g
161-0781	10% Tween 20, for easy pipetting, 1 L
161-0786	Bio-Safe Coomassie Stain, 1 L
163-2097	Proteomics Grade Water, 500 ml
170-7856	Molecular Imager FX Pro Fluorescent Imager, PC
170-7857	Molecular Imager FX Pro Fluorescent Imager, Mac
170-7850	Molecular Imager FX Pro Plus MultiImager, PC
170-7851	Molecular Imager FX Pro Plus MultiImager, Mac
170-8140	VersaDoc Model 4000 Imaging System, PC
170-8141	VersaDoc Model 4000 Imaging System, Mac
170-8070	ChemiDoc XRS System, PC
170-8071	ChemiDoc XRS System, Mac
170-9600	Quantity One 1-D Analysis Software
170-9630	PDQuest 2-D Analysis Software

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