Protocol

TD-P Revision 2.0

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Blazin' Bright™ Luminescent Protein Gel Stain Protocol

A Ready-To-Use Protein Gel Staining Method

Introduction

Blazin' Bright™ Luminescent Protein Gel Stain is a ready-to-use luminescent protein gel staining solution that is a safer and more effective replacement for traditional fluorescent staining. Blazin' Bright™ offers a faster alternative to protein staining (5-60 minutes) in a single step without the need for fixation (destaining is optional). The Blazin' Bright™ gel stains are aqueous based, there are no hazardous chemicals like methanol or acetic acid to worry about. Blazin' Bright™ protein gel stain is certified under CCR Title 22 as non-toxic to the environment for drain disposal after a simple pH neutralization step.

Blazin' Bright™ has a detection limit around 1-10 ng, depending on the detection method used (staining intensity varies between proteins) and does not cause gel shrinkage. Blazin' Bright™ staining is also fully compatible with mass spectrometry (MS) and Edman-based sequencing.

Performance Properties

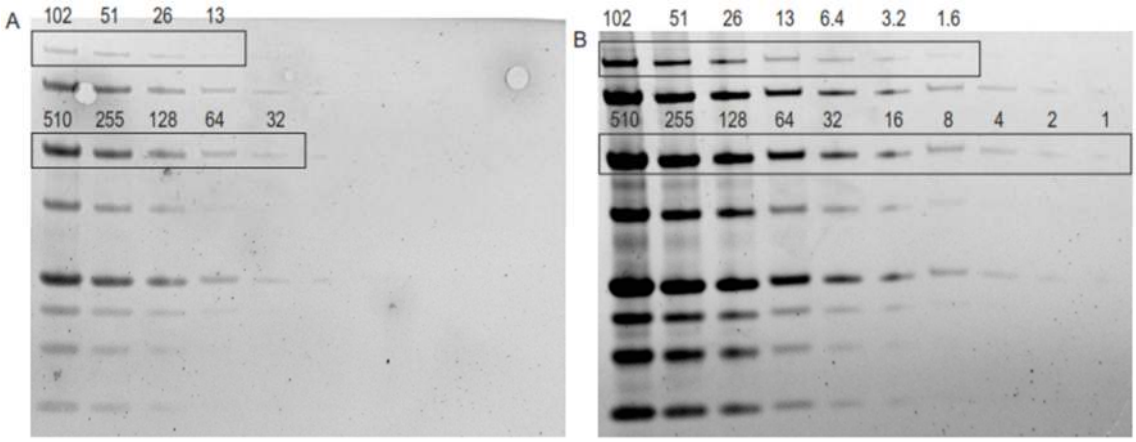

Figure 1. Blazin' Bright™ Luminescent Protein-stained SDS-PAGE gel. Two-fold dilutions of a protein standard were separated on a 1 mm thick 4-12% Bis-Tris MES mini-gel. The gel was stained with Blazin' Bright™ luminescent protein stain for 30 minutes without fixation, then imaged on a UV transilluminator with an ethidium bromide filter using a UVP GelDoc-It™ imaging system. A) Gel imaged immediately after staining. B) Gel imaged after overnight destain in water. Labels indicate approximate protein amounts (ng) in the boxed bands beneath.

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Spectral Properties

λ_{abs} / λ_{em} = ~480 (broad)/~610 nm

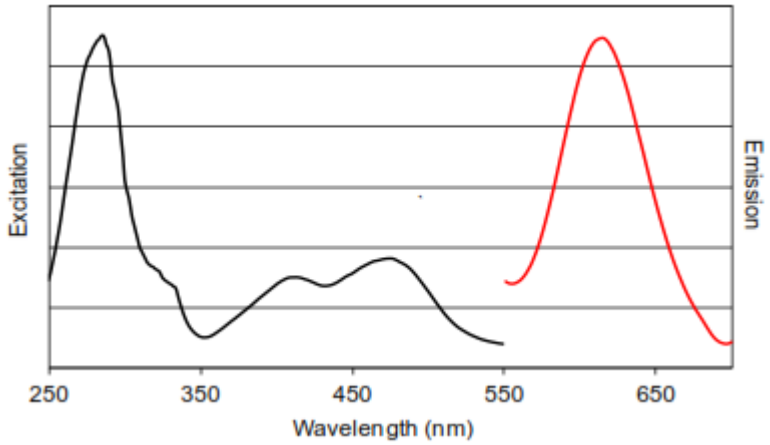

Figure 2. Excitation and emission spectra of Blazin' Bright™ luminescent protein dye

Storage/Handling

Store at room temperature. Product is stable for at least 6 months from date of receipt.

Materials

- Blazin' Bright™ Luminescent Protein Gel Stain (Catalog # P-820)

Method

The following protocol is optimized for 1 mm thick, 8 X 8 cm SDS-PAGE minigels.

1. Staining. After electrophoresis, place the unfixed gel in a clean container containing 25 ml of Blazin' Bright™ luminescent protein stain per mini-gel and incubate with gentle rocking at room temperature. Bands may start to be detectable after 5 minutes depending on the amount of protein present. For the best sensitivity, stain for 60 minutes.

   Note: The gel can be left in the staining solution overnight without overstaining.

   Note: For larger gels, scale up the volume of staining solution accordingly using the mini-gel size as a reference.

   Note: Blazin' Bright™ can also be used to stain fixed gels. Sensitivity can be increased by fixation with 45% methanol/10% acetic acid for 1 hour before staining, followed by destaining in water.

2. Destaining (optional). Destaining is not required, but can be done to reduce background. Gels can be destained in water for 2 washes of 5 minutes each or overnight with gentle rocking.

3. Imaging and Quantitation. The gel can be imaged with a variety of instruments. See Table 1 for a list of suitable excitation sources and emission filters.
    a. UV Transilluminator: A UV transilluminator with a 300 nm excitation and an ethidium bromide filter may be used for viewing/imaging fluorescence.

    b. LED-based Gel Viewer: Blue light LED-based gel boxes designed for safe viewing of DNA/RNA gels can also be used for viewing and imaging Blazin' Bright™ stained protein gels. Detection sensitivity may vary depending on device.

    c. Laser-based Gel Scanner: Blazin' Bright™ can be imaged on a gel scanner (such as a Typhoon® scanner) with 488 nm or 532 nm laser excitation with a detection window centered around 610 nm emission (such as the SYPRO® Ruby channel). Using 532 nm excitation may give lower background fluorescence compared to 488 nm excitation.

    Note: For downstream analysis such as sequencing or mass spectrometry, gel slices can be processed the same way as SYPRO® Ruby stained gels.

Table 1. List of suitable excitation sources and emission filters for Blazin' Bright™

Excitation sources/filters	Emission filters	
300 nm UV	Longpass	490 nm, 515 nm, 520 nm, 580 nm, 590 nm, 610 nm
365 nm UV	595±4.5 nm (monochromator, Molecular Devices)	
450±15 (filter)	Ethidium bromide filter	
470 nm blue LED	Bandpass	600 nm, 618 nm, 620 nm
473 nm laser	600±20 nm	
480 nm excitation interference filter (epi-illumination)	600±35 nm	
485±4.5 nm (monochromator)	610±35 nm	
488 nm laser	625± 15 nm	
532 nm laser	625±T15 nm, Texas Red filter (~630 nm bandpass)	
	640± 35 nm	

4. Disposal. Blazin' Bright™ is a 100% aqueous solution uniquely formulated using chemicals that qualify as food ingredients that can be disposed down the drain. It does not contain methanol and is classified as non-hazardous to the environment. However, the solution is acidic and must be neutralized before drain disposal. To neutralize, add 653 ul 1N sodium hydroxide per ml Blazin' Bright™ and mix well. Alternatively, you can add 26 mg sodium hydroxide pellets per ml Blazin' Bright™ and stir to dissolve completely.

Table 2. Related Products

GoldBio Catalog #	Product Name
P007	BLUEstain™ Protein ladder, 11-245 kDa
P008	BLUEstain™ 2 Protein ladder, 5-245 kDa
P-810	Blazin' Blue™ Protein Gel Stain
P-825	Blazin' Bright™ Luminescent UV Protein Gel Stain
H-350	Nickel NTA Agarose Beads
H-320	Nickel Agarose Beads (High Density)
G-725	GelRed™ Nucleic Acid Gel Stain, 10,000X in Water
G-745	GelGreen™ Nucleic Acid Stain Gel Stain, 10,000X in Water
S-100	Ultra HBC™ Streptavidin Agarose Resin