Protocol



TD-P Revision 2.0

Creation Date: 2/28/2017 Revision Date: 1/24/2019

Blazin' Bright™ Luminescent UV Protein Gel Stain Protocol A Ready-To-Use Protein Gel Staining Method

Introduction

Blazin' Bright™ Luminescent UV 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. Gels stained with our Blazin' Bright™ luminescent UV protein gel stain can be visualized rapidly (5-30 minutes) in a single step without 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™ Luminescent UV 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. Our Blazin' Bright™ Luminescent UV gives a better signal on a UV box than our normal Blazin' Bright™ luminescent protein gel stain, but is not compatible with a blue light illuminator or laser-based scanner.

Performance Properties

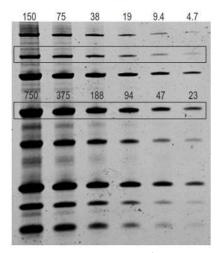


Figure 1. Blazin' Bright™ Luminescent UV-stained SDS-PAGE gel. Two-fold dilutions of a protein standard were separated on a 1 mm thick 4-12% Bis-Tris MES mini-gel. The gel was stained with Blazin' Bright™ luminescent UV protein stain for 60 minutes without fixation, then imaged on a UV transilluminator with an ethidium bromide filter using a UVP GelDoc-It™ imaging system. The gel was imaged immediately after staining. Labels indicate approximate protein amounts (ng) in the boxed bands beneath.



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Spectral Properties

 $\lambda_{abs}/\lambda_{em} = ^288 \text{ nm}/^603 \text{ nm}$

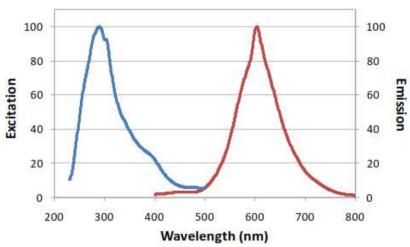


Figure 2. Excitation and emission spectra of Blazin' Bright™ luminescent UV protein dye

Storage/Handling

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

Materials

Blazin' Bright™ Luminescent UV Protein Gel Stain (Catalog # P-825)

Method

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

Staining. After electrophoresis, place the unfixed gel in a clean container containing 25 ml of Blazin' Bright™ luminescent UV 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 30-60 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.

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- 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 UV transilluminator and an ethidium bromide filter.

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

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 630 ul 1N sodium hydroxide per ml Blazin' Bright™ and mix well. Alternatively, you can add 25 mg sodium hydroxide pellets per ml Blazin' Bright™ and stir to dissolve completely.

Table 1. Related Products

GoldBio	Product Name
Catalog #	Floddet Name
P007	BLUEstain™ Protein ladder, 11-245 kDa
P008	BLUEstain™ 2 Protein ladder, 5-245 kDa
<u>P-810</u>	Blazin' Blue™ Protein Gel Stain
P-820	Blazin' Bright™ Luminescent Protein Gel Stain
<u>H-350</u>	Nickel NTA Agarose Beads
<u>H-320</u>	Nickel Agarose Beads (High Density)
<u>G-725</u>	GelRed™ Nucleic Acid Gel Stain, 10,000X in Water
<u>G-745</u>	GelGreen™ Nucleic Acid Stain Gel Stain, 10,000X in Water
<u>S-100</u>	Ultra HBC™ Streptavidin Agarose Resin

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