

Blazin' Blue™ Protein Gel Stain Protocol

A Ready-To-Use Protein Gel Staining Method

Introduction

Blazin' Blue™ Protein Gel Stain is a ready-to-use protein gel staining solution that is a safer and more effective replacement for traditional Coomassie stains. Blazin' Blue™ offers a faster alternative to protein staining (5-60 minutes) in a single step without the need for fixation or washing. Protein bands can be detected through visible blue staining or by near-infrared fluorescence. And since Blazin' Blue™ is aqueous based, there are no hazardous chemicals like methanol or acetic acid to worry about. Blazin' Blue™ 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' Blue™ has comparable sensitivity to the standard Coomassie Blue stains, with a detection limit around 10-20 ng, depending on the detection method used (staining intensity varies between proteins). Blazin' Blue™ staining is also fully compatible with mass spectrometry (MS) and Edman-based sequencing.

Performance Properties

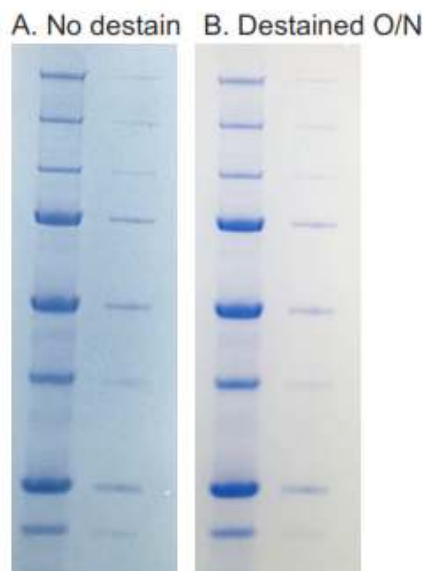


Figure 1. Blazin' Blue™-stained SDS-PAGE gel: (A) immediately after staining, or (B) after destaining in water overnight.

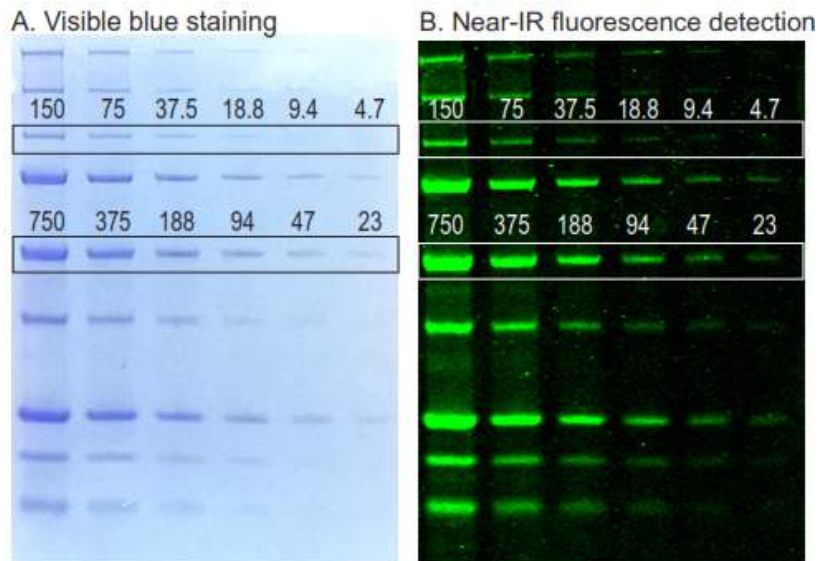


Figure 2. Blazin' Blue™-stained SDS-PAGE gel. Two-fold dilutions of Unstained Precision Plus Protein™ Standard (Bio-Rad) were separated on a 1 mm thick Novex® NuPage® 4-12% Bis-Tris MES mini-gel (Thermo Fisher). The gel was stained with Blazin' Blue™ for 60 minutes. A) Visible blue staining. B) Near-infrared fluorescence in the 800 channel of a LI-COR® Odyssey®. Labels indicate approximate amount of protein (ng) in the boxed bands beneath.

Spectral Properties

$$\lambda_{\text{abs}} / \lambda_{\text{em}} = \sim 610 / \sim 680 \text{ nm (broad)}$$

Materials

- Blazin' Blue™ Protein Gel Stain ([Catalog # P-810](#))

Method

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

1. Staining. Mix Blazin' Blue™ just before use by inverting the bottle several times. Place your unfixed gel in a clean container containing 25 mL of Blazin' Blue™ per mini-gel. Bands may start to appear in a few minutes, depending on the amount of protein present. For best sensitivity, incubate the gel for 60 minutes with gentle rocking.

Note: Blue particulates may be seen in the solution before or after adding your gel. This is normal and will not negatively affect staining.

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' Blue™ 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 (Figure 1). Gels can be destained in water for one hour to overnight with rocking.
3. Imaging and Quantitation. The gel can be photographed in visible light, or imaged using a standard densitometry-based imager. Blazin' Blue™ dye also emits near infrared fluorescence, allowing staining to be detected with a near-IR fluorescence gel scanner, such as the LI-COR[®] Odyssey[®] imaging system, in either the 700 nm or 800 nm channel (Figure 2).

Note: After staining, gels can be dried in cellophane according to standard protocols for Coomassie-stained gels.

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

4. Disposal. Blazin' Blue™ 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 743 ul 1N sodium hydroxide per ml Blazin' Blue™ and mix well. Alternatively, you can add 30 mg sodium hydroxide pellets per ml Blazin' Blue™ and stir to dissolve completely.

Associated Products

GoldBio Catalog #	Product Name
P007	BLUEstain™ Protein ladder, 11-245 kDa
P008	BLUEstain™ 2 Protein ladder, 5-245 kDa
P-820	Blazin' Bright™ Luminescent 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)
S-100	Ultra HBC™ Streptavidin Agarose Resin

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