Product Information

Brightein Protein Gel Stain，100×

Catalog Number: B6064

Product Size: 2 mL, 10 mL, 50 mL

Parameters

Appearance: Light yellow solution

Abs/Em = ~280 nm, ~450 nm (broad)/ 610 nm

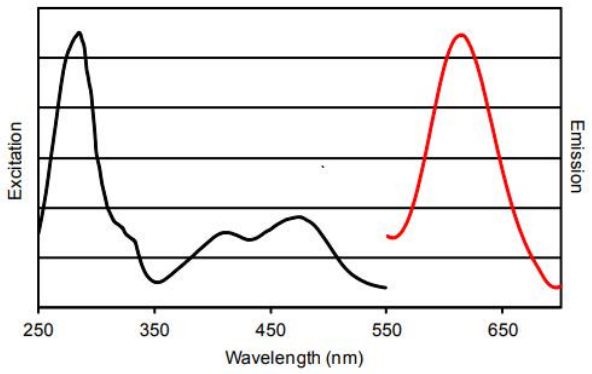

Fig.1 Emission and absorption spectra of Brightein

Storage

Store at room temperature or 4℃ and protect from light. When stored as directed, product is stable for at least 12 months.

Description

Brightein Protein Gel Stain is a luminescent dye designed for detecting proteins in SDS polyacrylamide (SDS-PAGE) gels. The dye can detect 1 ng protein or less, has compatibility with both UV and visible light excitation. With Brightein Protein Gel Stain Solution, protein fixation and staining is a single combined 90 min incubation step. Afterwards, the stained gel can be imaged immediately, destained, or simply washed in water before viewing/imaging. Finally, Brightein Protein Gel Staining is fully compatible with downstream protein analyses such as mass spectrometry and Edman-based sequencing. Brightein Protein Gel Stain is supplied as a highly concentrated 100 × solution, which is more economical and convenient for shipping, handling and storage.

Protocol

Preparation of 1 × Brightein dye solution:

Brightein 1 × staining solution is prepared by diluting 100 × with a combination of water, methanol and acetic acid. Use a clean bottle (preferably polypropylene or polyethylene) of a suitable size for preparing and storing your 1 × solution. Pour the vial of 100 × Brightein into the bottle, add the required amount of each solvent according to Table 1 and mix well. Use the diluted 1 × Brightein solution to rinse the original 100 × Brightein vial to ensure complete transfer of the 100 × solution. Purity of the solvents is not critical; reagents typically used for preparing protein gel fixation solution are suitable for preparing 1 × Brightein staining solution. The 1 × solution contains sufficient organic solvents to ensure both protein fixation and staining at the same time. Store the 1 × solution at room temperature or at 4℃ protected from light.

Table 1. Preparation of 1 × Brightein dye solution

Brightein 100×	diH_2O	Methanol	Acetic acid
2mL	116 mL	56 mL	26 mL
10 mL	580 mL	280 mL	130 mL
50 mL	2900 mL	1400 mL	650 mL

Preparation of Destaining Solution (optional):

Destaining after Brightein staining is not necessary; however, if desired, gels can be destaining by washing in de-ionized water for 20 minutes on a shaker (see staining procedure below). For

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even lower background and faster destaining, gels may be soaked in a destaining solution containing 30% methanol, 15% acetic acid and 55% water (see staining procedure below). To prepare approximately 100 mL destaining solution, mix 30 mL methanol, 15 mL acetic acid and 55 mL deionized water in a clean container.

Staining Protocol:

The following protocol is optimized for standard 1 mm thick, 8 cm × 8 cm SDS PAGE mini-gels.

Note: 1). Do not fix gels before protein staining with Brightein. Staining of pre-fixed gels with Brightein may not produce optimal results. 2). Do not pre-stain the gel with Coomassie Blue as Coomassie Blue may quench the fluorescence of Brightein stain. 3). Brightein will not stain proteins in non-denaturing polyacrylamide gels. If proteins do not need to be kept under non-denaturing conditions after electrophoresis, native gels can be soaked in 0.05% SDS/7.5% acetic acid for 30 minutes with shaking, then stained with Brightein as described below.

1. After electrophoresis, place the gel in a clean gel staining container (such as a polypropylene container) containing 80 mL of 1 × Brightein staining solution per mini-gel. For the best sensitivity, incubate the gel for at least 90 min with shaking. For rapid results, incubate for 30 minutes. For larger gels, scale up the volume of staining solution accordingly using the mini-gel size as a reference (i.e., V (mL) = 80 mL × (S/64), which S is the size of the gel in cm^2). Using an insufficient volume of staining solution may result in low signal.

Notes: 1) For large 2-D gels, use of a staining time longer than 90 min. may yield better results. 2) Carefully observe for any dye precipitation on the container wall. In case of dye precipitation due to insufficient staining solution, increase the staining time to 6 hours. Dye precipitation should not occur if the amount of staining solution is determined using V (mL) = 80 mL × (S/64).

2. Destaining is not required, but can be performed to reduce background. Remove Brightein staining solution and wash in 100 mL of destaining solution with shaking for 5 min. Decant the destaining solution, add at least 100 mL deionized water and agitate for at least another 5 min before viewing/imaging. Alternatively, destaining and rinsing can be accomplished in a single step by washing the stained gel in at least 100 mL deionized water for 20 minutes with shaking.

Note: The single-step destaining/rinsing in water may produce slightly higher background than the two-step destaining/rinsing procedure, but avoids the use of additional organic solvents. The single-step procedure is adequate for 1-D gels or applications where signal/noise ratio is relatively less demanding.

Viewing and Photographing the Gel:

Brightein has a UV excitation maximum at around ~280 nm and a broad visible excitation maximum centered around ~450 nm (Fig. 1). It emits bright red fluorescence at around ~610 nm. As a result, gels stained with the dye can be viewed using a standard 300 nm UV transilluminator (with ethidium bromide emission filter), a 470 nm blue LED transilluminator, or a laser scanner with a laser line at 450, 473, 488 or 532 nm. For maximum sensitivity, a 490 nm longpass filter should be used. A list of suitable excitation sources and emission filters is shown in Table 2.

The stained gel may be imaged using a photographic film or with a CCD camera. When using a Polaroid camera with Polaroid 667 black-and-white print film, the best result may be obtained using a 490 nm longpass emission filter. A CCD camera permits quantitative detection of protein bands/spots stained with the dye with a linear detection range spanning at least three orders of magnitude. Note that the data must be plotted in logarithm form, i.e. in the form of Log(luminescence Intensity) vs. Log(Protein Amount) in order to obtain the best linear fit. Finally, the exceptional photostability of Brightein

allows long exposure times for maximal sensitivity.

Table 2. List of suitable excitation sources and emission filters for Brightein.

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

Reuse of Brightein 1 × Staining Solution:

Brightein 1 × staining solution may be reused one to two more times. The second use of the staining solution may require 3 hours of staining time while the third use of the staining solution may require overnight staining.

Handling and Disposal:

Brightein 1 × contains an extremely small amount (1-5 ppm) of the dye, which is not listed as a hazardous substance in the US. However, since the solution alsocontains methanol and acetic acid, it should be handled with care. The 1 × waste staining solution can be collected with other flammable liquid for disposed by incineration.

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