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Product Information

Lumitein™ Protein Gel Stain, 100X

Catalog Number: 21002, 21002-1 or 21002-2

Size: 2 mL (21002), 10 mL (21002-1) or 50 mL (21002-2)

Molecular Information: Proprietary

Color and Form: Yellow solution

Spectral Properties: λ_{abs} = ~280 nm, ~450 nm (broad); λ_{em} = 610 nm (See Figure 1)

Storage and Handling

Lumitein 100X and 1X working solution can be stored protected from light at room temperature or at 4°C for at least 12 months from the date of receipt.

Product Description

Lumitein Protein Gel Stain is a luminescent dye designed for detecting proteins in SDS polyacrylamide (SDS-PAGE) gels. The dye has many of the same desirable features as SYPRO® Ruby gel stain, such as detection of 1 ng protein or less, compatibility with both UV and visible light excitation, excellent photostability and a linear range of detection of at least 3 orders of magnitude. However, Lumitein has some important advantages over SYPRO Ruby. Protein staining using Lumitein is far simpler and faster than with SYPRO Ruby. With Lumitein, protein fixation and staining is a single combined 90 minute incubation step. Afterwards, the stained gel can be imaged immediately, destained, or simply washed in water before viewing/imaging. While the linearity of protein quantitation with Lumitein extends for at least three log units, the linearity plot of Lumitein signal has a smaller slope compared to SYPRO Ruby. As a result, highly abundant proteins may appear brighter in SYPRO Ruby-stained gels than in Lumitein-stained gels. However, less abundant proteins in Lumitein-stained gels generally appear as bright as, or even brighter than those in SYPRO Ruby-stained gels. This slope difference may prove advantageous for 2-D gels stained with Lumitein because fluorescent spots of less abundant proteins are less likely to be overwhelmed by those of highly abundant proteins nearby. Finally, Lumitein protein gel staining is fully compatible with downstream protein analyses such as mass spectrometry and Edman-based sequencing using the same sample preparation protocols as for SYPRO Ruby gel stain.

Lumitein protein gel stain is supplied as a ready-to-use 1X staining solution or as a highly concentrated 100X solution, which is more economical and convenient for shipping, handling and storage.

Experimental Procedures

Preparation of Lumitein 1X Staining Solution

Lumitein 1X staining solution is prepared by diluting Lumitein 100X 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 1X solution. Pour the vial of Lumitein 100X into the bottle, add the required amount of each solvent according to Table 1 (below) and mix well. Use the diluted Lumitein 1X solution to rinse the original Lumitein 100X vial to ensure complete transfer of the 100X solution. Purity of the solvents is not critical; reagents typically used for preparing protein gel fixation solution are suitable for preparing Lumitein 1X staining solution. The 1X solution contains sufficient organic solvents to ensure both protein fixation and staining at the same time. Store the 1X solution at room temperature or at 4°C protected from light.

Table 1. Solvent volumes required to prepare 1X staining solution from Lumitein 100X.

Lumitein 100X	Deionized water	Methanol	Acetic acid
2 mL (cat# 21002)	116 mL	56 mL	26 mL
10 mL (cat# 21002-1)	580 mL	280 mL	130 mL
50 mL (cat# 21002-2)	2,900 mL	1,400 mL	650 mL

Preparation of Gel Destaining Solution (optional)

Destaining after Lumitein staining is not necessary; however, if desired, destaining may be accomplished by soaking the stained gels in de-ionized water for 20 minutes on a shaker (See staining procedure below). For even lower background and faster destaining, gels may be destained in a destaining solution containing 30% methanol, 15% acetic acid and 55% water. 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 X 8 cm SDS PAGE mini-gels.

Important:

- Do not fix gels before protein staining with Lumitein. Staining of pre-fixed gels with Lumitein may not produce optimal results
- Do not pre-stain the gel with Coomassie Blue as Coomassie Blue may quench the fluorescence of Lumitein stain.
- Lumitein 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 Lumitein as described below.

1. After electrophoresis, place the gel in a clean gel staining container (such as a polypropylene container) containing 80 mL of Lumitein 1X staining solution per mini-gel. For the best sensitivity, incubate the gel for at least 90 minutes 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 x (S/64), where S is the size of the gel in cm^2). Using an insufficient volume of staining solution may result in low signal.

   Notes:
   - For large 2-D gels, use of a staining time longer than 90 min. may yield better results.
   - 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 x (S/64).

2. Destaining is not required, but can be performed to reduce background. Remove Lumitein 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

Lumitein has a UV excitation maximum at around ~280 nm and a broad visible excitation maximum centered around ~450 nm (Figure 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 (below).

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, however, 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 Lumitein allows long exposure times for maximal sensitivity.

Notes:
- For handling gels, use powder-free gloves and/or rinse your gloves with water prior to the handling.
- Clean the glass surface of the imaging equipment before imaging the gel to avoid potential background from contamination.

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

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 longpass, 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.

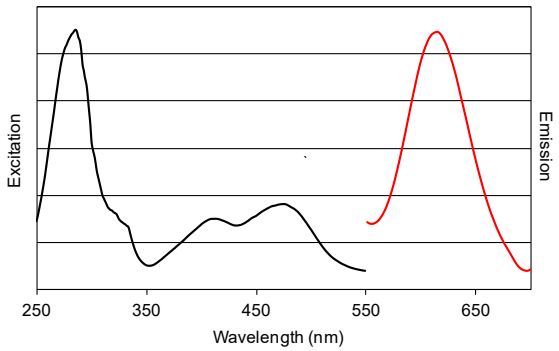

Figure 1. Excitation and emission spectra of Lumitein .

Reuse of Lumitein 1X Staining Solution

Lumitein 1X 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

Lumitein 1X 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 also contains methanol and acetic acid, it should be handled with care. The 1X waste staining solution can be collected with other flammable liquid for disposed by incineration.

Related Products

Catalog Number	Product Name	Unit Size
21001	Lumitein™ Protein Gel Stain, 1X	200 mL
21001-1	Lumitein™ Protein Gel Stain, 1X	1 L
21001-2	Lumitein™ Protein Gel Stain, 1X	5 x 1 L

Please visit our website at www.biotium.com to view our full selection of bioconjugates of our exceptionally bright and photostable CF™ dyes, including antibodies, antibody labeling kits, phalloidin, annexin V, nucleotides, and a-bungarotoxin, as well as classic fluorescent nucleic acid dyes.