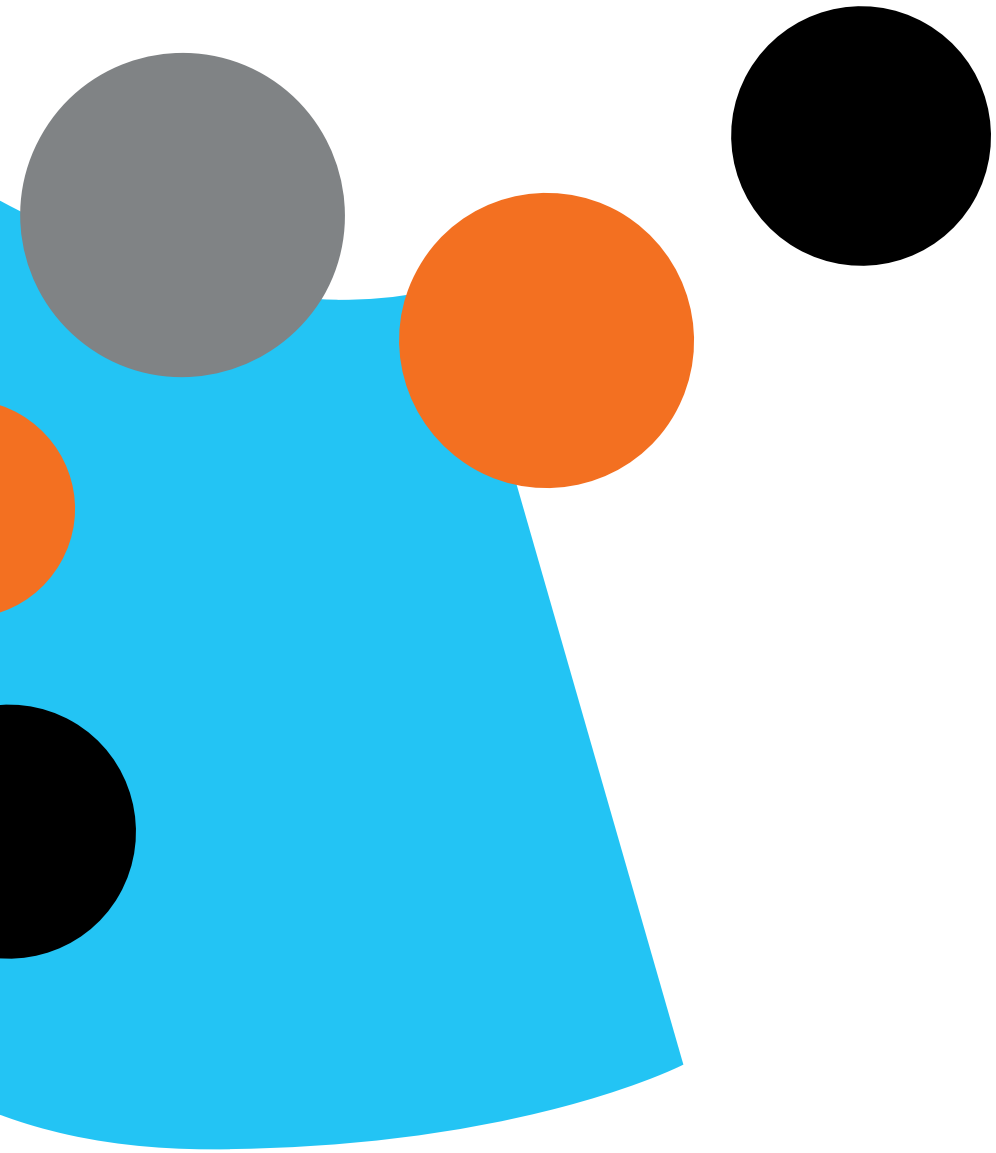
LavaBlue™

The Fluorescent Alternative to Colloidal Coomassie Blue



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Ordering

LB-011001 Lava Blue Protein Kit 1 mL (~ 20 minigel)

LB-011005 Lava Blue Protein Kit 5 mL (~ 100 minigel)

Order fro*m* *www.gelcompany.com*

Content

LavaBlue Protein Stain

LavaBlue is a fluorescent stain for 1-D and 2-D protein gels, developed as an alternative to colloidal Coomassie™ Blue stain. **LavaBlue** staining protocol is fast, simple and flexible, achieving benefits such as high sensitivity and linear dynamic range of proteins over traditional colorimetric stains. LavaBlue is suitable for use with transilluminator based imaging systems and is compatible with mass spectrometry

Quick Facts

Storage

On receipt, store Part A (LavaBlue stain) at room temperature in the original brown bottle to protect from light. When prepared, store solution 1 at room temperature and solution 2 in the fridge (2 – 8 °C).

Disposal

The generation of waste should be avoided or minimized wherever possible. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements.

Detection

The optimum excitation wavelength is around 390 nm (see figure 1). Suitable light source include common UV-transilluminators (UVA or UVB), black light or 405 nm solid state laser scanner.

The optimum emission wavelength is around 500 nm (figure 1). Suitable filters include 450 – 550 long pass filters or band pass filters, or set your system for SYBR™ green (515 nm band Pass).

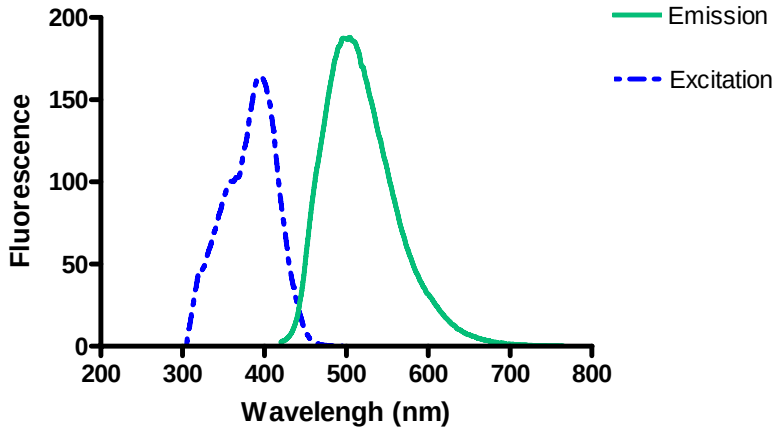

Figure 1. The fluorescence excitation and emission spectra of **LavaBlue** (1 μM) with bovine serum albumin (0.01 μM).

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Features

LavaBlue has the following features:

- **Linear dynamic range.** Fluorescence intensity of **LavaBlue**-stained bands is linear with protein quantity over at least over 2-orders of magnitude, enabling accurate quantification.
- **Simple and quick protocols.** A three-step staining protocol is complete in 2.5 h.
- **Compatibility with standard laboratory imaging equipment.** **LavaBlue**-stained gels can be visualized on a standard UV or a blacklight transillumintor, and imaged with Polaroid® or CCD cameras.
- **Low protein-to-protein variability.** **LavaBlue** stains SDS-protein complexes to generate a consistent staining pattern.
- **Sensitive.** Detects as little as 2.5 ng of protein per 1-D gels.
- **MS-compatibility.** Compatible with PMF down to 5 ng/band.
- **Ready-to-use.** No need to prepare the stain.
- **Cost-effective.** **LavaBlue** staining is less-expensive than other fluorescent stains and is comparable in price with colloidal Coomassie Blue staining.
- **Flexible protocol.** Staining protocols may be modified to have overnight staining to fit into your work flow without loss of performance.
- **Health and safety.** No acetic, phosphoric acid or methanol is used avoiding noxious fumes and simplifying disposal.

LavaBlue is not recommended for staining proteins to achieve the highest sensitivity, the greatest linear dynamic range and the highest MS coverage. For these applications, we recommend **LavaPurple™** protein gel stain (LP-011005).

Stability and Storage

LavaBlue (Part A) should be stored, protected from light, at room temperature. When stored properly, the stock solution is stable at least for 6 months.

Safe Handling and Disposal

All chemicals should be considered potentially hazardous. This product should only be handled by those persons trained in laboratory techniques, and used in accordance with the principles of good laboratory practice. Wear suitable protective clothing including laboratory coat, safety glasses and

disposable gloves. **LavaBlue** is a dilute DMSO solution of the active fluorophore. The diluted working solution is minimally hazardous and non-flammable; however the complete properties of the dye component have not been fully investigated. Part B contains citric acid which is classed as an irritant and contact with skin and eyes should be avoided as severe irritation may occur. Part C contains potassium chloride which is not classified as hazardous according to criteria of NOHSC.

Tips and Troubleshooting

- Ensure that the protocol is followed precisely.
- It is **essential** that solution 2 should be cold when added to the gel in the enhancement step. Ensure that the solution 2 is stored in the fridge (2 – 8 °C) until use. After use, return solution 2 in the fridge for future use.
- Ensure that a green lens (e.g. filter No. 58, Tiffan) is used for photography with Polaroid instant camera systems.
- For CCD systems, use a long pass (450 - 550 nm) filter or the emission filter for SYBR™ green.

Reagents and Equipment

LavaBlue Kit components

LavaBlue protein gel stain kits are supplied as 1000x concentrate of the stain in dimethylsulfoxide (DMSO), either as a single vial containing 1 mL (LB 011001) or as a single bottle containing 5 mL (LB 011005) of stock solution. LB 011001 and LB 011005 provides sufficient fluorophore to stain 20 and 100 polyacylamide minigels, respectively. For convenience, pre-weighed citric acid and potassium chloride powder packets are included in the kit for preparing solutions required for staining steps.

Code/size/No. of minigels

LB 011001: 1 mL size for 20 minigels
LB 011005: 5 mL size for 100 minigels

Contents of Parts

Part A: **LavaBlue** (1000x concentrate)
Part B: citric acid powder (10.1 g/packet)
Part C: potassium chloride powder (150 g/packet)

LB 011001 (20 minigels)

Part A: 1 mL of **LavaBlue** concentrate (1000x)
Part B: 2 packets of citric acid powder
Part C: 1 packet of potassium chloride powder

LB 011005 (100 minigels)

Part A: 5 mL of **LavaBlue** concentrate (1000x)
Part B: 10 packets of citric acid powder
Part C: 5 packets of potassium chloride powder

Solutions to be prepared for staining protocols

Solution 1, fixation and staining solution:
Use solution 1 for both fixation and staining steps.
To prepare 1 L of solution 1, place 850 mL of high purity water into a 1 L bottle then add the contents of citric acid packet 1 (Part B), and mix until dissolved. Add 150 mL of 100% ethanol and mix thoroughly. Store solution 1 at room temperature.

Solution 2, enhancement solution:
Place 800 mL of high purity water into a 1 L bottle then add the contents of the potassium chloride packet (Part C) and mix thoroughly until dissolved. Make the solution up to 1 L with high purity water. There is no need to adjust the pH of the solution. **Important**: Solution 2 should be cold when added to the gel in the enhancement step. Store solution 2 in the fridge (2 – 8 °C) until use.

Staining Protocol

Standard Protocol

1. Fixation (1 h): Pour 50 mL of solution 1 into a small plastic container. Place the minigel (8 cm x 11 cm x 1 mm) into the container and fix the gel for a minimum of 1 h with gentle rocking. Ensure that the gel is completely covered by the solution.

For a small format 2-D gels (13.3 cm x 8.7 cm x 1 mm), use 100 – 150 mL of solution 1, depending on the size of gel containers.

2. Staining (1 h): Decant solution 1 and replace with 50 mL of fresh solution 1. Add 50 µL of **LavaBlue** concentrate into the gel container and mix thoroughly by gentle rocking of the gel container. Cover the container with aluminum foil and stain the gel for a minimum of 1 h with gentle rocking.

For small format 2-D gels, use 100 – 150 mL of solution 1 and add 100 – 150 µL of **LavaBlue** concentrate.

3. Enhancement (0.5 h): Decant the staining solution and pour about 50 mL of chilled enhancement solution 2 into the tray. Cover the container with aluminum foil and enhance the gel for a minimum of 0.5 h with gentle rocking.

For the small format 2-D gels, use 100 – 150 mL of solution 2.

Storing stained gels

LavaBlue-stained gels should be stored in the enhancement solution at 4°C, protected from light. The fluorescence signal decreases over time, but the gels should retain a usable fluorescence signal for many weeks, depending on the amount of protein in the bands.

Imaging

LavaBlue-stained gels can be visualized using a standard UV-transilluminator (300 or 365 nm) or optimally a transilluminator with blacklight blue lamps (320 – 400 nm). The gels are best visualized while wearing yellow glasses (e.g. IRC right vision or Bolle™ viper safety glasses).

Imaging with Polaroid® GelCam

LavaBlue-stained gels can be visualized on a standard UV-transilluminator and photographed with Polaroid GelCam using Black and White Polaroid film (figure 2A). A dark green lens should be used to achieve the best sensitivity. The film can be scanned using a common desktop scanner and processed using a graphics program (e.g ImageJ, Photoshop, etc).

Imaging with CCD camera

Any common gel documentation system equipped with a standard UV-transilluminator (figure 2B) or an excitation filter for SYBR Green can be used. Please refer to figure 1 for optimal excitation and emission wavelengths.

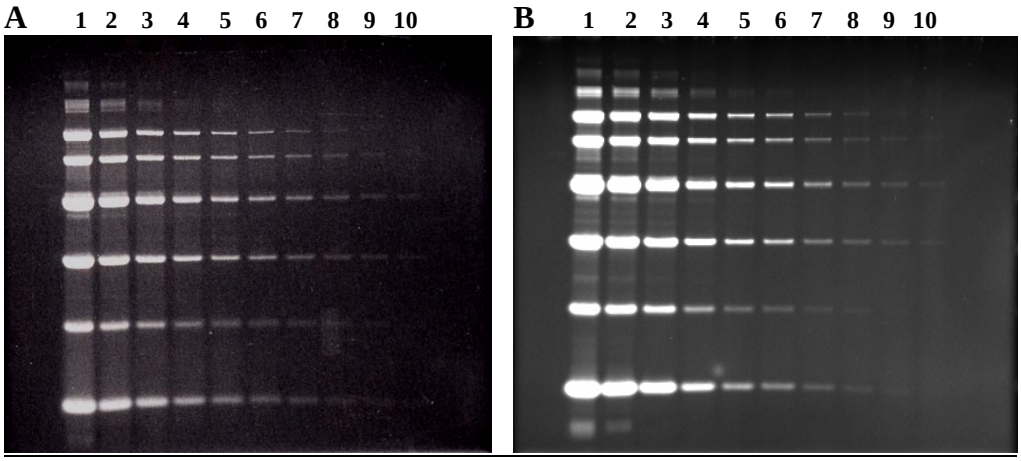

Figure 2. LavaBlue staining of a 2x serial dilution of the low molecular maker (GE Healthcare) using a standard protocol. **LavaBlue**-stained protein bands were photographed with Polaroid GelCam (A) using Tiffen N-58 green lens; f5.6; 2-3s exposure (A), and with a CDD camera (B), using G:BOX (Syngene; Ex/Em = UV transilluminator/short wave (450 – 550 nm) band pass). Lane 1 contains the two target proteins, BSA (2nd band) and CA (4th band) at a concentration of 1280 ng; lane 2, 640 ng; lane 3, 320 ng; lane 4, 160 ng; lane 5, 80 ng; lane 6, 40 ng, lane 7, 20 ng; lane 8, 10 ng; lane 9, 5 ng; lane 10, 2.5 ng

Optional Protocol

The **LavaBlue** protocol is flexible and it is possible to extend the staining step to overnight to fit into your workflow.

Related Products

gelcompany offers a range of related products including ultrasensitive gel stain, ultrasensitive blot stain, a protein quantification kit, peptide quantification kit, monitoring of proteolytic digestion and a live cell imaging reagent. For details of these and our new products visit our website at: *www.gelcompany.com*

Legal

LavaBlue can only be used for research applications in the life sciences.

Lava^{TM} is a trademark of Fluorotechnics.
Coomassie^{TM} is a trademark of Imperial Chemical Industries.
SYBR^{TM} is a trademark of Invitrogen.
Polaroid^{TM} is a trademark of The Polaroid Corporation.
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