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INSTRUCTION MANUAL

LavaPurple™

Protein Staining for Polyacrylamide Gels and Blotting Membranes

(Cat. No. 43373)

SERVA Electrophoresis

Contents

Ver. 08/11

1. LavaPurple™ Protein staining

1.1. Introduction

LavaPurple™ is based on a small naturally occurring fluorescent compound [1] that reversibly binds to lysine, arginine, and histidine residues in proteins and peptides [2] to yield an intensely red-fluorescent product. This unique mechanism provides sensitive quantification of proteins in 1 and 2D gels of all chemistries, on both PVDF and nitrocellulose blots [3, 4] and provides unparalleled compatibility with mass spectrometry [5].

[1] Bell, P.J.L. and Karuso, P. (2003) Epicocconone, a novel fluorescent compound from the fungus *Epicoccum nigrum*. Journal of the American Chemical Society. **125**, 9304.

[2] Coghlan, D. R., Mackintosh, J. & Karuso, P. (2005). Mechanism of reversible fluorescent staining of protein with Epicocconone. Organic Letters. **7**, 2401-240.

[3] Malmport, E., Mackintosh, J., Ji, H., Veal, D. & Karuso, P. (2005). Visualization of proteins electro-transferred on Hybond ECL and Hybond-P using Deep Purple Total Protein Stain. GE-Healthcare Life Science News. **19**, 12-13.

[4] Mackintosh, J.A., Choi, H.-Y., Bae, S.-H., Veal, D.A., Bell, P.J., Ferrari, B.C., van Dyk, D., Verrills, N.M., Paik, Y.-K. & Karuso, P. (2003). A fluorescent natural product for ultra sensitive detection of proteins in 1-D and 2-D gel electrophoresis. Proteomics. **3**, 2273-2288.

[5] Tannu, N.S. Sanchez Brambila, G.S., Kirby, P., Andacht, T.M. (2006) Effect of staining reagent on peptide mass fingerprinting from in-gel trypsin digestions: A comparison of Sypro Ruby and Deep Purple.Electrophoresis **27**, 3136 - 3143.

1.2. Storage

On receipt, store the stain in a freezer at -15 °C to -30 °C in the original brown bottle provided to protect from light.

1.3. Staining

Do not use metal trays, you can use dark or transparent plastic trays.

1.4. Detection

Excitation wavelengths: 405, 500 nm. Suitable light sources include green (543, 532 nm); blue (488 nm); violet (405 nm) or UVA.

Emission wavelength: The maximum emission is at wavelength of 610 nm regardless of what excitation source is used. Suitable filters include 610 nm band pass or 560 long pass.

1.5. Disposal

LavaPurple is based on epicocconone, a biodegradable natural product, in an environmentally safe solution that requires no special disposal procedures.

2. Features

- **Flexible: LavaPurple** is suitable for staining proteins separated by either 1D or 2D electrophoresis on native or denaturing gels of all chemistries. **LavaPurple** is also suitable for both PVDF and nitrocellulose blots.
- **Compatible:** Unique reversible staining by **LavaPurple** makes it fully compatible with downstream processing (MS, immuno-staining and Edman sequencing). **LavaPurple** shows higher MS compatibility than competing products [5].
- **Environmentally friendly: LavaPurple** is made from a biodegradable natural product that is safe and simple to dispose.
- **Health and safety:** No requirement to store and handle volatile, corrosive acetic acid, heavy metal based or toxic stains.
- **Simple and convenient:** The protocol is simple (4 steps) and quick (3 h).
- **Sensitive:** reliably provides ≥ 50 pg sensitivity, in gels, when tested on 14 standard proteins with a range of molecular weights.
- **Multiplex compatible:** with other fluorophores (e.g. Cy™ Dyes), other stains (e.g. Coomassie™, ProQ Diamond™).
- **Clean background and no speckling: LavaPurple** does not produce speckles and has low background fluorescence.
- **Stronger gels:** Staining with **LavaPurple** does not involve the use of high concentrations of organic solvents that result in fragile gels.

3. Staining of polyacrylamide gels

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 overalls, safety glasses and gloves. **LavaPurple** is a dilute solution of a natural organic dye in DMSO/acetonitrile. The diluted working solution is minimally hazardous and non-flammable; however the complete properties of the dye component have not been fully investigated.

3.1. Important to know before starting

- Ensure that the protocol has been followed accurately.
- **LavaPurple** degrades over time in high pH solutions and in bright light.
- For appropriate staining, it is important, that it occurs under basic conditions. It is a good idea to keep the used staining solution in a bottle and use it for a short pre-buffering of a gel between the fixing and the staining step.
- **Do not stain the gels for longer than the recommended time.**
- Ensure that **LavaPurple** has been brought to room temperature prior to adding to solution 2 to make the staining solution.
- **LavaPurple** is a very sensitive protein stain and for best results scrupulous cleanliness is required. Dust or particles in reagents used during staining may cause speckling. Detergents used for cleaning staining trays and bottles need to be completely removed by multiple rinses in high purity water. Use only analytical or higher grade chemicals and reagents. If speckling is still present filtering buffers may be required.
- Plastic trays that have been previously used for Sypro™ products, Coomassie™ or other stains may cause **LavaPurple** to speckle; therefore trays should be used only for **LavaPurple** stain or cleaned with detergent, water and methanol.

3.2. Buffers and Solutions

Solution 1 (fixation and acidification): Place 850 ml of high purity water into a 1 L bottle then add 10 g citric acid (SERVA Cat. No. 38640) and mix until dissolved. Add 150 ml of 100 % ethanol and mix thoroughly.
Please note: Due to volume contraction, the complete volume of this solution will be less than 1 L.

Solution 2 (staining buffer): Add 1 L of high purity water to a 1 L bottle and add 6.2 g boric acid (SERVA Cat. No. 15165). Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then weigh and add 3.85 g of sodium hydroxide (NaOH, p.a. quality) and continue stirring until fully dissolved.

Solution 3 (washing): Mix 850 ml of high purity water and 150 ml of 100 % ethanol in a 1L bottle.
Please note: Due to volume contraction, the complete volume of this solution will be less than 1 L.

Storage of solutions
Solutions 1-3 can be stored at room temperature and are stable for up to 6 months. Solutions should be free of precipitation and protected from airborne particulate matter as this will result in speckling on the gels.

3.3. Staining protocol

3.3.1. Fixation

- Fix gels in fixation **solution 1** for a minimum of 1hr with gentle rocking.
- For appropriate volumes see table 1. For gels thicker than 1 mm the fixation time should be extended to 1.5 h.

  The fixation time can be extended to overnight if required to decrease background or to fit into the workflow. Additional fixation washes may be used to decrease background levels even further, though this is generally not required.

- In parallel, the **LavaPurple** concentrate should be removed from -20 °C and allowed to warm to room temperature at this time.

3.3.2. Staining

Prepare the staining solution immediately prior to staining by thoroughly mixing the fully thawed concentrated stain and then diluting **1 part LavaPurple concentrate in 200 parts staining buffer solution 2**.
For appropriate volumes see table 1.

Please note: LavaPurple slowly degrades over time in the high pH buffer. If used immediately the staining solution may be re-used once for gel staining.

- Ensure the 1x staining solution is well mixed before adding to the gel.
- Remove the gels from the fixation solution 1 and place into the staining solution with gentle rocking.
- Stain for 1 h for 1.0 mm thick free-floating gels and for 1.5 h for 1.5 mm thick or 1 mm thick film-backed gels.

IMPORTANT: Minimize carry-over of the acidic fixation solution (a short pre-buffering can increase staining effectivenes).

- Extending the staining time up to 2 h will not affect results.
- Do not stain for longer than 2 h.

3.3.3. Washing

- Remove the gels from the staining solution and wash the gels by gentle rocking in the washing **solution 3** for 30 min. For appropriate volumes see table 1.
- For 1.5 mm gels or if high background fluorescence is experienced washing time should be increased to 45 min.

3.3.4. Acidification

- Remove the gels from the washing solution and acidify by placing them in solution 1 and rock gently for 30 min. For appropriate volumes see table 1.

This step may be repeated or extended up to overnight to reduce background staining). If left in the acidifier overnight, the gels should be protected from the light.

Table 1: **LavaPurple** staining of polyacrylamde gels

Gel type		***MiniGel***	***Standard Flatbed***	***Large Flatbed***	***Large gel (1 mm thick)***	
Process	**Solution**	**Volume per gel**				**Time ***
Fixing	Solution 1	100 ml	200 ml	300 ml	400 ml	**1 h**
Staining	Solution 2	0.5 ml dye in 100 ml	1 ml dye in 200 ml	1.5 ml dye in 300 ml	2 ml dye in 400 ml	**1 h**
Washing	Solution 3	100 ml	200 ml	300 ml	400 ml	**30 min**
Acidification	Solution 1	100 ml	200 ml	300 ml	400 ml	**30 min**

***** For 1.5 mm or 1 mm backed gels extend time by 50 %.

IMPORTANT:

- The gel may be fixed in solution 1 overnight with no negative effects.
- Bring dye concentrate to room temperature and thoroughly mixed prior to being added.
- The staining solution must be made fresh (not more than 30 minutes prior to use).
- Add LavaPurple concentrate to solution 2 before being poured onto the gel to prevent staining artifacts.
- It is not necessary to protect the gel from light.
- Do not stain longer than 3 h as signal will decrease after this time.
- If there is no time for scanning you can leave the gel in the acidifier solution 1 overnight.

3.4. Storage

Gel should be stored at 4°C protected from light in 1 % citric acid. For extended storage (up to 6 months) add **LavaPurple** (1:200) to the storage solution. Prior to imaging the gels should be rinsed (2 x 15 min) in washing solution 3. Acidifying in solution 1 for 15 min may be used to reduce background.

4. Staining of blotting membranes

For best results run the buffer front off the base of the gel during electrophoresis prior to transfer. Care should be taken to ensure that the membrane does not dry during the staining.

4.1. Buffers and solutions

The solutions are identical to those used for gel staining.

4.2. Staining protocol

Care should be taken to ensure that the membrane does not dry during the staining.

Washing: Following transfer, place the wet membrane in water and wash for 3 x 5 min. For small-sized blots use 50 ml for all steps, for large-sized blots use 200 ml.

Basification: Wash the blot in **solution 2** for 10 minutes.

Staining: For small blots add 250 µl **LavaPurple** to 50 ml of high purity water. For large blots add 1 ml of LavaPurple to 200 ml of high purity water. Stain in **Lava Purple** for 15–30 min. Blots should be placed ‘protein side’ down in the prepared stain.

Follow either the PVDF or nitrocellulose protocol from this point.

4.2.1. PVDF membrane

Acidification: Place the blot in **solution 1** and rock gently for 5 min.
For large blots use 400 ml. For small blots use 50 ml.
Please note: This treatment will cause the blot to appear green.

Washing: Rinse blot with 100 % methanol for 2–3 min until green background on blot has been completely removed. Multiple rinses may be required.

Drying: Dry for 2–3 min. To allow simultaneous drying of the blot on both sides it may be best to dry the blot on a wire mesh. Allow the blot to completely dry. The blot is then ready for imaging and further analysis.

4.2.2. Nitrocellulose

Washing: Place the blot in **solution 2** and rock gently for 5 min. Remove from **solution 2** and place into high purity water and rock gently for 5 min. Repeat the water washing.

Drying: Allow blot to completely dry. Your blot is now ready for imaging and further analysis.

4.3. Storage

PVDF and nitrocellulose blots should be stored dry, in the dark at room temperature.

4.4. De-staining

LavaPurple staining is reversible and the stain may be removed for subsequent analysis, for example by immuno staining.
LavaPurple may be removed from blots without significant removal of proteins by washing membranes overnight in 50 mM ammonium carbonate solution.

For rapid **de-staining of PVDF membranes**, wash in 50 % acetonitrile containing 30 mM ammonium carbonate for 15 min.

For rapid **de-staining of nitrocellulose membranes**, wash in 50 % ethanol (methanol may be used) containing 50 mM ammonium carbonate for 15 min.

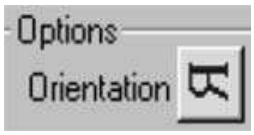
Note: The rapid de-staining protocols may result in loss of protein from the membrane.

5. Scanning of 2DGel flatbed NF precast gels

The gels are scanned with the gel surface down facing the platen directly after applying a few ml water on it (focal plane 0). During scanning a LF glass plate or the new Scan Frame (SERVA Cat. No. HPE-A22) is laid on the gel to avoid curling of the edges.

Do not apply „Press Sample“!

For accurate spot picking the scanning orientation must be flipped as shown here:



6. Troubleshooting

Low signal intensity and poor sensitivity

- The most common cause of low signal intensity is poor basification.
  Check the pH during the staining step; it should be between pH 9.5 and 10.5.
  Carry-over of acid from the fixation step is a common cause of poor basification and thus staining.

  Too acidic conditions turn the color of the staining solution into yellow!

  It is helpful to apply a short pre-buffering step with used staining solution prior to staining.
- Ensure you use the appropriate volume of solutions, smaller volumes can reduce staining effectiveness.
- Ensure that you use the stain at the recommended 1:200 dilution.
  Greater dilution will result in lower fluorescence intensity.
- Long exposure time and associated heating on CCD-based instruments may cause the stain to fade.
- Ensure you are using the correct filters, photomultiplier tube settings and light source on your scanner.
- Ensure the concentrated **LavaPurple** was brought to room temperature and thouroghly mixed prior to dilution to 1x.
- Ensure that the correct fixation solution was used.

High background

- Ensure that the stain was fully mixed into the borate buffer before adding to the gel.
- Ensure correct volumes for gel solutions have been used.
- Ensure only one gel per tray is stained. Multiple gels in one tray can result in an uneven background.
- Ensure gels are not handled or only with clean gloves to avoid contamination with dust and/or protein.
- For thicker (>1mm) or backed gels you may need to extend fixing, washing and acidification times.
- Ensure the concentrated **LavaPurple** was brought to room temperature and thoroughly mixed prior to dilution to 1x.

Boundary or negative staining

- Ensure you use a high quality SDS in the preparation and running of the gel.
- Extend the fixation time to overnight.
- Ensure you use sufficient fixation and washing solutions.
- Extend your washing time.

7. Order Information

Product	Size	Cat. No.
Chemicals/Reagents		
LavaPurple	25 ml	43373.01
	4 x 25 ml	43373.02
Citric acid·H_2O analytical grade	500 g	38640.01
	1 kg	38640.02
	5 kg	38640.03
Boric acid analytical grade	250 g	15165.02
	1 kg	15165.01
Accessories for Staining and Scanning		
Steel Tray + Grid + Lid for all 125 mm x 260 mm gels (150 mm x 300 mm x 60 mm)	1 piece	HPE-A19
Steel Tray + Grid + Lid for all large and Dalt gels (220 mm x 280 mm x 60 mm)	1 piece	HPE-A20
Steel Tray Multi 6 for up to 6 large gels (20 cm x 26 cm)	1 piece	HPE-A21
ScanFrame	1 piece	HPE-A22