LavaPurple
Total Protein Stain for Gels and Blots

gelcompany

Ordering

LP-011005 LavaPurple Protein Kit 5mL dilutes to 1L

LP-011025 LavaPurple Protein Kit 25mL dilutes to 5L

LP-011100 LavaPurple Protein Kit 100mL dilutes to 20L

LP-012005 LavaPurple Protein Stain only 5mL dilutes to 1L

LP-012025 LavaPurple Protein Stain only 25mL dilutes to 5L

LP-012100 LavaPurple Protein Stain only 100mL dilutes to 20L

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Content

LavaPurple Total Protein Stain

LavaPurple™ is based on a small naturally occurring fluorescent compound[1] that reversibly binds to lysine, arginine, and histidine residues in proteins and peptides to yield an intensely red-fluorescent product[2]. This unique mechanism provides sensitive quantification of proteins in 1 & 2D gels of all chemistries, on both PVDF and nitrocellulose blots[3-5] and provides unparalleled compatibility with Mass Spectrometry[6-8]

Quick Facts

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. The buffers and fixatives (parts A-C) in the kit can be stored at room temperature in a dry location.

Disposal

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

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.

See www.gelcompany.com/lavapurple_gel.php for spectra.

Features

- Flexible: LavaPurple Total Protein Stain 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[8].

- 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 (3h) and, in the kit, all the required buffers are provided in convenient packaging.

- Sensitive: reliably provides <50pg 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.

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 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. Part A contains citric acid, which is classed as an irritant and contact with skin and eyes should be avoided as severe irritation may occur. Part B contains boric acid which can cause irritation of the eyes and respiratory tract and prolonged exposure may cause dermatitis. Part C contains sodium hydroxide which is corrosive to the eyes, the skin and the respiratory tract.

Solutions and Buffers

Solution 1 (fixation and acidification): Place 850mL of high purity water into a 1L bottle then add the contents of part 'A', and mix until dissolved. Add 150mL of 100% ethanol and mix thoroughly.

Solution 2 (staining buffer): Add 1L of high purity water to a 1L bottle and add the contents of part 'B'. Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) then weigh* and add 3.85g of sodium hydroxide (part 'C') and continue stirring until fully dissolved.

Solution 3 (washing): Mix 850mL of high purity water and 150mL of 100% ethanol in a 1L bottle.

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.

*The small kit (LP011005) packet C contains 3.85g of sodium hydroxide

Tips

- Ensure that the protocol has been followed accurately.
- LavaPurple degrades over time in high pH solutions and in bright light. 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 the most sensitive protein stain available 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.
- The platen on laser scanners that have previously been used for Sypro products may also be a source of speckling and high background, and it may be necessary to clean the platen with a 10-30% solution of hydrogen peroxide to remove any stain residue.
- Ensure you set-up your imager according the manufacturers instructions. The correct setting of PMTs is particularly important in order to obtain optimal results.

Gelcompany is continually improving its service to customers. You can find regularly updated frequently asked questions at www.gelcompany.com. To get prompt advice from our technical experts contact technical support at support@gelcompany.com.

Gel Staining Protocol

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 or backed gels the fixation time should be extended to 1.5hr. 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. The LavaPurple concentrate should be removed from -20°C and allowed to warm to room temperature at this time.

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. Ensure the 1x staining solution is well mixed before adding to the gel. For volumes required refer to table 1. The staining solution degrades over time and should not be stored. Remove the gels from the fixation solution 1 and place into the staining solution with gentle rocking. Minimize carry-over of the acidic fixation solution. Stain for 1hr for 1.0mm thick free-floating gels and for 1.5hr for 1.5mm thick or backed gels.
Extending the staining time up to 2 hr will not affect results.
Do not stain for longer than 2hr.

Washing: Remove the gels from the staining solution and wash the gels by gentle rocking in the washing solution 3 for 30min. For 1.5mm gels or if high background fluorescence is experienced washing time should be increased to 45min.

Acidification: Remove the gels from the washing solution and acidify by placing them in solution 1 and rock gently for 30min.
(This step may be repeated or extended up to overnight to reduce background staining). If destaining overnight, the gels should be protected from the light.

Table 1: Volumes of solutions used for different gels sizes

SOLUTION / GEL DIMENSION	Fixation 1	Staining		Washing 3	Fixation 1
		Buffer 2	Lava Purple		
8cm x 11 cm x 1 mm (Mini-gels)	100mL	50mL	250µL	100mL	100mL
13.3 cm x 8.7 cm x 1 mm (small format 2D gels)	200mL	100mL	500µL	200mL	200mL
17 cm x 17 cm x 1 mm	1L	500mL	2.5mL	500mL	500mL
17 cm x 17 cm x 1.5 mm	1L	500mL	2.5mL	500mL	500mL
15 cm x 19 cm x 1 mm	1L	500mL	2.5mL	500mL	500mL
15 cm x 19 cm x 1.5 mm	1L	500mL	2.5mL	500mL	500mL
20 cm x 25 cm x 1 mm	1.5L	750mL	3.75mL	750mL	750mL
20 cm x 25 cm x 1.5 mm	1.5L	750mL	3.75mL	750mL	750mL

*Gels must be completely covered and free to move in the solution.

Blot Staining Protocol

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.

Washing: Following transfer, place the wet membrane in water and wash for 5min. For small-sized blots use 50mL volumes for all steps. For large-sized blots use 400mL volumes.

Follow either the PVDF or nitrocellulose protocol from this point.

PVDF

Staining: For small blots add 125µL LavaPurple concentrate to 50mL of solution 2. For large blots add 1mL of LavaPurple concentrate to 400mL of solution 2. Place blot protein side down in the prepared stain. Stain, with gentle rocking, for 15-30mins.

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

Washing: Rinse blot with 100% ethanol (methanol may be used) for 2–3min until green background on blot has been completely removed. 3 washes should be sufficient.

Drying: To allow the blot to dry evenly, hang from a peg or dry on wire mesh. Allow the blot to completely dry. The blot is then ready for imaging and further analysis.

Nitrocellulose

Staining: For small blots add 125µL LavaPurple concentrate to 50mL of solution 2. For large blots add 1mL of LavaPurple concentrate to 400mL of solution 2. Place blot protein side down in the prepared stain. Stain, with gentle rocking, for 15-30mins.

Washing: Place the blot in solution 2 and rock gently for 5min.

Remove from solution 2 and place into high purity water and rock gently for 5min. Repeat the water washing.

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

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 50mM ammonium carbonate solution (not supplied).

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

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

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

Imaging

LavaPurple Total Protein Stain may be imaged by standard fluorescence scanners and CCD camera systems such as the Typhoon™ (GE-Healthcare), Ettan DIGE™ Scanner (GE-Healthcare), FX Molecular Imager™ (BioRad), FLA 3000 (Fuji), ChemiGenius™ (Syngene), ImageQuant™ Imager (GE-Healthcare) or similar gel documentation systems.

Laser-scanning

For laser scanning based instruments we recommend the use of the green (532nm) light source and a long pass orange filter (LP 560nm). For exceptionally low backgrounds a red (610nm) band pass filter may be used. For many multiplex applications (such as DIGE) see www.gelcompany.com/lavapurple_gel.php. for more details.

Transilluminator-based CCD systems

We recommend that you use long wavelength UVA or black light blue lamps in combination with orange (LP 560nm) filters. Cooling the gel prior to imaging will increase the signal and help prevent bleaching.

Ettan DIGE imager

With the Ettan™ DIGE imager (GE-Healthcare) use the green (540/25 nm) light source with the orange (595/25 nm) filter. For many multiplex applications (such as DIGE) the violet excitation filter (390/20 nm) with the orange emission filter (595/25 nm) avoids cross talk (e.g. with the Cy2 and Cy3 signal).

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 15min) in washing solution (3). Acidifying in solution 1 for 15 minutes may be used to reduce background.

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

Image Analysis

Following generation of the digital image from your LavaPurple stained gel it is important to assess the quality of the image using suitable image analysis software. Advice on image analysis of LavaPurple stained gels can be found at www.gelcompany.com/lavapurple_gel.php

Multiple gel staining and high throughput

LavaPurple is particularly suited to automated high throughput systems and to the simultaneous staining of multiple gels in a multi-gel apparatus. For further details see: www.gelcompany.com/lavapurple_gel.php

Reuse and dilution

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

Spot picking:

For manual spot picking we recommend the use of blacklight blue lamps with orange glasses. This provides a safer option than UV-lamps for spot cutting and is closer to the excitation maxima of epicocconone. LavaPurple stained gels can also be subsequently stained with Coomassie Brilliant Blue or silver to visualise spots for manual spot picking. Details of colorimetric post staining can be found at: www.gelcompany.com/content/documents/manual_spot.pdf
Printed images have been successfully used as templates for cutting spots. An applications note describing this can be found at: www.fluorotechnics.com/lavapurple_gel.php

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 pH 10.5. Carry-over of acid from the fixation step is a common cause of poor basification and thus staining.
- 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 thoroughly mixed prior to dilution to 1x.

High background

- Ensure that the correct fixation solution was used.
- 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.

Options

Fixation and acidification solution (1):

- Ethanol 15% (v/v) in water can be replace by methanol 30% (v/v) in water.
- Citric acid 1% (w/v) in water can be replaced by acetic acid 7.5% (v/v) in water.
  Note: Acetic acid concentrations should not exceed 7.5%
- You may prepare your own buffers; details can be found at www.gelcompany.com/lavapurple_gel.php. All reagents should be of analytical grade or higher.

Washing solution (3)

- Ethanol 15% (v/v) in water can be replace by methanol 30% (v/v) in water.

We recommend that you consistently use either methanol or ethanol throughout the procedure.

Related Products

Gelcompany offers a range of related products including 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

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Notes