

# INSTRUCTION MANUAL

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## SERVA Purple HiSens

Protein Staining for Polyacrylamide Gels and Blotting Membranes

(Cat. No. 43408)



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# 1. SERVA Purple HiSens Protein staining

## 1.1. Introduction

**SERVA Purple HiSens** is a new synthetic fluorescent dye. It is a further development of the naturally occurring fluorescent compound Epicocconone <sup>[1]</sup> that reversibly binds to lysine, arginine, and histidine residues in proteins and peptides <sup>[2]</sup> 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 <sup>[3, 4, 5]</sup> and provides unparalleled compatibility with mass spectrometry <sup>[5]</sup>.

[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] Moritz C.P. et al. (2014) *Proteomics*, **14** (2-3), 162 – 168.

[6] 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.

SERVA Purple can only be used for research applications in life science.

## 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: 518 nm

Emission wavelength: 610 nm

Suitable filters include 610 nm band pass or 560 long pass.

## 2. Features

- **Flexible:** **SERVA Purple HiSens** is suitable for staining proteins separated by either 1D or 2D electrophoresis on native or denaturing gels of all chemistries. **SERVA Purple HiSens** is also suitable for both PVDF and nitrocellulose blots.
- **Compatible:** Unique reversible staining by **SERVA Purple HiSens** makes it fully compatible with downstream processing (MS, immuno-staining and Edman sequencing). **SERVA Purple** shows higher MS compatibility than competing products <sup>[5]</sup>.
- **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:** Sensitivity is up to < 20 pg/band
- **Multiplex compatible:** with other fluorophores (e.g. Cy™ Dyes), other stains (e.g. Coomassie®, ProQ® Diamond).
- **Clean background and no speckling:** **SERVA Purple HiSens** does not produce speckles and has low background fluorescence.
- **Stronger gels:** Staining with **SERVA Purple HiSens** does not involve the use of high concentrations of organic solvents that result in fragile gels.

ProQ® Diamond is a trademark of Invitrogen Life Technologies Corporation..

Cy™ is a trademark of GE Healthcare.

Coomassie® is a trademark of ICI Ltd.

### 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.

**SERVA Purple HiSens** is a dilute solution of a synthetic organic dye in DMSO, water-free. 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.
- **SERVA Purple HiSens** 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 (alternatively Solution 2) 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 (staining duration > 3 h will cause a strong decrease in sensitivity).**
- Ensure that **SERVA Purple HiSens** has been brought to room temperature prior to adding to solution 2 to make the staining solution.
- **SERVA Purple HiSens** 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<sup>®</sup> products, Coomassie<sup>®</sup> or other stains may cause **SERVA Purple HiSens** to speckle therefore trays should be used only for **SERVA Purple HiSens** stain or cleaned with detergent, water and methanol.

SYPRO<sup>®</sup> is a trademark of Invitrogen Life Technologies Corporation.

## 3.2. Buffers and Solutions

**Solution 1 (fixation & 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 pH 9.5):**

Dissolve 4.4 g  $\text{NaHCO}_3$  (SERVA cat. no. 30180) in 900 ml water (high purity), add 2 ml of 5 N NaOH and fill with high purity water to a final volume of 1 L. Dissolve the powder by mixing using a magnetic stirrer (may take several minutes) 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 **SERVA Purple HiSens** concentrate should be removed from -20 °C and allowed to warm to room temperature at this time.

#### 3.3.2. Buffering

- Incubate the gels 3 x 10 min in **Solution 2** while gently rocking.

#### 3.3.3. Staining

Prepare the staining solution immediately prior to staining by thoroughly mixing the fully thawed concentrated stain and then diluting **1 part SERVA Purple HiSens concentrate in 250 parts Solution 2.**

For appropriate volumes see table 1.

**Please note: SERVA Purple HiSens 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 **Solution 2** and place into the staining solution with gentle rocking.
- Stain the gels for 1 h.

#### 3.3.4. 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.

### 3.3.5. 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: **SERVA Purple HiSens** staining of polyacrylamde gels

Gel type		<i>MiniGel</i>	<i>Standard Flatbed</i>	<i>Large Flatbed</i>	<i>Large gel (1 mm thick)</i>	
Process	Solution	Volume per gel				Time *
<b>Fixing</b>	Solution 1	100 ml	200 ml	300 ml	400 ml	<b>1 h</b>
<b>Staining</b>	Solution 2	0.4 ml dye in 100 ml	0.8 ml dye in 200 ml	1.0 ml dye in 250 ml	1.6 ml dye in 400 ml	<b>1 h</b>
<b>Washing</b>	Solution 3	100 ml	200 ml	300 ml	400 ml	<b>30 min</b>
<b>Acidification</b>	Solution 1	100 ml	200 ml	300 ml	400 ml	<b>30 min</b>

#### 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 SERVA Purple HiSens 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 documentation 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 % (w/v) citric acid (storage solution). For extended storage (up to 6 months) add **SERVA Purple HiSens** (1:250) 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 let the buffer front run 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 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 **SERVA Purple HiSens** to 50 ml of high purity water. For large blots add 1 ml of SERVA Purple to 200 ml of high purity water. Stain in **SERVA Purple HiSens** 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

**SERVA Purple HiSens** staining is reversible and the stain may be removed for subsequent analysis, for example by immuno staining.

**SERVA Purple HiSens** 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. Documentation

For subsequent documentation either SERVA BlueImager (cat. no. BI-RGB) or the Typhoon Imaging System (GE Healthcare) can be used.

## 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 Solution 2 or 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 1:250 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 **SERVA Purple HiSens** was brought to room temperature and thoroughly mixed prior to dilution to 1x.
- Ensure that the correct fixation solution was used.

### High background

- The staining tray shouldn't be used for Coomassie staining before.
- 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 **SERVA Purple HiSens** 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 that sufficient fixation and washing solutions are used.
- Extend the washing time.

## 7. Order Information

<b>Product</b>	<b>Size</b>	<b>Cat. no.</b>
<b>Chemicals/Reagents</b>		
SERVA Purple	5 ml	43408.01
	25 ml	43408.02
Citric acid·H <sub>2</sub> O analytical grade	500 g	38640.01
	1 kg	38640.02
	5 kg	38640.03
NaHCO <sub>3</sub>	1 kg	30180.02
<b>Documentation</b>		
SERVA Bluemager	1 unit	BI-RGB