INSTRUCTION MANUAL

SERVA ProteinStain Fluo-Y

Sensitive Fluoresecent Protein Staining for Polyacrylamide Gels

(Cat. No. 35092)

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1. SERVA ProteinStain Fluo-Y

1.1. Introduction

SERVA ProteinStain Fluo-Y is a fast and sensitive fluorescent dye for visualization and quantitation of proteins separated by 1D- and 2D-SDS PAGE. The 100x stock solution can be easily diluted with water to a 1x staining solution. The stock solution (10 ml) is sufficient for preparing 1 L 1x staining solution. Gels stained with **SERVA ProteinStain Fluo-Y** may be directly visualized with a variety of different UV-based fluorescent imaging systems. The optimal excitation of **SERVA ProteinStain Fluo-Y** is at 330 nm / 390 nm. When bound to protein the maximum emission is at 570 nm. (see Fig. 1). It is also compatible with filters for ethidium bromide stain or SYPRO Ruby. The dye has a low fluorescence but when bound to proteins it emits a strong fluorescence (brigth golden color). For subsequent analysis, e. g. enzymatic digestion or mass spectrometry, the **SERVA ProteinStain Fluo-Y** can be easily removed from the proteins by immersing the gel in sufficient water. Stained gels may be stored in stain solution light-protected at +2 °C - +8 °C.

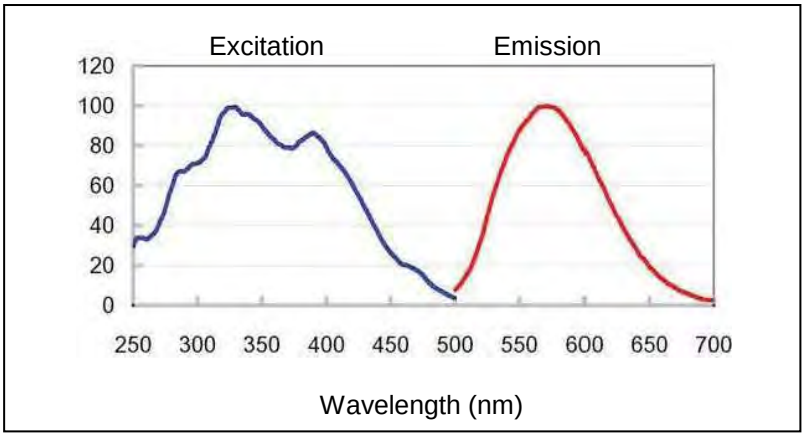

Fig. 1: Excitation and emission spectra of **SERVA ProteinStain Fluo-Y**

1.2. Additionally required lab equipment and reagents

- Staining containers: Glass trays are recommended.
- Imaging System: Gels are best imaged using a UV-based fluorescence imager capable of excitation near 330 nm and 390 nm as well as detection near 570 nm (also compatible with filters for ethidium bromide stain or SYPRO Ruby)
- Laboratory shaker or rocker for gel staining incubation steps
- Acetic acid (SERVA Cat. No. 45633)
- Ethanol (SERVA Cat. No. 11093)
- Filtered distilled or deionized water

1.3. Storage conditions

The recommended storage temperature is +2 °C to +8 °C. Under these storage conditions the unopened reagent is at least useable until: see expiry date on the label.

Please protect from light and avoid exposure to temperature greater than 37 °C.

2. Staining protocol

2.1. Required solutions

To prepare the required solutions, please use distilled or deionized water that has been filtered.

2.1.1. Fixing solution

The fixing solution consists of 40 % (v/v) ethanol and 7 % (v/v) acetic acid.

In consideration of safety and stain performance methanol is not recommended.

The quantity of fixing solution required depends on the number and the size of the gels to be stained. As a rule of thumb, the volume of the fixing solution should be equal to 10 – 15 times the volume of the gel.

Gel size	Volume of fixing solution
9 cm x 7 cm (mini gel)	100 ml
13 cm x 9 cm	200 ml
16 cm x 16 cm	500 ml
26 cm x 23 cm	1 L

2.1.2. Staining solution

Important:
The 1x staining solution is recommended to be freshly prepared prior to its intended use.

To prepare the staining solution (1x) the 100x stock solution is diluted 1:100 with water (1 volume stock solution + 99 volumes dH_2O).

The quantity of 1x staining solution required depends on the number and the size of the gels to be stained. As a general guide, the volume of the staining solution should be equal to 10 times the volume of the gel.

Gel size	Volume of staining solution
9 cm x 7 cm (Minigelformat)	60 ml
13 cm x 9 cm	120 ml
16 cm x 16 cm	300 ml
26 cm x 23 cm	600 ml

2.2. Staining and Detection

Fixation Place gel in a clean glass or plastic tray with fixing solution. Cover the tray, place it on a rocker/shaker and agitate gently.

Standard	Rapid
60 min at room temperature (RT)	Microwave to boil and then agitate ar RT for 15 min

<u>Please note:</u> Either shortened or prolonged fixation time may reduce sensitivity.

Staining Carefully pour off the fixing solution and add the 1x staining solution. Cover the tray, place it on a rocker/shaker and agitate gently.

Standard	Rapid
min. 45 min at RT	15 - 45 min at RT (30 min of staining might be sufficient for general applications)

Destaining Destaining is not necessary. However, it might be helpful to remove excess dye from the gel surface by quickly (1 – 5 min) rinsing the gel with clean water

<u>Important:</u> Prolonged wash might largely reduce signals.

Detection Excitation: UV light (max. 330 nm / 390 nm)
Emission: best at 570 nm

Also compatible with filters for ethidium bromide stain or SYPRO Ruby.

Storage of stained gel in 1x staining solution is possible at +2 °C - +8 °C. Then staining intensity persists or even increases. For long-term storage, gels should be placed in sealable plastic bags with 5 – 10 ml of staining solution and stored light protected at +2 °C - +8 °C.

3. Compatibility of SERVA ProteinStain Fluo-Y staining with subsequent analysis

The bound **SERVA ProteinStain Fluo-Y** dye can be easily removed from the protein by keeping the gel in plenty of pure water or PBS (phosphate buffered saline) for hours. Subsequent analysis, e. g. enzymatic digestion, mass spectrometry or other proteomics applications can be better performed by using destained (stain-free) protein to minimize experimental interference with bound dye molecules.

4. Ordering Information

Product	Size	Cat. No.
Ethanol undenatured absolute analytical grade	250 ml	11093.01
	1 L	11093.02
	2.5 L	11093.03
Acetic acid 100 % analytical grade	1 L	45633.01
	2.5 L	45633.02

5. Troubleshooting

Problem	Possible Cause	Remedy
Poor staining sensitivity	Too short or prolonged fixation period	Follow the recommendations for fixation
	Too long destain/wash	Do not destain or wash the gel in water for more than 5 min. For long-term storage, keep the stained gel in staining solution (1x) at +2 °C - +8 °C
	Insufficient staining time	Stain sensitivity maximizes after 45 min
	Dirty containers for staining	Make sure that the staining trays and other equipment have been thoroughly cleaned
	Insufficient stain volume	Follow the recommendations for stain volume appropriate to the gel size
	Reuse of the stain	Reuse is not recommended
High staining background	Excess dye remained on the gel surface	Quickly rinse the gel with clear water immediately before imaging Staining sensitivity maximized after 45 min
	Too much time in staining solution	Reduce time of staining if needed
	Dirty containers for staining	Make sure that the staining trays and other equipment have been thoroughly cleaned
	Reagent impurities	Use high quality reagents for fixing and staining solution

Problem	Possible Cause	Remedy
Speckles in the gel image	Particles from reagents and environment during operation	Ensure that the gel is prepared without particulate material and that the staining trays are thoroughly cleaned
		Use powder-free gloves and forceps to handle gels only by the edges
		Limit exposure of gels and staining solution to open air
Uneven staining	Insufficient shaking during staining	Make sure that the gel is well agitated and immersed during staining
No detectable signals for protein bands or spots	Error of imaging system	Check instrument settings and instructions
	No protein on gel	Stain with Coomassie or silver to verify the protein load on the gel