

INSTRUCTION MANUAL

SERVA CSF Silver Staining Kit **Sensitive Staining of Proteins in Polyacrylamide Gels**

(Cat. No. 43398.01)



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1. SERVA CSF Silver Staining Kit

1.1. Introduction

The silver staining kit form SERVA allows protein detection on polyacrylamide gels with high sensitivity. It is particularly well suitable for detection of oligoclonal IgG bands in cerebrospinal fluid (CSF). The kit bases on silver nitrate.

1.2. Kit components

Component	Amount
Fixing Solution I	1 L
Ethanol denatured for Fixing Solution II and Wash Solution	800 ml
Fixing Solution III	400 ml
Solution A	2x 55 ml
Solution B	2x 55 ml
Solution C	300 µl
Solution D	1.2 ml
Solution E	25 g
Spoon for ca. 1.5 g portions	1 piece

1.3. Additionally required reagents

In addition to the kit the following reagents are required:

- Glycerol (85 %)

1.4. Storage conditions

The recommended storage temperature for the kit is +15 °C to +30 °C. Under these storage conditions the unopened reagent is at least useable until: see expiry date on the label.

Please note that Solution E is provided as dry powder in the kit.

Component	Temperature
Fixing Solution I	+15 °C to + 30 °C
Ethanol denatured for Fixing Solution II and Wash Solution	+15 °C to + 30 °C
Fixing Solution III	+15 °C bis + 30 °C
Solution A	+15 °C to + 30 °C
Solution B	+15 °C to + 30 °C
Solution C	+15 °C to + 30 °C
Solution D	+15 °C to + 30 °C
Solution E	Dry powder: +15 °C to + 30 °C

2. Staining of IEF gels (FocusGels)

2.1. Required solutions

One gel (12 cm x 25 cm) can be stained using 200 ml of each solution in the various steps.

- Fixing Solution for Fixingstep II: (30 % (v/v) Ethanol) 60 ml Ethanol, denatured ad 200 ml dH₂O
- Fixing Solution for Fixingstep III: 80 ml Fixing Solution III ad 200 ml dH₂O
- Wash Solution: (5 % (v/v) Ethanol) 10 ml Ethanol, denatured ad 200 ml dH₂O
- Staining Solution: Prepare 150 ml dH₂O
Add 20 ml Solution A & mix well
While mixing add 20 ml Solution B
Add dH₂O to a final volume of 200 ml
- Developer: 50 µl Solution C
200 µl Solution D
ad 200 ml dH₂O
- Stop Solution: (1 % (m/v) Glycin) 3 g Solution E (2x 1.5 g with the spoon)
ad 300 ml dH₂O
- Conservation: 12 ml Glycerol (85 %)
ad 200 ml dH₂O

Please note:

When using an automated gel stainer, air-bubbles below the gel should be avoided. The gel could be positioned and fixed with the help of magnetic stir bars.

Step	Solution (200 ml per gel)	Time
1. Fixing I	Fixing Solution I	20 min
2. Fixing II (1)	Fixing Solution for Fixingstep II: 30 % (v/v) Ethanol	10 min
3. Fixing II (2)	Fixing Solution for Fixingstep II: 30 % (v/v) Ethanol	10 min
4. Washing (1)	Wash Solution: 5 % (v/v) Ethanol	10 min
5. Washing (2)		10 min
6. Fixing III	Fixing Solution for Fixingstep III	20 min
7. Washing (1)	Wash Solution: 5 % (v/v) Ethanol	10 min
8. Washing (2)		10 min
9. Rinsing (1)	200 ml dH ₂ O	10 min
10. Rinsing (2)		10 min
11. Rinsing (3)		10 min
12. Staining	Staining Solution: 20 ml Solution A + 20 ml Solution B ad 200 ml dH ₂ O	30 min
13. Rinsing (1)	200 ml dH ₂ O each	2 min
14. Rinsing (2)		5 min
15. Developing	Developer: 50 µl Solution C+ 200 µl Solution D ad 200 ml dH ₂ O	2 to 3 min (by sight)
16. Stopping	Stop Solution: 1 % (w/v) Glycine	5 min
17. Rinsing (1)	200 ml dH ₂ O each	5 min
18. Rinsing (2)		5 min
19. Preservation	Preservation solution	5 min
20. Drying	Air-dry the gel, then roll the gel cover sheet supplied with the gel onto the gel surface	Several hours

2.2. Required Solutions for using the SERVA BlueStain

One gel (12 cm x 25 cm) can be stained using min. 250 ml of each solution in the various steps.

- Fixing Solution for Fixingstep II: (30 % (v/v) Ethanol) 80 ml Ethanol, denatured
ad 250 ml dH₂O
- Fixing Solution for Fixingstep III: 100 ml Fixing Solution III
ad 250 ml dH₂O
- Wash Solution: (5 % (v/v) Ethanol) 12.5 ml Ethanol, denatured
ad 250 ml dH₂O
- Staining Solution: Prepare 180 ml dH₂O
Add 25 ml Solution A & mix well
While mixing add 25 ml Solution B
Add dH₂O to a final volume of 250 ml
- Developer: 62.5 µl Solution C
250 µl Solution D
ad 250 ml ddH₂O
- Stop Solution: (1 % (m/v) Glycin) 3 g Solution E (2x 1.5 g with the spoon)
ad 300 ml dH₂O
- Conservation: 18 ml Glycerol (85 %)
ad 300 ml dH₂O

NOTE: The staining protocol is already installed in the SERVA BlueStain. For further details, please see the instruction manual for the stainer.

3. Ordering information

Product	Size	Cat. No.
Glycerol from plant	1 L	23176.01