INSTRUCTION MANUAL

SERVA HPE Silver Staining Kit

Sensitive Protein Staining for SDS Polyacrylamide Gels

(Cat. No. 43395)

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1. SERVA HPE 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 staining film-backed HPE gels after one- and two-dimensional separations. The kit bases on silver nitrate, not on the ammoniacalic reaction. When it is applied according to **protocol A** the staining is **compatible with mass spectrometry (MS) analysis**. When **protocol B** is applied, the proteins will become crosslinked in the gel matrix; this allows **higher sensitivity of detection**. It is fully compatible with the Automated Gel Stainer (GE Healthcare).

1.2. Kit components

Component	Amount
Citric acid	10 g
Formaldehyde (37 %)	5 ml
Glycine	10 g
Sodiumthiosulfate	0.5 g
Glutaraldehyde (25 %)	100 ml
Solution A	100 ml
Solution B	200 ml
Solution C	20 ml
Solution D	100 ml
Solution E	Dry powder sufficient for 10 ml solution

1.3. Additionally required reagents

In addition to the kit the following reagents are required:

- Trichloroacetic acid
- Ethanol (95 %)
- 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. As soon as it is reconstituted in water, the solution should be store at +4 °C to +8 °C (short term, 1 week stable) or at -15 °C to -30 °C (long term, 6 months stable) in aliquots.

All other solutions in the kit can be stored at +15 °C to +30 °C. Please see the table below for further details.

Component	**Temperature**
Citric acid	+15 °C to + 30 °C
Formaldehyde (37 %)	+15 °C to + 30 °C
Sodiumthiosulfate	+15 °C to + 30 °C
Glycine	+15 °C to + 30 °C
Glutaraldehyde (25 %)	+15 °C to + 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
	Reconstituted in ddH_2O: +4 °C to +8 °C (1 week stable) -15 °C to -30 °C (aliquoted; 6 months stable)

2. Protocol A: MS compatible staining

2.1. Required solutions

The volumes given in the protocol are sufficient for staining one gel (19 cm x 25 cm).

- Solution 1, 500 ml:
  (15 % v/v Ethanol/1 % w/v Citric acid)
  75 ml Ethanol
  5.0 g Citric acid
  ad 500 ml dH_2O

- Solution 2, 250 ml (stir into 50 ml dH_2O):
  25 ml Solution A
  25 ml Solution B
  81 mg Sodiumthiosulfat
  79 ml Ethanol
  ad 250 ml dH_2O

- Solution 3, 250 ml (stir into 100 ml dH_2O):
  Please note: This solution contains silver.
  5 ml Solution C
  25 ml Solution B
  ad 250 ml dH_2O
  325 µl Formaldehyde (37 %)

- Solution 4, 250 ml (stir into 180 ml dH_2O):
  25 ml Solution D
  325 µl Formaldehyde (37 %)
  250 µl Solution E
  ad 250 ml dH_2O

- Solution 5, 250 ml:
  (1 % w/v Glycine)
  2.5 g Glycine
  ad 250 ml dH_2O

- Solution 6, 250 ml:
  15 ml Glycerol (85 %)
  ad 250 ml dH_2O

Step	Solution	Incubation time
1. Fixing	Solution 1	45 min
2. Fixing	Solution 1	45 min
3. - 6. Washing	250 ml dH_2O each step	4 x 10 min
7. Sensitizing	Solution 2	30 min
8. - 11. Washing	250 ml dH_2O each step	4 x 5 min
Before staining perform „Droplet Test“: 100 µl Solution 3 + 100 µl Solution 4		
12. Silvering	Solution 3	45 min
13. - 15. Rinsing	250 ml dH_2O each step	3 x 1 min
16. Developing	Solution 4	2 bis 4 min (visual control)
17. Stopping	Solution 5	30 min
18. Preserving	Solution 6	30 min
19. Drying	Air-dry the gel, then roll the gel cover sheet supplied with the gel onto the gel surface.	Several hours

3. Protocol B: Higher sensitivity staining

3.1. Required solutions

The volumes given in the protocol are sufficient for staining one gel (19 cm x 25 cm).

- Solution 1, 500 ml:
  (15 % v/v Ethanol/1 % w/v Citric acid)
  75 ml Ethanol
  2.5 g Citric acid
  ad 500 ml dH_2O

- Solution 2, 250 ml (stir into 50 ml dH_2O):
  25 ml Solution A
  25 ml Solution B
  81 mg Sodiumthiosulfat
  25 ml Glutaraldehyde (25 %)
  79 ml Ethanol
  ad 250 ml dH_2O

- Solution 3, 250 ml (stir into 100 ml dH_2O):
  Please note: This solution contains silver.
  5 ml Solution C
  25 ml Solution B
  ad 250 ml dH_2O
  325 µl Formaldehyde (37 %)

- Solution 4, 250 ml (stir into 180 ml dH_2O):
  25 ml Solution D
  325 µl Formaldehyde (37 %)
  250 µl Solution E
  ad 250 ml dH_2O

- Solution 5, 250 ml:
  (1 % w/v Glycine)
  2.5 g Glycine
  ad 250 ml dH_2O

- Solution 6, 250 ml:
  15 ml Glycerol (85 %)
  ad 250 ml dH_2O

Step	Solution	Incubation time
1. Fixing	Solution 1	45 min
2. Fixing	Solution 1	45 min
3. - 6. Washing	250 ml dH_2O each step	4 x 10 min
7. Sensitizing	Solution 2	30 min
8. - 11. Washing	250 ml dH_2O each step	4 x 5 min
Before staining perform „Droplet Test“: 100 µl Solution 3 + 100 µl Solution 4		
12. Silvering	Solution 3	45 min
13. - 15. Rinsing	250 ml dH_2O each step	3 x 1 min
16. Developing	Solution 4	2 bis 4 min (visual control)
17. Stopping	Solution 5	30 min
18. Preserving	Solution 6	30 min
19. Drying	Air-dry the gel, then roll the gel cover sheet supplied with the gel onto the gel surface.	Several hours

4. Ordering information

Product	Size	Cat. No.
Reagents/Chemicals		
Ethanol undenatured 96 %	1 L	11094.01
	2.5 L	11094.02
Gylcerol from plant	1 L	23176.01