Staining Procedures

1. **SERVA Blue G (Coomassie®[1)] Brilliant Blue G-250) or SERVA Blue R (Coomassie®[1)] Brilliant Blue R-250)**
2. **Protein Staining: SERVA Blue W**
3. **Protein Staining: SERVA Violet 17**
4. **Silver Staining Optimized for SERVALYT™ PRECOTES™**
5. **Silver Staining for SDS PAGE and Native PAGE**

1. SERVA Blue G (Coomassie®[1)] Brilliant Blue G-250) or SERVA Blue R (Coomassie®[1)] Brilliant Blue R-250)

Stock solution I:	0.2 % SERVA Blue G rsp.R i.e. 100 mg SERVA Blue G (cat.no. 35050) or 100 mg SERVA Blue R (cat.no. 35051) dissolve in 50 ml of 90 % Ethanol (cat.no. 11094)		
Stock solution II:	20 % acetic acid		
Staining steps:			
Fixation:	Ethanol	40 ml	30 min
	Glacial acetic acid	10 ml	
	Water	50 ml	
Staining:	Stock solution I	50 ml	20 min
	Stock solution II	50 ml	
Destaining I:	Fixation solution:		30 sec
Destaining II:	Ethanol	20 ml	visual inspect.
	Glacial acetic acid	10 ml	
	Water	70 ml	
Rinsing:	Water demin.		2x5 min

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2. Protein Staining: SERVA Blue W

Fixation solution:	20 % Trichloroacetic acid (cat.no. 36910) 20 g in 100 ml H_2O	
Stock solution:	100 mg SERVA Blue W (cat.no. 35053) (dissolve for 10 minutes using a magnetic stirrer) in 250 ml H_2O demin.	
Destaining solution:	20 ml fixing acid in 1 l H_2O demin.	
Staining steps:		
Fixation of the gel:	Fixation solution (200 ml)	20 min.
Rinsing:	H_2O demin. (200 ml)	30 sec.
Staining:	250 ml staining solution 5 ml fixation solution (to be added immediately before staining)	10 min.
Destaining:	destaining solution (200 ml)	ca. 2 x 10 min.
Neutralization:	H_2O demin.	2 x 5 min.
Drying:	in a jet of warm air or at room temperature	

3. Protein Staining: SERVA Violet 17

Fixation solution:	20 % trichloroacetic acid (w/v) (cat. no. 36910)
Stock solution I:	1 g SERVA Violet 17 (cat. no. 35072) to 250 ml distilled H_2O
Stock solution II:	20 % phosphoric acid (w/v)
Destaining solution:	3 % phosphoric acid (w/v)
Staining steps:	
Fixation:	20 min in 20 % TCA solution (200 ml)
Rinsing:	1 min in destaining solution (200 ml)
Staining:	Mix stock staining solutions I and II in equal quantities shortly before use Stain for 10 minutes on the agitator
Destaining:	2 - 3 times for 10 min in destaining solution until background is clear
Washing:	2 x 2 min. in distilled water
Drying	in a jet of warm air or at room temperature

The volume of solutions or staining mixture required for one IEF gel (e.g. SERVALYT™ PRECOTE™) is 100 ml.

4. Silver Staining (Optimized for SERVALYT™ PRECOTES™)

1) Poehling, H.-M. & Neuhoff, V. (1982) Electrophoresie **2,** 141-147
2) Hochstrasser, D.F., Patchornik, A. & Merril, C.R. (1988) Anal. Biochem. **173**, 412-423)

Important hints for silver staining:

- Use only absolutely clean vessels. The use of vessels soiled with colourants such as Coomassie®[1)] etc. may affect the results (stainless steel or glass staining vessels are recommended)
- The gel should be kept in constant motion in the respective solution by means of a shaker.
- Ensure that the gel is **always covered** with the respective solution (100 - 200 ml solution depending on the size of the staining vessels are sufficient).
- Staining solution and developing solution should be prepared **just before use.**
- The following sequence in preparing the staining solution should be kept:

  a) Dissolve the silver nitrate in 60 ml H_2O
  b) Add 1.5 ml ammonium hydroxide 25 %
  c) Add 10.5 ml NaOH 0.1 M
  d) fill up to 100 ml with H_2O

  The staining solution should be as clear as water.

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Fixing solution I	150 ml ethanol (Cat.No. 11094) 17.3 g sulfosalicylic acid (Cat.No. 35706) 285 ml trichloroacetic acid 20 % (Cat.No. 36910) ad 500 ml H_2O
Fixing solution II	500 ml ethanol 100 % 100 ml acetic acid ad 1000 ml H_2O
Fixing solution III	40 ml glutaraldehyde 25 % (Cat.No. 23115) 60 ml H_2O
Washing solution	50 ml ethanol ad 1000 ml H_2O
Staining solution	400 mg $AgNO_3$ (Cat.No. 35110) 1.5 ml ammonium hydroxide 25 % 10.5 ml NaOH 0.1 N ad 100 ml H_2O
Developing solution	10 ml citric acid 0.05 % (Cat.No. 38640) 100 µl formaldehyde 37-40 % ad 100 ml H_2O
Stop solution	10 ml acetic acid ad 100 ml H_2O

Staining Steps	
Fixing solution I:	1 x 20 minutes
Fixing solution II:	2 x 10 minutes
Washing solution:	2 x 10 minutes
Fixing solution III:	1 x 20 minutes
Washing solution:	2 x 10 minutes
Rinsing:	3 x 10 minutes
Staining solution:	1 x 30 minutes
Rinsing:	1 x 5 minutes
Developing solution:	ca. 2 minutes (visual inspection)
Stop solution:	1 x 5 minutes
Rinsing:	2 x 5 minutes
Air drying at room temperature	

5. Silver Staining for SDS PAGE and Native PAGE

(using SERVA Silver Staining Kit Cat. No. 35076.01 and SERVA Silver Staining Kit Cat. No. 35077.01)

The kits contain:

For SDS PAGE (Cat.No. 35076.01)	For native PAGE (Cat. No.35077.01)
500 ml Fixing Solution	500 ml Trichloroacetic acid
25 x 30 mg Sodium thiosulfate . 5 H_2O	25 x 30 mg Sodium thiosulfate . 5 H_2O
250 ml Silver nitrate solution	250 ml Silver nitrate solution
1.5 ml Formaldehyde	1.5 ml Formaldehyde
500 ml Sodium carbonate solution	500 ml Sodium carbonate solution
500 ml Citric acid solution	500 ml Citric acid solution

Staining Protocol:

For the following instructions please use 100 ml solution per staining step per gel (125 x 125 mm). The time for incubation and wash steps is optimized for acrylamide gels of 0.3 - 0.5 mm thickness, for thicker gels please double the time. The use of a shaker is recommended in all steps of detection. Gentle motion (50 - 100 rpm) of the gel in the solution will help to optimize fixation, staining and destaining steps. Silver staining is a highly sensitive method. Please use dest. water and clean staining dishes (ideally made of glass) to reduce background. For reproducibility please keep within given incubation and washing time.

Step		Solution	Incubation
1.	Fixing (native) Part-No. 35077-6 Fixing (SDS) Part-No. 35076-6	Trichloroacetic acid * 100 ml (> 5x reusable!) Fixing solution 100 ml (> 5x reusable!)	≥ 20 min.
2.	Wash	30 % (v/v) Ethanol	2 x 10 min.
3.	Pre-Treatment Part-No. 35077-5 Part-No. 35076-5	Sodium thiosulfate . pentahydrate Dissolve 30 mg aliquot in 100 ml H_2O dest.	1 min.
4.	Wash	H_2O dest.	3 x 10 sec. (stop watch)
5.	Stain Part-No. 35077-1 Part-No. 35076-1	Silver nitrate solution ** 10 ml ad 100 ml H_2O dest.	15 min.
6.	Wash	H_2O dest.	2 x 10 sec. (stop watch)
7.	Develop Part-No. 35077-2 + 35077-4 Part-No. 35076-2 + 35076-4	Sodium carbonate solution 20 ml ad 100 ml H_2O dest. + 50 µl Formaldehyde 37%	by view, approx. 1-5 min.
8.	Wash	H_2O dest.	1 x 10 sec.
9.	Stop Part-No. 35077-3 Part-No.. 35076-3	Citric acid solution 20 ml ad 100 ml H_2O dest.	5 min.
10.	for long term storage	2 % (w/v) Glycerol in H_2O dest.	10 min.

** please dispose chloride hydrocarbon expertly*

*** please dispose silver nitrate expertly*

Safety Precautions:
TCA solution is corrosive. Please follow the instructions for handling hazardous substances. It is strongly advised to wear protective glasses, gloves and clothing during all steps of the staining procedure.

Literature References:

SERVA Blue G (cat.no. 35050):

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7. Dutta, R. and Inouye, M.: Reverse Phosphotransfer from OmpR to EnvZ in a Kinase/Phosphatase Mutant of EnvZ (EnvZN347D), a Bifunctional Signal Transducer of Escherichia coli; (1996) J. Biol. Chem. **271** (3): 1424-1429
8. Wragg, S. et al: Kinetic Analysis of a Recombinant UDP-N-acetyl-D-galactosamine:Polypeptide N-Acetylgalactosaminyltransferase; (1995) J. Biol. Chem.**270** (28): 16947-16954

Coomassie Brilliant Blue R-250 (cat. no. 17525):

1. Diakowski, W, and Sikorski, A.F.: Interaction of Brain Spectrin (Fodrin) with Phospholipids; (1995) Biochemistry **34(40),**13252-13258
2. Doussière, J. et al: Photoaffinity Labeling and Photoinactivation of the O_2-Generating Oxidase of Neutrophils by an Azido Derivative of FAD b (1995) Biochemistry 34(5), 1760-1770

SERVA Blue R (cat. no. 35051)

1. Papov, V.V. et al: Hydroxyarginine-containing Polyphenolic Proteins in the Adhesive Plaques of the Marine Mussel Mytilus edulis (1995) J. Biol. Chem. 1995; **270 (34):** 20183-20192

SERVA Blue W (cat. no. 35053)

1. Jensen, T.H. et al: Intermolecular Binding Sites of Human Immunodeficiency Virus Type 1 Rev Protein Determined by Protein Footprinting(1995) J. Biol. Chem.; **270 (23):** 13777-13784