

Silver Stain Kit

Part #0080-0183

The Investigator Silver Stain Kit uses a modification of the Rabilloud¹ procedure which has the advantages of high sensitivity and high signal to noise. The procedure may be modified for non-destructive silver staining by omission of the glutaraldehyde in Fixative 2 Solution; this is useful when analysis by mass spectrometry is to be performed. This procedure uses silver nitrate (as opposed to silver diammine) as the silvering agent and is a relatively quick method. Unlike silver diammine methods, this method is quantitative when the development time is kept to 10 minutes or less. This allows spots to be quantified with densitometers or other scanning methods. For a review of silver staining methods, see the article by Rabilloud, *T. in Electrophoresis*, 13, 1992, 429-439.

Staining Procedure:

Reagents Needed

The silver staining procedure requires the common laboratory reagents and equipment (not supplied) listed below in addition to those in the kit:

- Ethanol
- Acetic Acid
- 18 MOhm water
- Laboratory rotary shaker
- Staining trays (Catalog No. 0080-0189)

STEP 1:

Prepare all Solutions:

Once prepared, the contents of the kit will provide 4 L of each solution. This is enough to stain 10 large (22 X 22 cm) gels with 400 ml solution per gel. These solutions can be stored at room temperature for two weeks.

I. Fixative 1 Solution (4 L)

Ethanol (1.6 L)

Acetic acid (400 ml)

Mix ingredients and qs to 4 L with 18 MOhm water.

Final Concentration

40% EtOH

10% Acetic Acid

II. Fixative 2 Solution (4 L)

Contents of Glutaraldehyde bottle

Contents of Fixative 2 bottle

Ethanol (1200 ml)

Mix ingredients and qs to 4 L with 18 MOhm water.

Final Concentration

8 mM Potassium tetrathionite

829 mM Sodium Acetate

0.5% glutaraldehyde

30% EtOH

¹ Rabilloud, T., 1992, *electrophoresis*, 13, 429-439

III. Silver Nitrate Solution (4 L)

Contents of Silver bottle

Formaldehyde (1 ml)

Mix ingredients and qs to 4 L with 18 MOhm water.

Final Concentration

11.7 mM Silver Nitrate

0.009% Formaldehyde

IV. Developer Solution (4 L)

Contents of Developer bottle

Formaldehyde (0.6 ml)

Final Concentration

63 mM Sodium thiosulfate

0.2 M Potassium carbonate

0.005% Formaldehyde

Fill a large beaker with approximately 3.5 L of 18 MOhm water and stir briskly. While stirring, add contents of Developer Bottle slowly until it is all dissolved. Add formaldehyde and bring the level up to 4 L with 18 MOhm water.

V. Stop Solution (4 L)

Contents of Stop Bottle

Acetic acid (80 ml)

Mix ingredients and qs to 4 L with 18 MOhm water.

Final Concentration

0.4 M Tris

2% Acetic Acid

STEP 2:

Silver Staining:

All steps can be performed at room temperature. Gently agitate the trays on a rotary shaker at low speed during all steps.

1. Fix the gels in **Fixative 1** for 1 hour.
2. Place the gels in **Fixative 2** for 1 hour or up to overnight. The fixative solution may appear yellowish after this step.
3. Wash the gels for 15 minutes in 18 MOhm water. Repeat 3 more times with fresh water each time.
4. Place gels in **Silver Nitrate Solution** for 45 min. (Gels can remain in silver solution for up to 48 hours if necessary.)
5. Wash gels for 1 minute in 18 MOhm water.
6. Place gels in **Developer Solution**. A small amount of brownish precipitate will be observed during this step. Leave gels in developer until spots appear (approximately 5-8 minutes). Gels may be left in developer for an extended period of time (up to 30 minutes) to bring up faint spots.
7. Place gels in **Stop Solution** for 10 minutes.

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