Mass Spectrometry Compatible Silver Staining

1. Protocol usage

This protocol is used for silver staining both 1D and 2D SDS-PAGE gels. The staining method is mass spectrometry compatible; it doesn't affect peptide elution from the gel or disturb ionization in a mass spectrometer. Detection limit of this method is 1–5 ng of protein per spot or band.

2. Principle

Silver staining is based on the affinity of the proteins for a cation, here silver. Other substances, like SDS, which also have a high affinity for silver, must be removed prior to staining. This means that fixation step is required. The sensitivity of the staining reaction can be enhanced by use of sensitizers, here sodium thiosulphate, between fixation and silver impregnation.

3. Reagents and equipment

Plastic or glass trays, rinsed with ethanol and wiped with laboratory tissue
Disposable gloves
Platform shaker
Water, MilliQ (MQ)
Ethanol, Altia ETAX A
Acetic acid (*, Fluka 45731, (purity >99,8%)
Sodium thiosulphate pentahydrate(*, Riedel-de Haën 31459, (purity >99,5%)
Silver nitrate(* (purity >99,8%)
Potassium carbonate(* (purity >99%)
Formaldehyde(* (>36,5%)
Tris(*, Ducheta T1501, (purity >99,9%)
*) Supplier of these chemicals can vary, but the purity grade should be the mentioned one or better.

4. Solutions

<u>Fixation solution</u>

Mix together 300 ml ethanol, 100 ml acetic acid and 600 ml water. This solution can be stored in room temperature for at least 1 month.

<u>Rinsing solution</u>

Mix together 200 ml ethanol and 800 ml water. This solution can be stored in room temperature for at least 1 month.

Sensitization solution

Weigh 300 mg sodium thiosulphate pentahydrate in 1 l water. Prepare this solution fresh.

Silver solution

Weigh 2 g silver nitrate in 1 l water. Prepare this solution fresh.

Development solution

Weigh 30 g potassium carbonate and 15 mg sodium thiosulphate pentahydrate in 1 l water. Add 700 µl of 37% formaldehyde. Prepare this solution fresh.

Stop solution

Mix together 25 ml acetic acid and 975 ml water. Weigh 50 g Tris base and dissolve it in this solution with a magnetic stirrer. This solution can be stored in room temperature for at least 3 months.

Note: Never use formaldehyde or glutaraldehyde in the fixation or silver solutions because this will fix the proteins permanently in the gel!

5. Instructions

Note: Remember always to wear gloves to protect gels from keratin contamination. Wipe the trays carefully with ethanol to remove all old silver spots. Never use same trays for staining and immunoblotting to avoid BSA contamination, which causes trouble in mass spectrometry analysis.

Use 100 ml of silver solution for a big gel (20 x 20 cm). A suitable amount for the rest of the solutions is about 200 ml.

5.1. Gel fixation

After a gel run, place the gel in glass or plastic tray in fixation solution. Place the tray on a platform shaker and shake gently for minimum 1 hour and maximum about 18 hours.

5.2. Silver staining

After fixation rinse the gel for 15 minutes with rinsing solution and 15 minutes with water on a platform shaker.

Sensitize the gel with sensitizing solution for exactly 1,5 minutes on the platform shaker. Do not exceed the time! Rinse the gel then twice for 20 seconds with plenty of MQ water.

Stain the gel with silver solution for 30 minutes on the platform shaker. Remember to collect the used silver solution in a waste container. Rinse the gel for 20 seconds with water.

Develop the gel in the development solution by shaking it gently on the platform shaker until the spots almost have a desired intensity. This usually takes 2-5 minutes. Try to add the stopper slightly before desired stain intensity is reached.
Note that background of the gel should stay clear.

Stop the development by shaking the gel gently for 2 minutes in stop solution. Rinse the gels with water 2 times for 10 min.
The gel can be stored in water for several days or dried between cellophane sheets (see Work description MS compatible gel drying).

6. References

O'Connell et al. Electrophoresis 18 (1997) 349-359.

7. Troubleshooting

The most common problems and solutions for them:

Problem	Cause	Solution
Dark or uneven background	Poor water quality	Use ultra pure water of >18 megaohm/cm resistance.
	Staining trays not clean	Wash trays better to remove all old silver spots.
	Improper washing between steps	Do not skip or reduce any washing steps.
	Gels are not completely submerged during staining	Perform all steps using a shaker to improve even staining.
Poor band development or low sensitivity	Loss of silver ions from the gel	Limit the wash after staining to exactly 20 s.
	Stainer or developer solutions not prepared properly	Make sure that the solutions are prepared correctly using ultra pure water.
Stained gels are too dark	Stopper not added to the gel at the appropriate time	Be sure that you add the stopper slightly before

		desired stain intensity is reached.
	Gels are overloaded	Decrease protein load on the gel.
Large dark spots on the gel	Improper gel handling	Wear always gloves. If you need to touch the gel, use corners of the gel.
Presence of a 50-68 kDa band across the gel	Keratin contamination	Wear gloves all the time. Rinse all wells of the gel with ultra pure water before sample loading.
Long time for band development needed resulting in dark background	Some proteins may need longer fixation time	Increase the time for fixation to 2 h or overnight.
	Low protein load	Be sure that at least 1-5 ng of protein is loaded on the gel.