MS COMPATIBLE SILVER STAINING METHODS

Commercially Available Silver Stain kits

The following three commercial available kits provide high sensitivity in the mass spectrometric analysis:

1. FASTsilver Gel Staining Kit from Calbiochem (VWR # CALB341298-1)
2. Focus-FastSilver™ staining kit from Geno Technology Inc (VWR CAT # 82021-376)
3. ProteoSilver Silver Stain Kit from Sigma (PROT-SIL2)

Silver Stain Protocol

This is the protocol that we recommend. It provides the highest sensitivity when mass spectrometry is used to identify proteins using spots from Silver stained gels.
This procedure provides a method of visualizing proteins in SDS-PAGE gels. It is more sensitive than Coomassie Blue staining and can be used for both 1D and 2D gels.

Environmental and safety issues

All work should be performed in a fume hood. Read safety sheets for Acetic acid, EtOH 96%, Silver nitrate, Formaldehyde. Collect silver nitrate solutions in waste container.

Materials required	**Supplier**	**Cat. No.**
Acetic acid (HAc)	Merck KGaA	1.00063.1000
EtOH 99%	Merck KGaA	1.00983.1000
Silver nitrate	VWR	52384-25
$Na_2S_2O_3 \cdot 5H_2O$	VWR	302355E
Na_2CO_3	VWR	16392-1
Formaldehyde min 35%	VWR	52531-1

Procedure

Notes: Wear gloves at all stages and always keep gel containers closed so as to avoid keratin contamination of the gels.

1. Incubate the gel in Fixer (40% EtOH, 10% HAc, 50% H_2O) for 1 h.
2. Wash the gel in H_2O for at least 30 min or overnight. Extended washing may reduce the background staining of the gel. Make as many changes of the water as possible.
3. Sensitise the gel in 0.02% $Na_2S_2O_3$ (0.04 g $Na_2S_2O_3$, 200 ml H_2O) for **only 1 min** (longer incubations may interfere with mass spectrometry analysis).
4. Wash gel in H_2O for 3 x 20 sec.
5. Incubate gel in silver nitrate solution (0.1% $AgNO_3$ [0.2 g, 200 ml H_2O], 0.02% formaldehyde [add 40 µl just before use]) for 20 min at 4 ºC (the staining will be enhanced if the incubation is performed with cold silver nitrate solution).
6. Wash the gel in H_2O for 3 x 20 sec.
7. Place the gel in a fresh gel chamber to avoid residual of $AgNO_3$.
8. Wash the gel in H_2O for 1 min.
9. Incubate the gel in developer (3% Na_2CO_3 [7.5 g, 250 ml H_2O], 0.05% formaldehyde [add 125 µl just before use]). Observe the colour and change solution when the developer turns yellow. Terminate when the staining is sufficient.
10. Wash the gel in H_2O for 20 sec.
11. Terminate staining in 5% HAc for 5 min.
12. Leave the gel at 4 ºC in 1% HAc for storage.
13. If the gel is to be processed by MS wash it in water for 3 x 10 min to ensure complete removal of HAc.