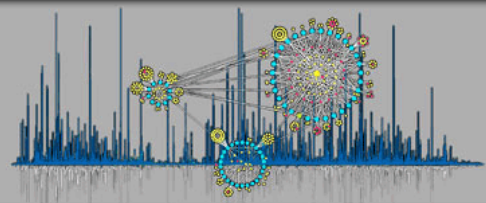


SOP	department of protein science (prot)		
TITLE	Silver Staining Protocol (MS compatible)		
CATEGORY	Staining	AUTHOR	Core Facility Proteomics
VERSION #	4.1	DATE	1.12.2010

REAGENTS

Fixation Solution: 50 % MeOH, 12% HAc

Detailed recipes each for 2 Gels: 100 ml MeOH, 76 ml H₂O, 24 ml Hac,

Wash: 50 % EtOH

150 ml EtOH, 127 ml H₂O

Sensitizing: 0.02 % (0,2 g/l) Na₂S₂O₃

20 mg Na₂S₂O₃, 100 ml H₂O

Staining with Silver nitrate: 2 g/L AgNO₃, 0,075 % Formaldehyd (37 %)

200 mg AgNO₃, 100 mL H₂O, 75 µL Formaldehyd (37 %)

Developer: 60 g/L Na₂CO₃, 5 mg/L Na₂S₂O₃, 0,05 % Formaldehyd (37 %)

6g Na₂CO₃, 97,5 ml H₂O, 2,5 ml Sensitizer, 50 µL Formaldehyd (37 %)

Stop solution: 0.5 % Glycin

1 g Glycin, 200 ml H₂O

Storage Solution: 20 % EtOH, 2% Glycerin

20 ml EtOH, 77,5 ml H₂O, 2,5 ml Glycerin (80 %)

Please Note: Add Formaldehyd just shortly before usage!

PROCEDURE

- Fix gel with fixation solution: 2x 30 min for bigger gels, 2x 15 min for Minigels
- Wash gel 3 times with 50 % EtOH for 20 min. each
- Sensitize gel with 0.02 % $\text{Na}_2\text{S}_2\text{O}_3$ for 1-2 min.
- Wash gel with H_2O for 5 min.
- Stain gel with staining solution for 20 min.
- Wash gel: H_2O for 1 min.
- Develop gel with developing solution until spots are visible (and background doesn't get too dark; depending on spot intensities few sec. to 5-10 min.)
- Stop staining with 0.5 % Glycine for 15 min.
- Store the gels in storage solution at 4 °C