MS-Compatible Silver staining of proteins in SDS- and native-PAGE

A newer **Gel staining protocol**

Chevallet M et al. (2008) Sweet silver: A formaldehyde-free silver staining using aldoses as developing agents, with enhanced compatibility with mass spectrometry. Proteomics, 8:4853

An older **Gel staining protocol** *(adapted from Blum et al. Electrophoresis, 8, 93-99, 1987)*

Prepare all solutions fresh!

This is an easy staining protocol and is compatible with mass spectrometric protein analysis. The stain is useful for protein concentrations ranging from <1ug to >1ng. If the staining does not work, for whatever reason, then gels can easily be destained and restained again (see below).

	solution		V = 100 ml	operation	time
1	sol A	50% methanol, 10% acetic acid		fix	30 min
2	sol B	50% methanol		incubate	15 min
3		milli-Q H_2O		Wash, several changes of H_2O	1 hour
4	**sol. C**	**Sodium thiosulphate ($Na_2S_2O3x5H_2O$)**	**0.2 g/L fresh!**	Incubate	120 sec
5		milli-Q H_2O		wash 3 times	2 x 30 sec
6	sol D	silver nitrate ($AgNO_3$)	0.2 g/100 mL	Incubate	>25 min
7		milli-Q H_2O		wash 3 times	3 x 60 sec
8	sol E	Sodium carbonate (Na_2CO_3) 37% HCOH Na_2S_2O3 x $5H_2O$ (Sol C)	3 g/100 mL 25ul/100mL 2mL/100mL	develop	max 10 min
9	sol F	Na_2-EDTA	(14g / L)	stop develop	10 min
10		milli-Q H_2O		wash	

Destaining protocol

- Dissolve 0.4g Potassium ferricyanide ($K_3Fe(CN)_6$) in 200ml Sodium thiosulphate (0.2 g/L, **sol. C** above)

Destain:

- destain in ferricyanide solution (**sol. C**) with several changes until no bands are visible; the gel will have a yellow hue!
- wash gel 4-5 times for 15 min with milli-Q H_2O until gel is transparent and has no background colour.
- stain gel again starting with **sol. C** from gel staining protocol.