

Silver staining proteins for SDS-PAGE and native-PAGE gels

(adapted from Blum et al. *Electrophoresis*, 8, 93-99, 1987)

Everything Fresh!

This is an easy staining protocol and is compatible with mass spectrometric protein analysis. Glutaraldehyde protocol can not be used for MS 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).

Gel staining protocol

	solution		V = 100 ml	operation	time
1	sol A	50% methanol 5% acetic acid		fix	30 min
2	sol B	50% methanol		incubate	15 min
3		milli-Q H ₂ O		wash times	5 x 5 min
4	sol. C	sodiumthiosulphate (Na ₂ S ₂ O ₃ .5H ₂ O)	0.2 g/L fresh!	Incubate	60 sec
5		milli-Q H ₂ O		wash times	2 x 60 sec
6	sol D	silver nitrate (chilled to 4C) (AgNO ₃)	0.2 g/100 ml	Incubate	25 min
7		milli-Q H ₂ O		wash times	2 x 60 sec
8	sol E	sodium carbonate anhydrous (Na ₂ CO ₃) 37% HCOH	3 g/100 ml 25ul/100ml	develop	max 10 min
9	sol F	Na ₂ -EDTA	(14g / L)	stop develop	10 min
10		milli-Q H ₂ O		wash	2X1min

Destaining protocol

- Dissolve 0.4g potassium ferricyanide ($K_3Fe(CN)_6$) in 200ml sodiumthiosulphate (0.2 g/L, [solution c](#) above)

Destain:

- destain 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 [solution c](#) from gel staining protocol.