

Silver staining (Shevchenko) for proteomic- samples

SOP

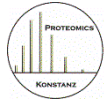
Date 03-04-2013

Version 1

1. Materials/Solutions:

For best results prepare the solutions fresh, just before use!

	Chemicals	for 2 small. gels	for 4 small gels	for 6 small gels
Solution 1	Ethanol	40 ml	80 ml	120 ml
	Acetic Acid	10 ml	20 ml	30 ml
	Ad Milli-Q-H ₂ O up to	100 ml	200ml	300ml
Solution 2	Ethanol	45 ml	90ml	135 ml
	Ad Milli-Q-H ₂ O up to	150 ml	300ml	450 ml
Solution 3	Na ₂ S ₂ O ₃ x 5 H ₂ O	0,01g	0,02g	0,03 g
	Ad Milli-Q-H ₂ O up to	50 ml	100ml	150 ml
Solution 4* <i>wrap with aluminium foil, lightsensitiv</i>	AgNO ₃	0,1 g	0,2g	0,3 g
	Ad Milli-Q-H ₂ O up to	50 ml	100ml	150 ml
Solution 5 = Developing -Solution	Na ₂ CO ₃	3 g	6g	9 g
	Na ₂ S ₂ O ₃ x 5 H ₂ O = Solution 3	1,2 ml	2,5ml	3,7 ml
	Formaldehyde (37%)	67,5 µl	135µl	202,5 µl
	Ad Milli-Q-H ₂ O up to	50 ml	100ml	150 ml
Solution 6 = STOP- Solution	EDTA (Stock 250 mM)	10 ml	20ml	30 ml
	Acetic Acid	0,5 ml	1 ml	1,5 ml
	Ad Milli-Q-H ₂ O up to	50 ml	100ml	150 ml



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2. Staining Procedure:

Step	Time [min]	Solution
1	30	Solution 1: 40% EtOH, 10% Acetic Acid
2	30	Solution 1
3	20	Solution 2: 30% EtOH
4	20	Solution 2
5	20	Solution 2
6	1	Solution 3: 0,2 g/l $\text{Na}_2\text{S}_2\text{O}_3 \times 5\text{H}_2\text{O}$
7	1	MilliQ H_2O
8	1	MilliQ H_2O
9	20	Solution 4: 2 g/l AgNO_3
10	1	MilliQ H_2O
11	1	MilliQ H_2O
12		Solution 5: 6% Na_2CO_3 /5 mg/l $\text{Na}_2\text{S}_2\text{O}_3 \times 5\text{H}_2\text{O}$ / 0,05% Formaldehyde
13		Solution 6: STOP solution

Step 12 and 13):

- just before developing in step 12 (Solution 5), fill the STOP-Solution in a second dish
- discard the MilliQ H_2O in the first dish
- carefully fill in the staining solution in the first dish
- gentle rock the dish until all bands/spots are developed and to avoid that the upcoming "clouds" stick on the gel
- transfer the gel in the STOP-Solution, incubate for $\geq 30\text{min}$, RT
- wash gel with MilliQ H_2O