Protein Silver Staining Kit 1004-14

The **gel**company Protein Silver Staining Kit allows protein detection on polyacrylamide gels with high sensitivity combined with MS-compatibility.

The kit components dilute to a staining volume of 1L. For *Standard Format* gels (125 x 255 mm) 200mL and for *Large Format* gels (200 x 255 mm) 400 mL volumes per gel should be used.

Gelcompany Protein Silver Staining Kit:

- Is suitable for a wide variety of different gel types and chemistries including: IEF-FocusGels, 1D-, 2D-SDS, including gels on a film supports.
- Provides equisite sensitivity.
- Is fully compatibility with mass spectrometry (slight modification to the protocol).
- Is based on simple to use and reliable silver nitrate not on the ammoniacalic reaction.
- Allows post-staining of fluorescently labelled or stained proteins for manual spot picking.
- Is compatible with the Hoefer Automated Gel Stainer (GE-Healthcare).

Storage:

On reciept the kit should be stored in its original container in a dry place at room temperature. Part E is provided as a dry powder in a plastic centrifuge tube. When required Part E should be disolved by adding 10 mL of deionised water. Once disolved Part E is stable for 1 week at 4 - 8°C. If the complete kit is not used within 1 week we recommend aliquoting Part E and storing at -20°C.

Kit contents:

Citric acid	for Fixation step	10 g pre-weighed in sachet
Formaldehyde (37%)	for Silver and Developing step	5 mL
Na-thiosulphate	for Sensitiser step	0.5 g in reaction cup
Glutaraldehyde (25%)	for Sensitiser step	100 mL
Solution A	for Sensitiser step	100 mL
Solution B	for Sensitiser and Silver step	200 mL
Solution C	for Silver step	20 mL
Solution D	for Developing step	100 mL
Solution E	for Developing step	0.2 g pre-weight dry powder Make to 10 mL with deionised water.

Additional requirements (not supplied):

Acetic Acid (HAc; glacial)
Glycerol (85%)
Ethanol (> 95%) If only denatured ethanol is available use MEK (methyl ethyl ketone).
Hiqh quality deionised water.
Trichloroacetic acid (analytical grade > 99%) - for Focus IEF-Gel staining only.

Protocols:

Protein Silver Staining Kit Protocol

The gelcompany Protein Silver Staining Kit is particularly well suitable for detection oligoclonal IgG bands in cerebrospinal fluid and provides equisite sensitivity.

Method 1: Focus IEF Gels

In addition to the kit you will require trichloroacetic acid (TCA) prepared as a 77% solution***, ethanol (> 95%), acetic acid (glacial) and glycerol (85%). Use only pA quality reagents. If only denatured ethanol is available use MEK (methyl ethyl ketone) denatured ethanol.

Step	Solution	Solution in deionised water for 1 FocusGel	Final-Volume*	Time
1. Fixing	1	40 mL of a 77% solution*** of trichloroacetic acid	200 mL	45 min
2. Rinsing		deionised water	200 mL	5 min
3. Washing (1)	2	100 mL ethanol + 20 mL acetic acid	200 mL	40 min
4. Washing (2)	3	10 mL ethanol + 14 mL acetic acid	200 mL	20 min
5. Rinsing		deionised water	200 mL	Rinse briefly
6. Sensitizing	4	Stir into 100 mL deionised water: 20 mL Solution A + 20 mL Solution B + 20 mL glutaraldehyde	200 mL	15 min
7.-9. Rinsing		deionised water	3 x 200 mL	3 x 20 min
10. Silvering	5	Stir into 100 mL deionised water: 4 mL Solution C + 20 mL Solution B + 260 µL Formaldehyde (37%) *(Before Silvering perform the "Droplet Test": Mix 100 µL Silvering and 100 µL developing solution.)*	200 mL	40 min
11.-13. Rinsing		deionised water	200 mL	Rinse briefly
14. Developing	6	Stir into 100 mL deionised water: 20 mL Solution D + 260 µL of formaldehyde (37%) + 200 µL Solution E	200 mL	2 - 4 min**
15. Stopping	7	20 mL acetic acid pA	200 mL	30 min
16. Preserving	8	12 mL of glycerol (85%)	200 mL	30 min
17. Drying		Air-dry the gel, then roll the gel cover sheet supplied with gel onto the gel surface.		Several hours

* Make up to the final volumes (above) using high quality deionised water.

** Determine when developed visually.

*** To prepare a 77% solution of TCA we recommend adding 300 mL of water to a 1 Kg bottle of TCA and dissolving completely.

Warning: Trichloroacetic acid, Acetic acid, Formaldehyde and Glutaraldehyde are hazardous chemicals. Please ensure you read the MSDS and take appropriate safety precautions.

gelcompany

Protein Silver Staining Kit Protocol
Method 2: SDS Gels for higher sensitivity

In addition to the kit you will require ethanol (> 95%), acetic acid (glacial), formaldehyde (37%) and glycerol (85%). Use only pA quality reagents. If only denatured ethanol is available use MEK (methyl ethyl ketone) denatured ethanol The difference to the mass spectrometry compatible protocol is the addition of glutaraldehyde which reduces protein losses during the washing steps by cross-linking of the proteins in the matrix.
Both gels on '*Standard'* and '*NF'*-backings can be used. For best results with this silver staining kit use gels on gelcompany *'Standard'* backings.

Step	Solution	Solution in deionised water for 1 *Standard format* gel	Final Volume*	Time
		For a *Large Format gel* DOUBLE the quantities below		
1. Fixing†	1	80 mL ethanol + 20 mL acetic acid + 2 g citric acid	200 mL	45 min
2.-5. Rinsing		deionised water	4 x 200 mL	4 x 10 min
6. Sensitizing	2	Stir into 50 mL deionised water: 20 mL Solution A + 20 mL Solution B + 65 mg Na-Thiosulphate + 20 mL Glutaraldehyde (25%) + 63 mL Ethanol and fill up to 200 mL with deionised water	200 mL	30 min
7.-10. Rinsing		deionised water	4 × 200 mL	4 x 5 min
11. Silvering	3	Stir into 100 mL deionised water: 4 mL Silvering solution C + 20 mL Solution B fill up to 200 mL with deionised water + 260 µL Formaldehyde pA (37%) *(Before Silvering perform the "Droplet Test":* *Mix 100 µL Silvering and 100 µL developing solution.)*	200 mL	45 min
12.-14. Rinsing		deionised water	3 x 200 mL	3 x 1min
15. Developing	4	Stir into 180 mL deionised water: 20 mL Solution D + 260 µL Formaldehyde pA (37%) + 200 µL Solution E	200 mL	2 - 3 min**
16. Stopping/ Preserving	5	20 mL acetic acid + 24 mL glycerol (85%) ††For NF-gels only stop with 2 mL acetic acid + 24 mL glycerol	200 mL	30 min
17. Drying		Air-dry the gel, then roll the gel cover sheet supplied with gel onto the gel surface.		Several hours

* Make up to the final volumes (above) using high quality deionised water.
** Determine when developed visually.
† Do not store film-backed gels over night in the fix containing 40% ethanol, this causes curling of the film-backed gels. For overnight fixing use a modified fixing solution containing only 15% ethanol.
†† NF-gels are stopped with a lower concentrated acetic acid to avoid development of CO_2 gas bubbles.

Warning: Acetic acid, Formaldehyde and Glutaraldehyde are hazardous chemicals. Please ensure you read the MSDS and take appropriate safety precautions.

gelcompany

Protein Silver Staining Kit Protocol

Method 3: SDS Gels Mass Spec. compatible

In addition to the kit you will require ethanol (> 95%), acetic acid (glacial) and glycerol (85%). Use only pA quality reagents. If only denatured ethanol is available use MEK (methyl ethyl ketone) denatured ethanol.
Both gels on '*Standard'* and '*NF'*-backings can be used. For best results with this silver staining kit use gels on gelcompany *'Standard'* backings.

Step	Solution	Solution in deionised water for 1 *Standard Format* Gel	Final Volume*	Time
		For a *Large Format gel* DOUBLE the quantities below		
1. Fixing†	1	80 mL ethanol + 20 mL acetic acid + 2 g citric acid	200 mL	45 min
2.-5. Rinsing		deionised water	4 x 200 mL	4 x 10 min
6. Sensitizing	2	Stir into 50 mL deionised water: 20 mL Solution A + 20 mL Solution B + 65 mg Na-Thiosulphate + 63 mL Ethanol fill up to 200 mL with deionised water	200 mL	30 min
7.-10. Rinsing		deionised water	4 × 200 mL	4 x 5 min
11. Silvering	3	Stir into 100 mL deionised water: 4 mL Silvering solution C + 20 mL Solution B fill up to 200 mL with deionised water + 260 µL Formaldehyde pA (37%) *(Before Silvering perform the "Droplet Test": Mix 100 µL Silvering and 100 µL developing solution.)*	200 mL	45 min
12.-14. Rinsing		deionised water	3 x 200 mL	3 x 1 min
15. Developing	4	Stir into 180 mL deionised water: 20 mL Solution D + 260 µL Formaldehyde pA (37%) + 200 µL Solution E	200 mL	2 - 3 min**
16. Stopping/ Preserving	5	20 mL acetic acid pA + 24 mL glycerol (85%) ††For NF-gels only stop with 2 mL acetic acid pA + 24 mL glycerol	200 mL	30 min
17. Drying		Air-dry the gel, then roll the gel cover sheet supplied with gel onto the gel surface.		Several hours

* Make up to the final volumes (above) using high quality deionised water.
** Determine when developed visually.
† Do not store film-backed gels over night in the fix containing 40% ethanol, this causes curling of the film-backed gels. For overnight storage use a modified fixing solution containing only 15% ethanol.
†† NF-gels are stopped with a lower concentrated acetic acid to avoid development of CO_2 gas bubbles.
Warning: Acetic acid and Formaldehyde are hazardous chemicals. Please ensure you read the MSDS and take appropriate safety precautions.

gelcompany

Protein Silver Staining Kit Protocol

Weights and solutions for more SDS Gels

Only use high quality de-ionized water for silver staining.

Step	Time	2 *Standard Format* gels 1 *Large Format* gel	3 *Standard Format* gels -	4 *Standard Format* gels 2 *Large Format* gels
1. Fixing†	45 min	160 mL ethanol + 40 mL acetic acid + 4 g citric acid fill up to 400 mL with H_2O	240 mL ethanol + 60 mL acetic acid + 6 g citric acid fill up to 600 mL with H_2O	320 mL ethanol + 80 mL acetic acid + 8 g citric acid fill up to 800 mL with H_2O
2.-5. Rinsing	4 x10 min	4 x 400 mL H_2O	4 x 600 mL H_2O	4 x 800 mL H_2O
6. Sensitizing	30 min	100 mL H_2O + 40 mL Solution A + 40 mL Solution B (+ 40 mL Glutaraldehyde††) + 130 mg Na-Thiosulphate + 126 mL Ethanol fill up to 400 mL with H_2O	150 mL H_2O + 60 mL Solution A + 60 mL Solution B (+ 60 mL Glutaraldehyde††) + 195 mg Na-Thiosulphate + 189 mL Ethanol fill up to 600 mL with H_2O	200 mL H_2O + 80 mL Solution A + 80 mL Solution B (+ 80 mL Glutaraldehyde††) + 260 mg Na-Thiosulphate + 252 mL Ethanol fill up to 800 mL with H_2O
7.-10. Rinsing	4 x 5 min	4 x 400 mL H_2O	4 x 600 mL H_2O	4 x 800 mL H_2O
11. Silvering	45 min	200 mL H_2O + 8 mL Solution C + 40 mL Solution B fill up to 400 mL with H_2O + 520 µL Formaldehyde *(Before Silvering perform the "Droplet Test": Mix 100 µL Silvering and 100 µL developing solution.)*	300 mL H_2O + 12 mL Solution C + 60 mL Solution B fill up to 600 mL with H_2O + 780 µL Formaldehyde *(Before Silvering perform the "Droplet Test": Mix 100 µL Silvering and 100 µL developing solution.).*	400 mL H_2O + 16 mL Solution C + 80 mL Solution B fill up to 800 mL with H_2O + 1.04 mL Formaldehyde *(Before Silvering perform the "Droplet Test": Mix 100 µL Silvering and 100 µL developing solution.)*
12.-14. Rinsing	3 x 1min	3 x 400 mL H_2O	3 x 600 mL H_2O	3 x 800 mL H_2O
15. Developing	2 - 3 min (visual control)	40 mL Solution D fill up to 400 mL with H_2O + 520 µL Formaldehyde + 400 µL Solution E	+ 60 mL Solution D fill up to 600 mL with H_2O + 780 µL Formaldehyde + 600 µL Solution E	+ 80 mL Solution D fill up to 800 mL with H_2O + 1.04 mL Formaldehyde + 800 µL Solution E
16. Stopping/ Preserving	30 min	40 mL HAc + 48 mL glycerol fill up to 400 mL with H_2O ‡For NF-gels stop with 4 mL acetic acid + 48 mL glycerol	60 mL HAc + 72 mL glycerol fill up to 600 mL with H_2O ‡For NF-gels stop with 6mL acetic acid + 72 mL glycerol	80 mL HAc + 96 mL glycerol fill up to 800 mL with H_2O ‡For NF-gels stop with 8 mL acetic acid + 96 mL glycerol
17. Drying		Air-dry the gel, then roll the gel cover sheet supplied with gel onto the gel surface.		

† Do not store film-backed gels over night in the fix containing 40% ethanol, this causes curling of the film-backed gels. For overnight storage use a modified fixing solution containing only 15% ethanol.

†† Do not add glutaradehyde for mass spectrometry compatible silver staining.

‡ NF-gels are stopped with a lower concentrated acetic acid to avoid development of CO_2 gas bubbles.

Warning: Acetic acid, Formaldehyde and Glutaraldehyde are hazardous chemicals. Please ensure you read the MSDS and take appropriate safety precautions.