technical manual
automated gel staining

Automated Staining of Polyacrylamide Gels with Processor Plus

Protocol Guide for Silver and Coomassie Staining

Amersham Biosciences

tm 80-6343-34/Rev. C1/11-00

Page finder

Staining Principles

1.1 Silver staining principles 1

- Process description
- Explanation of the steps in the silver staining process
- Reagents used in silver staining
- Hazardous substance considerations
- Pitfalls and troubleshooting

1.2 Coomassie staining principles 5

Staining Protocols

2.1 DNA silver staining 7

- Preparation of reagents
- **Protocol 1** DNA silver staining, 1-mm-thick unbacked gels and 0.5-mm-thick gels on plastic backing

2.2 Protein silver staining—SDS and native 8

- Preparation of reagents
- Preparation and use of stock solutions
- **Protocol 2** Protein silver staining, 0.75- and 1-mm-thick unbacked gels and 0.5-mm-thick gels on plastic backing
- **Protocol 3** Protein silver staining, 1.5-mm-thick unbacked gels

2.3 Protein silver staining—IEF 11

- Preparation of reagents
- **Protocol 4** Protein silver staining, 0.5- and 1-mm-thick IEF gels on plastic backing

2.4 Protein Coomassie staining—SDS and native 13

- Preparation of reagents
- **Protocol 5** Protein Coomassie staining, 1-mm-thick unbacked gels and 0.5-mm-thick gels on plastic backing
- **Protocol 6** Protein Coomassie staining, 0.75-mm-thick unbacked gels
- **Protocol 7** Protein Coomassie staining, 1.5-mm-thick unbacked SDS-Polyacrylamide gels

2.5 Protein Coomassie staining—IEF 15

- Preparation of reagents
- **Protocol 8** Protein Coomassie staining, 0.5- and 1-mm-thick IEF gels on plastic backing
- **Protocol 9** Cleaning

Ordering Information 18

Staining Principles

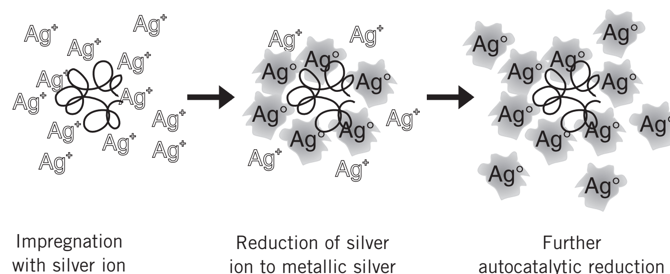
1.1 Silver staining principles

Silver staining is the most sensitive method for permanent staining of proteins or nucleic acids in polyacrylamide gels. It creates a record of the electrophoresis result that can be viewed without any special equipment. It is, however, a complex, multistep process, and many variables can influence the result. High-purity reagents and precise timing are necessary for reproducible, high-quality results.

Automation of the silver staining process with the Hoefer™ Processor Plus™ and the use of PlusOne™ Silver Staining Kits eliminate most of the variables associated with silver staining. Precise control of timing and the use of standard, prepackaged reagents provide exceptional reproducibility. The user is also freed from the necessity to be present throughout this once-tedious procedure.

Process description

In silver staining, polyacrylamide gels are impregnated with soluble silver ion (Ag^+) and developed by treatment with a reductant. Macromolecules in the gel promote the reduction of silver ion to metallic silver (Ag^0), which is insoluble and visible, allowing bands containing protein or nucleic acid to be seen. The initial deposition of metallic silver promotes further deposition in an autocatalytic process, resulting in exceptionally high sensitivity.



Impregnation with silver ion — Reduction of silver ion to metallic silver — Further autocatalytic reduction

There are many variations of the silver staining process, but the process developed for PlusOne Silver Staining Kits represents an optimization for convenience, sensitivity, reproducibility, and overall speed of the process.

Explanation of the steps in the silver staining process

The silver staining process consists of the following steps: Fixing, sensitization, silver impregnation, development, and stopping and gel preservation. Water washes are also included between some of the steps.

Fixing

In the fixing step, the gel is treated with acid. This renders the macromolecules in the gel insoluble and prevents them from diffusing out of the gel during subsequent staining steps. Substances in the gel that interfere with silver staining, such as buffers, ions, denaturants, detergents, or carrier ampholytes, are washed out of the gel during this step.

Sensitization

The gel is treated with reagents that chemically modify proteins, rendering them more reactive toward silver, and reagents that accelerate the subsequent reduction of silver ion. This step greatly enhances the sensitivity of silver staining for protein but is not necessary when silver staining DNA. Excess sensitization reagent results in a high level of background staining, so the gel is washed thoroughly with distilled or deionized water following the sensitization step.

Silver impregnation

In this step the gel is treated with silver nitrate. Mildly acidic conditions prevent silver ion from being reduced to metallic silver. The gel is briefly washed following this step to remove excess silver from the gel surface.

Development

The development solution contains formaldehyde, which reduces silver ion to metallic silver. This reaction proceeds only at high pH, so sodium carbonate is included to render the development solution alkaline.

Stopping and gel preservation

The stopping solution prevents further reduction of silver ion. The preserving solution contains glycerol, which prevents the gel from cracking during drying. These two steps are combined in the PlusOne DNA Silver Staining Kit.

Reagents used in silver staining

Benzenesulphonic acid, acetic acid, trichloroacetic acid (TCA)

These acids are used in the fixing solutions to render the protein or DNA in the gel insoluble.

Glutardialdehyde (glutaraldehyde)

This compound reacts covalently with protein, covering it with aldehyde groups that can react with silver. This greatly enhances the sensitivity of silver staining. Glutardialdehyde also cross-links protein molecules to one another, further preventing their diffusion out of the gel.

Sodium thiosulphate (NaS_2O_3)

This compound serves a dual purpose. It serves as a source of sulphide ion (S^{2-}), which reacts directly with silver, accelerating and enhancing development. Thiosulfate ion also forms a complex with free silver ion and prevents its reduction to metallic silver. This reduces background staining.

Silver nitrate ($AgNO_3$)

This compound is the source of the silver ions that are reduced to metallic silver.

Formaldehyde (H_2CO)

This compound serves as the reductant to convert silver ion (Ag^+) to metallic silver (Ag^0). It is oxidized to formic acid in the process.

Sodium carbonate (Na_2CO_3)

This compound shifts the pH to approximately 12, which allows development ($Ag^+ \rightarrow Ag^0$) to proceed.

EDTA

This compound complexes silver ion strongly, preventing its further reduction to metallic silver in the stopping solution.

Hazardous substance considerations

Many of the reagents used in silver staining (e.g. TCA, glutardialdehyde, formaldehyde, and silver nitrate) are hazardous substances. Automation of silver staining limits user exposure to these substances and simplifies their separate collection for proper disposal.

Pitfalls and troubleshooting

The high sensitivity of silver staining comes at the cost of susceptibility to interference from a variety of sources.

Exceptional cleanliness must be practiced in preparing the electrophoresis apparatus, in subsequent handling of the gel, and in preparation of the staining tray. All equipment used for running and staining the gel must be cleaned with detergent and rinsed thoroughly, as detergents can interfere with silver staining. Clean gloves should be worn when handling the electrophoresis apparatus, the gel, and the staining tray.

High-quality water must be used to prepare the reagents, as impurities have a strong effect on silver staining. Ideally, the water used should either be glass distilled or deionized. For best results use water with a resistivity of ≥5 MΩ.

Temperature also affects silver staining, with higher temperatures promoting faster development and darker background. This effect is more pronounced when staining with the PlusOne DNA Silver Staining Kit than with the PlusOne Silver Staining Kit, Protein. Protocols presented in this Protocol Guide are intended to be used within a normal range for room temperature (20–27 °C). If the ambient temperature falls outside of this range, one can compensate by adjusting the developing time (see "Silver stain troubleshooting" below).

Silver stain troubleshooting

Problem	Cause	Remedy
Bands develop poorly or not at all	Staining solutions not prepared correctly	Check the silver staining kit instructions and prepare solutions again
	Ambient temperature below 20 °C	Lengthen development time by 25%
	Temperature of solution too low	Allow solutions to come to room temperature
	Plates not clean	Clean plates with detergent
	Gel too thin for protocol	Use appropriate staining protocol or shorten wash duration prior to development
Background is excessively dark	Gel too thick for protocol	Use appropriate staining protocol or lengthen wash duration after sensitization
	Water impure	Use deionized water whenever possible
	Interfering substances not completely washed out	Repeat fixing step
	Ambient temperature above 27 °C	Shorten development time by 25%
Bands lighter than background	Overloading	Reduce the amount of protein loaded onto the gel
Smearing or blackening in lanes in which protein is loaded	Overloading	Reduce the amount of protein loaded onto the gel
Silver mirror reaction	Dirty tray	Clean staining tray with detergent and rinse thoroughly
Gel sticks to tray	Insufficient volume	Increase the amount of solution delivered to the tray
	Solution characteristics not optimal	Substitute methanol for ethanol in the fixing solution or add Tween™ 20 (1% v/v) to the sensitization solution

Automated staining using PlusOne Silver Staining Kits is best performed on gels ranging in thickness between 0.75 and 1.5 mm if unbacked, or between 0.5 and 1 mm if gels are on plastic backing. Thinner gels stain less efficiently due to insufficient retention of silver ion. Thicker gels stain less efficiently due to incomplete penetration of staining reagents. Also, the background is higher due to incomplete washing out of reagents between steps.

Gels containing less than 8% polyacrylamide or a cross-linker concentration of less than 2.5% of the total acrylamide concentration may temporarily stick to the staining tray, especially following steps that include ethanol in the solution. These gels normally start moving freely within 2 to 3 min of normal rocking motion. Occasional sticking of this type generally does not affect staining results, but it can be minimized by either increasing the amount of solution delivered to the tray, substituting methanol for ethanol in the fixing solution, or adding Tween 20 to the sensitizing solution (final concentration 1% v/v). These modifications do not affect staining results.

1.2 Coomassie staining principles

Coomassie staining of protein gels is based on binding of the dye Coomassie Blue R350, which binds nonspecifically to virtually all proteins. The gel is impregnated with a solution of the dye. Dye that is not bound to protein diffuses out of the gel during the destain steps. The staining protocols presented in this Protocol Guide also include a fixing step prior to staining to wash SDS or carrier ampholytes out of the gel. This results in more-rapid staining and allows the staining solution to be reused.

Although Coomassie staining is approximately 50-fold less sensitive than silver staining, it is widely used as a convenient alternative. Coomassie Blue dye binds to protein stoichiometrically, so this staining method is preferable when relative amounts of protein are to be determined by densitometry.

Coomassie stain troubleshooting

Problem	Cause	Remedy
Bands poorly stained	Insufficient staining time	Double the staining time
Blue background	Gel insufficiently destained	Repeat last destain step

Staining Protocols

The Hoefer Processor Plus unit is preprogrammed with eight protocols for using Amersham Biosciences staining kits and reagents, including one protocol for silver staining of DNA gels and seven protocols for silver or Coomassie™ staining of protein gels. Applications for each protocol and the approximate total processing time are summarized below.

Preprogrammed staining protocols

Staining protocol	Applications	Number of steps	Approximate total time (min)
1 DNA silver stain	DNA gels — 1 mm unbacked gels, 0.5 mm backed gels	5	100
2 Protein silver stain	SDS and native — 0.75 and 1 mm unbacked gels, 0.5 mm backed gels, Immobiline™ DryPlates	14	160
3 Protein silver stain	SDS and native — 1.5 mm unbacked gels	14	180
4 Protein silver stain	IEF — 0.5 and 1 mm backed gels	18	300
5 Protein Coomassie stain	SDS and native — 1 mm unbacked gels, 0.5 mm backed gels	8	320
6 Protein Coomassie stain	SDS and native — 0.75 mm unbacked gels	8	250
7 Protein Coomassie stain	SDS and native — 1.5 mm unbacked gels	8	460
8 Protein Coomassie stain	IEF — 0.5 and 1 mm backed gels	9	350
9 Cleaning	Remove residual reagents	9	4.5

Note: The silver staining protocols have been optimized for sensitivity. In many cases, the staining procedure can be accelerated by shortening the fixing, sensitization, or silver steps. Such changes usually reduce sensitivity, however.

The following sections describe all steps for each protocol, including port assignments. Please note that port assignments are as consistent as possible throughout, to minimize contamination of the reagent tubing and to prevent incompatible reagents from mixing when switching to a different protocol. Assignments are as follows:

IN-ports

port 0 deionized or distilled water

port 1 fixing solution

port 2 sensitizing solution (silver) or destain solution (Coomassie)

port 3 staining solutions that discolor tubing

port 4 developing solution (silver) or preserving solution (Coomassie)

port 5 stop solution

port 6 preserving solution (silver)

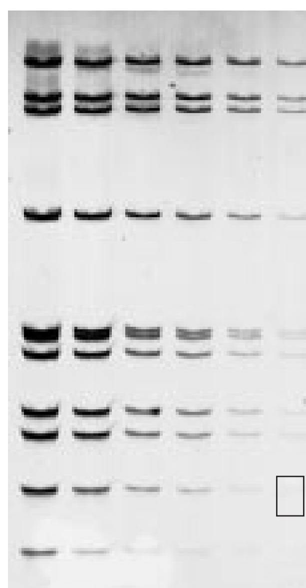
OUT-ports

port 7 generally reserved for relatively nontoxic waste

ports 8 and 9 toxic wastes as indicated in the footnote of each table

2.1 DNA silver staining

Silver staining is a highly sensitive method for visualizing DNA separated in polyacrylamide gels. The PlusOne DNA Silver Staining Kit is a convenient adaptation of this technique that gives reproducible results and sensitivity below 50 pg per band.



Lane: 6

Sensitivity of DNA staining A GeneGel Clean (15%) was loaded with serial dilutions of ΦX 174 DNA *Hae* III digest. The gel was stained in Processor Plus using the PlusOne DNA Silver Staining Kit. The faintest DNA band that can be seen is at 118 bp in lane 6 (15 pg).

Preparation of reagents

Prepare solutions as described in the instructions for the PlusOne DNA Silver Staining Kit. When using the 19 × 29 cm tray, 175 ml of each staining solution is recommended. When using the 29 × 35 cm tray, 325 ml of each staining solution is recommended.

All solutions should be prepared using the purest water available (distilled or deionized). Staining solutions should be allowed to warm to room temperature if they have been refrigerated. The fixing, silver, and stopping solutions are stable at room temperature for at least 1 mo after preparation. (If the gel is not stained immediately after running, it can be stored for up to 1 d in fixing solution.) The developing solution should be prepared immediately prior to use.

❶

Protocol: DNA silver staining, 1-mm-thick unbacked gels and 0.5-mm-thick gels on plastic backing

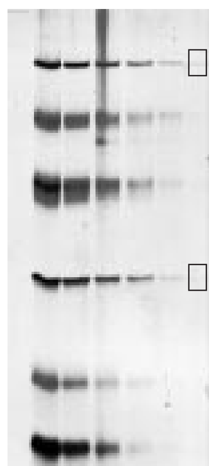
The Hoefer Processor Plus unit is preprogrammed with a basic PlusOne DNA silver staining protocol for staining 1-mm-thick unbacked DNA gels or 0.5-mm-thick DNA gels on plastic backing (DNA ExcelGel™ or CleanGel™).

DNA silver staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution**	1	8	30
2	**Silver solution**	3	9	30
3	**Water**	0	7	1
4	**Developing solution**	4	8	6
5	**Stopping/preserving solution**	5	7	30 (and hold)[2]

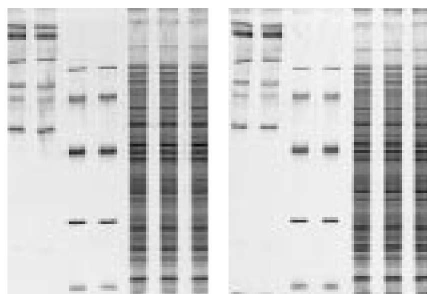
[1] Port assignments for waste material in this protocol are: port 7—waste water, glycerol, acetic acid, and sodium acetate; port 8—waste containing benzenesulphonic acid or formaldehyde; and port 9—waste containing silver. None of these solutions should be recycled.

[2] Hold on the last step so that the gel remains in the stopping/preserving solution.



Lane: 6

Sensitivity of protein staining A 12.5% ExcelGel was loaded with serial dilutions of Amersham Biosciences High Molecular Weight SDS standards and stained in the Processor Plus using the PlusOne Silver Staining Kit, Protein, at 24 °C. The faintest bands that can be seen are phosphorylase b (~0.22 ng) and carbonic anhydrase (~0.29 ng) in lane 6. As is true with most gel staining techniques, limits of sensitivity are somewhat protein specific.



Reproducibility of staining between gels Two 1-mm-thick vertical gels were loaded and run identically with *E. coli* extract. The gels were then stained in two different Processor Plus units, using solutions from the same PlusOne Silver Staining Kit, Protein.

2.2 Protein silver staining—SDS and native

Silver staining is a highly sensitive method for visualizing proteins separated in SDS-polyacrylamide gels. The PlusOne Silver Staining Kit, Protein, is a convenient adaptation of this technique that gives reproducible results and sensitivity below 1 ng per band for most proteins.

Preparation of reagents

Prepare solutions as described in the instructions for the PlusOne Silver Staining Kit, Protein, or prepare stock solutions as described below for convenience and reagent economy. In either case, all solutions should be prepared using the purest water available (distilled or deionized), and formaldehyde and glutardialdehyde should be added just prior to the staining procedure. Staining solutions should be allowed to warm to room temperature if they have been refrigerated.

Preparation and use of stock solutions

Stock solutions are stable at room temperature for up to 1 mo. All solutions should be prepared using the purest water available (distilled or deionized).

Sensitizing solution stock

1. Dissolve 5 sachets of sodium acetate (from the kit) in 800 ml of water and 375 ml of ethanol.
2. Add 50 ml of sodium thiosulphate solution (from the kit). Add water to bring the volume to 1 250 ml.
3. Immediately before staining, transfer the required amount (175 ml for the 19 × 29 cm tray, 325 ml for the 29 × 35 cm tray) into a reagent bottle and add 1/200 vol 25% of glutardialdehyde (from the kit)—0.875 ml or 1.625 ml, respectively.

Silver solution stock

1. Mix 125 ml of silver nitrate solution (from the kit) with 1 125 ml water.
2. Immediately before staining, transfer the required amount (175 ml for the 19 × 29 cm tray, 325 ml for the 29 × 35 cm tray) into a reagent bottle and add 1/2 500 vol 37% of formaldehyde (from the kit)—70 µl or 130 µl, respectively.

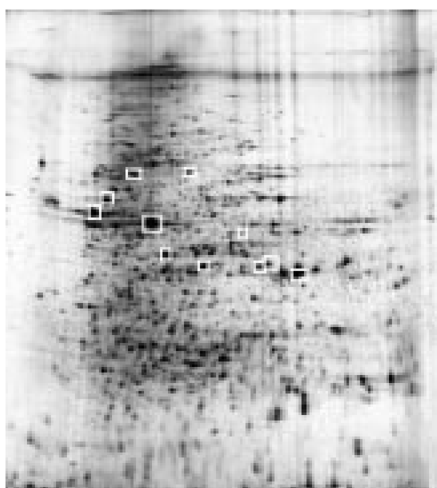
Developing solution stock

1. Dissolve 5 sachets of sodium carbonate (from the kit) in 1 200 ml of water. Add water to bring the volume to 1 250 ml.
2. Directly before staining, transfer the required amount (175 ml for the 19 × 29 cm tray, 325 ml for the 29 × 35 cm tray) into a reagent bottle and add 1/5 000 vol of 37% of formaldehyde (from the kit)—35 µl or 65 µl, respectively.

Stop solution stock

Dissolve 5 sachets of EDTA (from the kit) in 1 200 ml of water, then add water to bring the volume to 1 250 ml.

Immunoblot of glioblastoma multiforme brain tumor cell culture extract separated by 2-D electrophoresis. The blot was incubated with mouse anti-p53 primary antibody (1:30 000) and then goat anti-mouse horseradish peroxidase conjugate (1:5 000). Immunodetection was performed with the ECL detection kit. The human cell culture extract and monoclonal antibody were generously provided by Dr. Mike Harrington, Huntington Medical Research Institute, Pasadena, CA.



2-D gel of glioblastoma multiforme brain tumor cell culture extract stained with PlusOne Silver Staining Kit, Protein, using silver staining protocol 2 of the Processor Plus. p53 antigen that were detected on the 2-D blot above are boxed.

Solutions required for silver staining SDS and native polyacrylamide gels in Hoefer Processor Plus

Solution	Volume (ml) 19 × 29 cm tray	Volume (ml) 29 × 35 cm tray
Fixing solution (40% ethanol, 10% acetic acid)	175	325
Water	1 400	2 600
Sensitizing solution (from kit)	175	325
Silver solution (from kit)	175	325
Developing solution (from kit)	175	325
Stopping solution (from kit)	175	325
Preserving solution for unbacked gels (30% ethanol, 4% glycerol)	175	325
Preserving solution for backed gels (8.7% glycerol)	175	325

If the gel is not stained immediately after electrophoresis, it can be stored for up to 14 d in fixing solution. Note that this may result in the loss of small or acid-soluble polypeptides.

❷

Protocol: Protein silver staining, 0.75- and 1-mm-thick unbacked gels and 0.5-mm-thick gels on plastic backing

The Hoefer Processor Plus unit is preprogrammed with a silver staining protocol for unbacked 0.75- and 1-mm-thick SDS and native polyacrylamide gels, or 0.5-mm-thick SDS and native polyacrylamide gels on plastic backing (ExcelGel or CleanGel products). This protocol can also be used to stain Immobiline™ DryPlates.

Protein silver staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution**	1	7	30
2	**Sensitizing solution**	2	8	30
3	**Water**	0	7	5
4	**Water**	0	7	5
5	**Water**	0	7	5
6	**Silver solution**	3	9	20
7	**Water**	0	7	1
8	**Water**	0	7	1
9	**Developing solution**	4	8	4
10	**Stopping solution**	5	7	10
11	**Water**	0	7	5
12	**Water**	0	7	5
13	**Water**	0	7	5
14	**Preserving solution**	6	7	30 (and hold)[2]

[1] Port assignments for waste material in this protocol are: port 7—waste water, ethanol, acetic acid, EDTA, and glycerol; port 8—waste containing formaldehyde or glutardialdehyde; and port 9—waste containing silver. None of these solutions should be recycled.

[2] Hold on the last step so that the gel remains in the preserving solution.

❸

Protocol: Protein silver staining, 1.5-mm-thick unbacked gels

The Hoefer Processor Plus unit is preprogrammed with a silver staining protocol for unbacked 1.5-mm-thick SDS and native polyacrylamide gels.

Protein silver staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution**	1	7	30
2	**Sensitizing solution**	2	8	30
3	**Water**	0	7	10
4	**Water**	0	7	10
5	**Water**	0	7	10
6	**Silver solution**	3	9	30
7	**Water**	0	7	1
8	**Water**	0	7	1
9	**Developing solution**	4	8	8
10	**Stopping solution**	5	7	10
11	**Water**	0	7	5
12	**Water**	0	7	5
13	**Water**	0	7	5
14	**Preserving solution**	6	7	30 (and hold)[2]

[1] Port assignments for waste material in this protocol are: port 7—waste water, ethanol, acetic acid, EDTA, and glycerol; port 8—waste containing formaldehyde or glutardialdehyde; and port 9—waste containing silver. None of these solutions should be recycled.

[2] Hold on the last step so that the gel remains in the preserving solution.

2.3 Protein silver staining—IEF

Silver staining can also be used to stain proteins separated by isoelectric focusing (IEF) in carrier ampholyte–containing gels (Ampholine™ PAGplate or CleanGel IEF). Carrier ampholytes interfere strongly with silver staining, so additional steps are necessary to ensure that the carrier ampholytes are removed from the gel.

Preparation of reagents

Sensitizing, silver, developing, and stopping solutions are prepared as described in the instructions for the PlusOne Silver Staining Kit, Protein. Alternatively, see Section 2.2, "Preparation and use of stock solutions," for convenient and economical stock solution handling. With either method, always add formaldehyde and glutardialdehyde to the appropriate solutions immediately prior to starting the staining procedure.

This protocol requires additional reagents as listed below. All solutions should be prepared using the purest water available (distilled or deionized). Staining solutions should be allowed to warm to room temperature if they have been refrigerated.

Solutions required for silver staining IEF gels in Hoefer Processor Plus

Solution	Volume (ml) 19 × 29 cm tray	Volume (ml) 29 × 35 cm tray
Fixing solution #1 (20% [w/v] trichloroacetic acid [TCA])	175	325
Fixing solution #2 (50% methanol, 10% acetic acid)	175	325
5% methanol	700	1300
Water	1 225	2 275
Sensitizing solution (from kit)	175	325
Silver solution (from kit)	175	325
Developing solution (from kit)	175	325
Stopping solution (from kit)	175	325
Preserving solution for backed gels (8.7% glycerol)	175	325

❹

Protocol: Protein silver staining, 0.5- and 1-mm-thick IEF gels on plastic backing

The Hoefer Processor Plus unit is preprogrammed with a silver staining protocol for carrier ampholyte–containing IEF gels on plastic backing.

Protein silver staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	Fixing solution #1	7	9	45
2	Fixing solution #2	1	9	30
3	5% methanol	8	9	15
4	5% methanol	8	9	15
5	Sensitizing solution	2	9	30
6	5% methanol	8	9	30
7	5% methanol	8	9	30
8	Water	0	9	5
9	Water	0	9	5
10	Silver solution	3	9	20
11	Water	0	9	1
12	Water	0	9	1
13	Developing solution	4	9	4
14	Stopping solution	5	9	10
15	Water	0	9	5
16	Water	0	9	5
17	Water	0	9	5
18	Preserving solution	6	9	30 (and hold)[2]

[1] Port 9 is used for all waste. None of the solutions should be recycled.

[2] Hold on the last step so that the gel remains in the preserving solution.

2.4 Protein Coomassie staining—SDS and native

The Hoefer Processor Plus unit is also well suited for Coomassie staining of SDS, native, and 2-D gels. Although this method is approximately 50-fold less sensitive than silver staining, it is widely used as a convenient alternative. Because the Coomassie Blue dye is bound stoichiometrically by protein, this staining method is preferable when relative amounts of protein are to be determined by densitometry.

Preparation of reagents

In addition to the other solutions listed in the table below, prepare a 0.2% (w/v) stock solution of Coomassie Blue R350 in 60% methanol. This is most conveniently done using PhastGel™ Blue R tablets: Dissolve 1 tablet of PhastGel Blue R in 80 ml of distilled water and stir for 5 to 10 min. Add 120 ml of methanol and stir until all of the dye dissolves, then filter the solution. This stock solution can be stored for 7 to 21 d at 4 °C. Prior to staining, prepare a 0.02% stain solution: Mix 1 part of filtered stock solution to 9 parts of methanol:acetic acid:distilled water (3:1:6).

Solutions required for Coomassie staining SDS and native polyacrylamide gels in Hoefer Processor Plus

Solution	Volume (ml) 19 × 29 cm tray	Volume (ml) 29 × 35 cm tray
Fixing solution (40% methanol, 10% acetic acid)	175	325
Destain solution (25% ethanol, 8% acetic acid)	875	1 625
0.02% Coomassie Blue	175	325
Preserving solution (25% ethanol, 8% acetic acid, 4% glycerol)	175	325

❺

Protocol: Protein Coomassie staining, 1-mm-thick unbacked gels and 0.5-mm-thick gels on plastic backing

The Hoefer Processor Plus unit is preprogrammed with a Coomassie staining protocol for 1-mm-thick unbacked gels, or 0.5-mm-thick SDS-polyacrylamide gels on plastic backing (ExcelGel or CleanGel products).

Protein Coomassie staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution**	1	8	20
2	**Destain solution**	2	9	2
3	**0.02% Coomassie Blue**	3	3	60
4	**Destain solution**	2	9	10
5	**Destain solution**	2	9	30
6	**Destain solution**	2	9	80
7	**Destain solution**	2	9	80
8	**Preserving solution**	4	9	30 (and hold)[2]

[1] Port assignments for waste material in this protocol are: port 8—waste containing methanol; and port 9—waste destain solution. Port 4 is used both for pump in and pump out of 0.02% Coomassie Blue, which can be recycled up to three times.

[2] Hold on the last step so that the gel remains in the preserving solution.

❻

Protocol: Protein Coomassie staining, 0.75-mm-thick unbacked gels

The Hoefer Processor Plus unit is preprogrammed with a Coomassie staining protocol for 0.75-mm-thick unbacked SDS-polyacrylamide gels.

Protein Coomassie staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution**	1	8	15
2	**Destain solution**	2	9	1.5
3	**0.02% Coomassie Blue**	3	3	45
4	**Destain solution**	2	9	7.5
5	**Destain solution**	2	9	22.5
6	**Destain solution**	2	9	60
7	**Destain solution**	2	9	60
8	**Preserving solution**	4	9	30 (and hold)[2]

[1] Port assignments for waste material in this protocol are: port 8—waste containing methanol; and port 9—waste destain solution. Port 4 is used both for pump in and pump out of 0.02% Coomassie Blue, which can be recycled up to three times.

[2] Hold on the last step so that the gel remains in the preserving solution.

7

Protocol: Protein Coomassie staining, 1.5-mm-thick unbacked SDS-Polyacrylamide gels

The Hoefer Processor Plus unit is preprogrammed with a Coomassie staining protocol for 1.5-mm-thick unbacked gels.

Protein Coomassie staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution**	1	8	30
2	**Destain solution**	2	9	3
3	**0.02% Coomassie Blue**	3	3	90
4	**Destain solution**	2	9	15
5	**Destain solution**	2	9	45
6	**Destain solution**	2	9	120
7	**Destain solution**	2	9	120
8	**Preserving solution**	4	9	30 (and hold)[2]

[1] Port assignments for waste material in this protocol are: port 8—waste containing methanol; and port 9—waste destain solution. Port 4 is used both for pump in and pump out of 0.02% Coomassie Blue, which can be recycled up to three times.

[2] Hold on the last step so that the gel remains in the preserving solution.

2.5 Protein Coomassie staining—IEF

The Hoefer Processor Plus can be used for Coomassie staining of 0.5- or 1-mm-thick carrier ampholyte–containing IEF gels (Ampholine PAGplate or CleanGel IEF). Although this method is less sensitive than silver staining, it is widely used as a convenient alternative. Because the Coomassie Blue dye is bound stoichiometrically by protein, this staining method is preferable when relative amounts of protein are to be determined by densitometry.

Preparation of reagents

The solutions used for Coomassie staining of IEF gels differ from the staining solution used for SDS and native polyacrylamide gels in two ways: A 20% TCA solution is used as a fixative, and copper sulfate ($CuSO_4$) is added to the Coomassie Blue solution.

Prepare a 0.2% (w/v) stock solution of Coomassie Blue R350 in 60% methanol. This is most conveniently done using PhastGel Blue R tablets. Dissolve 1 tablet of PhastGel Blue R in 80 ml of distilled water and stir for 5 to 10 min. Add 120 ml of methanol and stir until all of the dye dissolves. Filter the solution. This stock solution can be kept for 7 to 21 d at 4 °C. Prior to staining, prepare a 0.02% stain solution: Mix 1 part of filtered stock solution to 9 parts of methanol:acetic acid:distilled water (3:1:6). Add copper sulphate ($CuSO_4$) to 0.1% (w/v) to this solution.

Solutions required for Coomassie staining of IEF gels in Hoefer Processor Plus

Solution	Volume (ml) 19 × 29 cm tray	Volume (ml) 29 × 35 cm tray
Fixing solution #1 (20% TCA)	175	325
Fixing solution #2 (methanol, 10% acetic acid)	175	325
Destain solution (25% ethanol, 8% acetic acid)	875	1 625
0.02% Coomassie Blue, 0.1% $CuSO_4$	175	325
Preserving solution (25% ethanol, 8% acetic acid, 4% glycerol)	175	325

8

Protocol: Protein Coomassie staining, 0.5- and 1-mm-thick IEF gels on plastic backing

The Hoefer Processor Plus unit is preprogrammed with a Coomassie staining protocol for 0.5- or 1-mm-thick carrier ampholyte–containing IEF gels on plastic backing (Ampholine PAGplate or CleanGel IEF).

Protein Coomassie staining protocol

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Fixing solution #1**	5	7	20
2	**Fixing solution #2**	1	8	30
3	**Destain solution**	2	9	2
4	**0.02% Coomassie Blue, 0.1% $CuSO_4$**	3	3	60
5	**Destain solution**	2	9	10
6	**Destain solution**	2	9	30
7	**Destain solution**	2	9	80
8	**Destain solution**	2	9	80
9	**Preserving solution**	4	9	30 (and hold)[2]

[1] Port assignments for waste material in this protocol are: port 7—waste containing TCA; port 8—waste containing methanol; and port 9—waste destain solution. Port 4 is used both for pump in and pump out of 0.02% Coomassie Blue, which can be recycled up to three times.

[2] Hold on the last step so that the gel remains in the preserving solution.

9

Protocol: Cleaning

The cleaning protocol should be used after each staining process to ensure that residual reagents are removed from reagent lines. Reagent lines 1 through 9 are placed in the sink or waste receptacle. Line 0 is placed into a container of water.

The programme draws solution through line 0 into the tray and empties the tray through lines 1 through 9 in succession.

Cleaning all reagent lines

Step #	Solution	IN-port	OUT-port[1]	Time (min)
1	**Distilled water**	0	1	0.5
2	**Distilled water**	0	2	0.5
3	**Distilled water**	0	3	0.5
4	**Distilled water**	0	4	0.5
5	**Distilled water**	0	5	0.5
6	**Distilled water**	0	6	0.5
7	**Distilled water**	0	7	0.5
8	**Distilled water**	0	8	0.5
9	**Distilled water**	0	9	0.5

Ordering Information

Hoefer Processor Plus Base unit, reagent tubing, and protocol key (order tray pack separately)	80-6444-04

Gel staining tray options

Staining Tray Pack 19 × 29 cm Complete with gel staining tray base, PTFE-coated tray, and lid	80-6444-80
Staining Tray Pack 29 × 35 cm Complete with gel staining tray base, PTFE-coated tray, and lid	80-6445-18

Staining Kits

PlusOne DNA Silver Staining Kit	17-6000-30
PlusOne Silver Staining Kit, Protein	17-1150-01
Coomassie tablets (40), PhastGel Blue R	17-0518-01

Blot processing tray options

Blot Processing Tray Pack Complete with tray base, disposable mini and standard trays, lid, reagent bottles and rack, and waste bottle cap	80-6444-23

Accessories for blot processing

Blot Processing Mini Tray Disposable mini trays (3/pk)	80-6444-42
Blot Processing Standard Tray Disposable standard trays (3/pk)	80-6444-61

Ampholine, CleanGel, ExcelGel, Hoefer, Immobiline, PhastGel, Processor Plus and PlusOne are trademarks of Amersham Biosciences Limited or its subsidiaries.

Amersham and Amersham Biosciences is a trademark of Amersham plc.

Tween is a trademark of ICI Americas Inc.

Coomassie is a trademark of ICI plc.

© 1999 Amersham Biosciences Inc.
All rights reserved.

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group that supplies them. A copy of these terms and conditions is available on request.

Printed in the USA.

Amersham Biosciences UK Limited
Amersham Place Little Chalfont
Buckinghamshire England HP7 9NA

Amersham Biosciences AB
SE-751 84 Uppsala Sweden

Amersham Biosciences Inc.
800 Centennial Avenue PO Box 1327
Piscataway NJ 08855 USA

Amersham Biosciences Europe GmbH
Munzinger Strasse 9 D-79111 Freiburg Germany

www.amershambiosciences.com

Automated Staining of Polyacrylamide Gels with Hoefer Processor Plus • Protocol Guide for Silver and Coomassie Staining