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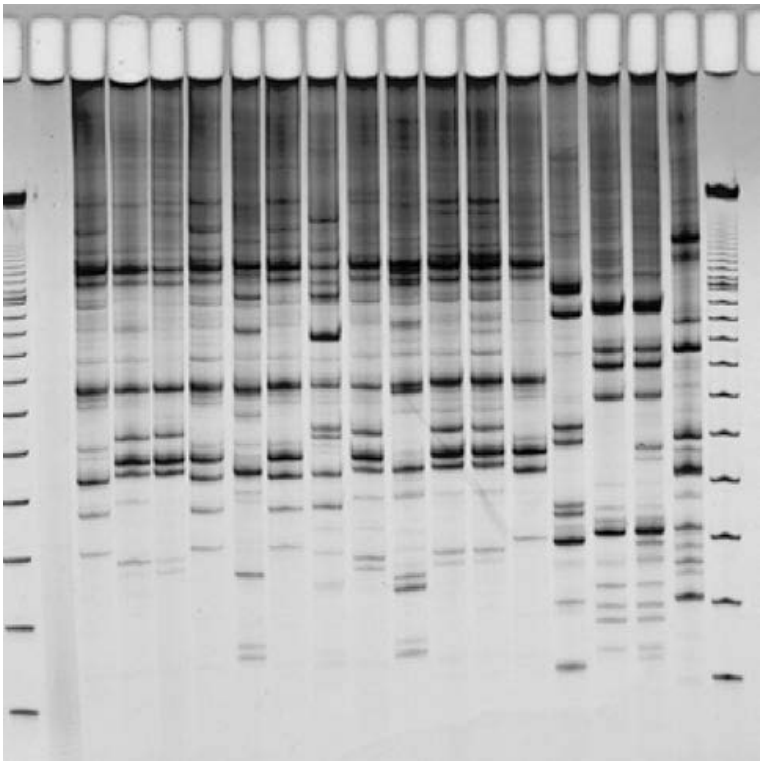
DNA Silver Staining Kit

Instructions for Use



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1 Introduction

PlusOne DNA Silver Staining Kit contains all essential components for staining of nucleic acids in polyacrylamide gels. To avoid transportation of large solution volumes, most of the solutions are in a 5x concentrated form, ready to use after a simple dilution. The high sensitivity of the visualization technique allows detection of nucleic acids down to 20–50 pg DNA/band. The method is reliable and reproducible and gives a gel with distinct bands on an essentially colourless background ready for drying and/or evaluation in about 1.5 h.

The formulation of the kit* minimizes production of toxic waste and optimizes sensitivity and robustness making the kit especially suitable for use in automatic staining equipment: Hoefer Automated Gel Stainer and GeneStain Automated Gel Stainer. In addition, the kit also provides the method of choice for standard manual silver staining of DNA in polyacrylamide gels.

The kit performs best on 0.5–1.0 mm thick plastic-backed gels, or 0.75–1.5 mm thick un-backed gels.

The procedure described in this instruction is based upon the use of 125 ml solution per process step. Each kit contains material for 20 processes. When using the 125 ml tray in Hoefer Automated Gel Stainer or GeneStain Automated Gel Stainer this gives the following processing capacity for different gel sizes:

Gel Size	Gels/process	Gels/kit
7 × 8 cm (SE250 minigels)	1–4	80
14 × 16 (SE 600 type)	1	20
12.5 × 26 cm (ExcelGel™, CleanGel)	1	20
11 × 12.3 cm (GeneGel)	2	40

* Patent pending

2 Kit contents and technical data

Kit contents

DNA Silver Staining Kit (Product code 17600030) contains the following items:

Item	Composition	Quantity
Fixing solution, 5×	Benzene sulphonic acid; 3.0% w/v in 24% v/v ethanol	500 ml
Staining solution, 5×	Silver nitrate; 1.0% w/v Benzene sulphonic acid; 0.35% w/v	500 ml
Sodium carbonate solution, 5×	Sodium carbonate; 12.5% w/v	500 ml
Formaldehyde; 37%	Formaldehyde; 37% w/v in water	4 ml
Sodium thiosulphate; 2%	Sodium thiosulphate; 2% w/v in water	4 ml
Stopping & Preserving solution, 5×	Acetic acid, 5% v/v Sodium acetate; 25% w/v Glycerol; 50% v/v	500 ml
Instructions	Booklet	1
Short instructions	Laminated sheet	1

When used in Hoefer Automated Gel Stainer or GeneStain Automated Gel Stainer the kit content is sufficient for staining 20 standard size gels (14×16 cm or 12×24 cm) or 80 minigels (7×8 cm).

Technical data

Sensitivity:	20-50 pg DNA per band
Usage temperature:	20°C to 27°C
Storage:	10°C to 30°C
Shelf life:	1 year at recommended storage conditions

Precautions: The chemicals in this kit should not be discarded via public waste water systems. Please dispose of these chemicals properly. We recommend that you collect and dispose of the silver containg waste separately. Consult your local regulations for more information. Read the warning text on the label of each bottle.

3 Protocol and procedure for silver staining

General information

This protocol is optimized for precast GeneGel, CleanGel and ExcelGel media from Cytiva, but will also work on most other gel types.

When used in Hoefer Automated Gel Stainer or GeneStain Automated Gel Stainer instruments the protocol will provide excellent results for gels in sizes from 7×8 cm up to 18×24,5 cm (ExcelGel SDS 12-14) in thicknesses of 0.75–1.5 mm for unbacked gels and 0.5–1.0 mm plastic-backed gels. Gels outside of these dimensions may require modified procedures.

All procedures are based on all reagents used at a temperature of 20°C to 27°C. Using reagents outside this temperature range will require modification of development times for optimal results. If the temperature is below 20°C, extend the development time to 8–10 min. If the temperature is above 27°C, reduce development time to 4 min.

For manual use we recommend gentle shaking in all steps on an automated shaker like Red Rotor Orbital Shaker equipped with Staining Tray 1 or Staining Tray 2 (see *Chapter 5 Recommended equipment and accessories, on page 11*). These trays have polished surfaces of SIS 2332 grade stainless steel that do not interfere with the silver staining technique.

For maximal convenience and reliability we recommend automated staining in programmable Hoefer Automated Gel Stainer or GeneStain Automated Gel Stainer with preset programs.

Chemicals required:

- Chemicals included in the DNA Silver Staining Kit, plus:
- Ethanol (24%) (Prepare in advance)
- Distilled or deionized water

Equipment required:

- Five Reagent vessels taking 125 ml solution
- Measuring cylinders; 25 and 100 ml
- Micropipette for delivering 125 ml
- Automated staining equipment or Staining tray (preferably stainless steel) and rotating table for manual staining
- Bottles

Reagent preparation

For staining volumes of 125 ml, prepare the 5 reagents listed below in clean glass bottles. Adjust component volumes appropriately if staining in volumes other than 125 ml.

Reagent	Components required from Kit	Other components
1. Fixing solution	25 ml Fixing solution, 5×	100 ml 24% ethanol
2. Staining solution	25 ml Staining solution, 5×	100 ml water
3. Developing solution	25 ml Sodium carbonate, 5× 125 µl Sodium thiosulphate 125 µl Formaldehyde[1]	100 ml of water
4. Stopping & Preserving solution	25 ml Stopping & Preserving solution, 5×	100 ml water
5. Water for washing		> 125 ml

[1] IMPORTANT: The Developing solution with formaldehyde is very unstable and should be prepared immediately before use. When performing the staining in any of the automated gel stainers, it should be prepared as the last step before starting the staining process. Please also note that the cloudy precipitate of polymerised formaldehyde that sometimes develops during normal storage does not affect staining performance.

Automated staining procedure

Automated staining in Hoefer Automated Gelstainer or GeneStain Automated Gel Stainer.

Step	Action
1	Attach the staining reagent bottles to IN-ports and the waste bottles to OUT-ports according to the *Table 3.1, on page 7* and *Table 3.2, on page 7* below. Make sure the suction pipes penetrate down to the bottom of the vessels to allow all reagent solution to be taken up.
2	Make sure all solutions are at the proper temperature (20°C to 27°C).
3	Position the gel(s) in the staining tray with or without the aid of the magnets.
4	Choose the pre-programmed DNA Silver Staining protocol.
5	Start the protocol.
6	Retrieve the stained gel after ca 1.5 h.

Table 3.1: Hoefer Automated GelStainer Protocol #1: DNA Silver Stain

Step	Solution	IN-port	OUT-port	Processing step time
1	Fixing solution	1	8	30 min
2	Silver solution	3	9	30 min
3	Water (washing)	0	7	1 min
4	Developing solution	4	8	6 min
5	Stopping & Preserving solution	5	7	30 min & hold

Table 3.2: GeneStain Automated GelStainer: DNA Silver Staining protocol

Step	Solution	IN-port	OUT-port	Processing step time
1	Fixing solution	1	5	30 min
2	Silver solution	2	6	30 min
3	Water (washing)	0	7	1 min
4	Developing solution	3	8	6 min
5	Stopping & Preserving solution	4	9	30 min & hold

Manual staining procedure

1. Make sure that the temperature of the reagents are within the range of 20°C to 27°C. If not, adjust the temperature or modify the reaction times for the corresponding steps (see *General information, on page 5*).
2. Use gloves and perform all steps with gentle shaking of the staining tray.
3. Be careful that the gel is evenly wetted with the entire solution volume during all process steps.

Procedure:

Step	Action
1	**Fixing** Soak the gel in fix solution 30 min (or longer, over night OK)
2	**Silver impregnation** Replace fixing solution with 30 min silver solution. Incubate

Step	Action
3	**Washing** Pour off silver solution. Add water 1 min
4	**Development** Pour off water Add Developing solution 6 min
5	**Stopping & preserving** Soak gel in Stopping & Preserving solution At least 30 min (Over night OK)

4 Troubleshooting guide

This Troubleshooting Guide is primarily for manual staining but most parts are also relevant for automated staining. For more comprehensive information on staining, please consult *Protocol Guide 80634334*. For problems when using automated staining, please also consult the User Manual for the relevant instrument.

Problem	Cause	Remedy
Bands do not develop	Temperature of reagent solution too low	Keep temperature of between 20°C and 27°C
	Gel not washed thoroughly enough	Repeat the washing procedure
	Did not fix properly	Check fixing solution or fixing properties of your particular nucleic acid(s)
	Protein samples contaning reducing agents have been run on the gel(s)	Run and stain DNA samples separately
Background is excessively dark (over-developed)	Too long exposure to developing solution	Use no more than 10–15 minutes for development of bands at 21°C to 25°C
	Metal from magnets contaminating reagent solutions (Automated staining only)	Exchange magnets with damaged coating
	Water impure	Use distilled or deionized water
Silver mirror reaction	Insufficient washing with water	Extend the time of washing after staining
	Dirty tray	Replace the chamber for development with a new clean one
	Insufficient shaking	During the development step, shake the tray sufficiently to prevent the gel from sticking to the bottom of the tray

Gels come loose from backing	Gels left in fix for too long	Reduce fixing time: A minimum of 30 min required. Over night fixing usually OK.

5 Recommended equipment and accessories

Product		Product code
Hoefer, Automated Gel Stainer	230 V	80633004
	115 V	80633023
GeneStain Automated Gel Stainer	230 V	80633042
	115 V	80634467
Red Rotor Orbital Shaker, 25×35 cm	115 V	80609691
	230 V	80609710
Red Rotor Orbital Shaker, 50×50 cm	115 V	80609805
	230 V	80609824
Staining Tray 1 with lid and removable gel holder (60×150×300 mm)		18101808
Staining Tray 2 with lid (60×260×320 mm)		18101809
Cellophane Sheets		80112938

6 Precast gels and related products

GenePhor gel media kits	Product code
GeneGel Excel 12.5/24 Kit	17600014
GeneGel Clean 15/24 Kit	17600013
MultiPhor™ II gel media kits	
ExcelGel DNA Analysis Kit	17119807
DNA Fragment Analysis Kit	17119806
CleanGel media for MultiPhor II	
CleanGel 25S	18103154
CleanGel 36S	18103155
CleanGel 48S	18103156
GelPool for rehydrating the gel	18103158
PaperPool for soaking electrode strips	18103159

DNA Molecular weight markers

Product name	Size range (bp)	Product code
ø×174-Hinc II digest	79–1,049	27404001
ø×174-Hae III digest	72–1,342	27404401
λ-Hind III digest	2,027–23,130	27404801
λ-Hind III/ø×174-Hinc II digest	79–23,130	27405201
λ-Hind III/ø×174-Hae III digest	72–23,130	27405401
DRIgest III (lyophilized)	72–23,130	27406001

7 Literature

Protocol Guide “Automated Silver and Coomassie Straining of Polyacrylamide Gels” Product code 80634334.

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