Dodeca™ Silver Stain Kit Instruction Manual

Catalog #161-0480
Catalog #161-0481

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Table of Contents

Section 1
Introduction

The Dodeca™ silver stain kit is an easy-to-use kit for the detection of nanogram levels of proteins in polyacrylamide gels. The kit is formulated with concentrated aqueous solutions that enable the user to quickly prepare volumes of working solutions that may be easily tailored to the number of gels to be stained. Furthermore, the kit is designed to be compatible with the high-throughput Dodeca stainer by facilitating reagent preparation and instrument cleaning.

The Dodeca silver stain kit is based on the method described by Sinha et al. (2001) in which protein-bound silver ions are chemically reduced to form visible metallic silver. Additionally, no protein-modifying glutaraldehyde is used, and only minimal concentrations of the reducing agent, formaldehyde, which is required for silver stain development. Consequently, this procedure yields silver-stained proteins that are compatible with mass spectrometry for protein identification analysis.

Reference

Sinha P et al., A new silver staining apparatus and procedure for matrix-assisted laser desorption/ionization-time of flight analysis of proteins after two dimensional electrophoresis, Proteomics 1, 835–840 (2001)

Section 2
Specifications

	Catalog #	
	161-0481	**161-0480**
Maximum Dodeca stainer volume	7 L	10 L
Component solutions		
Sensitizer concentrate (SC)	700 ml	1 L
Background reducer concentrate (BRC)	75 ml	125 ml
Silver reagent concentrate (SRC)	140 ml	200 ml
Development buffer concentrate (DBC)	700 ml	1 L
Image developer concentrate (IDC) (contains formaldehyde)	2.25 ml	2.25 ml
Quick reference card	1 each	1 each

Kit storage temperature	Ambient
Optimum kit operating temperature	21–25°C
Bovine serum albumin (BSA) staining sensitivity	≥0.5 ng
Shelf life	12 months

Table 1. Recommended Dodeca silver stain kit and Dodeca stainer for various gel sizes.

Kit Size	Stainer Size	Tray Size	Gel Sizes (W x L)	Bio-Rad Gel Formats
Dodeca silver stain kit, large	Large Dodeca stainer	Large staining tray	25.6 x 23 cm	PROTEAN® Plus precast gels
(161-0480)	(165-3400)		25 x 20.5 cm	PROTEAN Plus handcast gels (require one attachment per tray, catalog #165-3417)
Dodeca silver stain kit, small	Small Dodeca stainer	Small staining tray	20 x 20.5 cm	PROTEAN Plus handcast gels
(161-0481)	(165-3401)		18 x 20 cm	PROTEAN® II XL handcast and precast gels
			16 x 20 cm	PROTEAN II xi handcast gels
			16 x 16 cm	PROTEAN II xi handcast and precast gels
			13.3 x 8.7 cm	Criterion™ gels (up to 24 gels, require one attachment per tray, catalog #165-3418)

Section 3
Reagent Preparation

Additional Materials Needed

In addition to the components in the Dodeca silver stain kit, the following materials are required:

- Reagent-grade ethanol and glacial acetic acid
- Deionized, distilled, or high purity water. Water of ≥18 MΩ-cm resistivity is recommended
- Dodeca stainer (catalog #165-3400 or 165-3401), or glass or plastic staining trays
- When not using a Dodeca stainer, all steps in the procedure require agitation of the gel in solution — for best results, a shaker table is recommended

Warnings and Precautions

Read each label in the kit before beginning the procedure.

Always wear powder-free laboratory gloves when handling gels to prevent protein contamination.

The image developer concentrate contains formaldehyde and should be used in properly ventilated areas. Avoid contact with skin. In case of contact with eyes, flush with copious amounts of water and contact a physician.

See Material Safety Data Sheets for additional information about each solution.

Dispose of waste material according to local regulations.

Working Solution Volumes

For best results, prepare each working solution no more than 24 hr before use. Add the image developer concentrate to the developing working solution within 15 minutes of use. Note: The volume corresponding to water (Table 2, 3 and 4) indicates the final volume of the working solution.

Please refer to the appropriate section below to determine the proper working solution volumes for staining 1) 12 PROTEAN gels in a Dodeca stainer, 2) fewer than 12 gels in a Dodeca stainer, or 3) individual gels in a user-provided staining container.

1) **Dodeca stainer with 12 trays.** When using the full capacity of the Dodeca stainer, prepare each working solution as directed by Table 2. Please note that the cover tray is not counted.

Table 2. Dodeca stainer solution volumes.

Working Solutions	Reagents	Small Dodeca Stainer 12 Trays	Large Dodeca Stainer 12 Trays
Fixing	Ethanol	2.8 L	4.0 L
	Glacial acetic acid	700 ml	1.0 L
	Water	To 7 L	To 10 L
Sensitizing	SC (10×)	700 ml	1.0 L
	BRC (100×)	70 ml	100 ml
	Ethanol	2.1 L	3.0 L
	Water	To 7 L	To 10 L
Staining	SRC (50×)	140 ml	200 ml
	Water	To 7 L	To 10 L
Developing	DBC (10×)	700 ml	1.0 L
	IDC (5,000×)	1.4 ml	2.0 ml
	BRC (20,000×)	350 µl	500 µl
	Water	To 7 L	To 10 L
Stopping	Glacial acetic acid	350 ml	500 ml
	Water	To 7 L	To 10 L

2) **Dodeca stainer with less than 12 trays.** Although the provided volumes of the concentrates are optimized to stain 12 PROTEAN gels (or 24 Criterion gels) in a Dodeca stainer, fewer gels also may be stained. However, because of inherent dead volumes in the Dodeca stainer (as with most instruments), a slightly higher solution volume per tray ratio is required for optimal results. Using Table 3, the required working solution volumes are calculated by taking the number of staining trays used (not including the cover tray) multiplied by the volume listed in the appropriate "Per Tray" column, and then adding the corresponding volume listed under the adjacent "Plus" column. For example, six PROTEAN Plus gels stained using six trays in the large Dodeca stainer require (6 trays x 750 ml) + 1.5 L, for a total volume of 6.0 L for each working solution. Silver staining with fewer than four trays is not recommended.

Table 3. Dodeca stainer volumes for less than 12 trays.*

Working Solutions	Reagents	Small Dodeca Stainer 4–11 Trays		Large Dodeca Stainer 4–11 Trays	
		Per Tray	Plus	Per Tray	Plus
Fixing	Ethanol	200 ml	400 ml	300 ml	600 ml
	Glacial acetic acid	50 ml	100 ml	75 ml	150 ml
	Water	To 500 ml	To 1 L	To 750 ml	To 1.5 L
Sensitizing	SC (10×)	50 ml	100 ml	75 ml	150 ml
	BRC (100×)	5 ml	10 ml	7.5 ml	150 ml
	Ethanol	150 ml	300 ml	225 ml	450 ml
	Water	To 500 ml	To 1 L	To 750 ml	To 1.5 L
Staining	SRC (50×)	10 ml	20 ml	15 ml	30 ml
	Water	To 500 ml	To 1 L	To 750 ml	To 1.5 L
Developing	DBC (10×)	50 ml	100 ml	75 ml	150 ml
	IDC (5,000×)	100 µl	200 µl	150 µl	300 µl
	BRC (20,000×)	25 µl	50 µl	37.5 µl	75 µl
	Water	To 500 ml	To 1 L	To 750 ml	To 1.5 L
Stopping	Glacial acetic acid	25 ml	50 ml	37.5 ml	75 ml
	Water	To 500 ml	To 1 L	To 750 ml	To 1.5 L

*Please refer to page 6 for further details

3) Individual gels. Prepare working solutions according to Table 4. To stain multiple gels, multiply the solution volumes by the appropriate number of gels. The working solution volumes listed below are recommended volumes — the actual required volume may vary depending on the dimensions of the staining container used. For all cases, sufficient volumes of working solution should be prepared to completely submerge the gel to be stained.

Table 4. Suggested working solution volumes for individual gels.

Working Solutions	Reagents	Individual Mini or Criterion™ Gels (250 ml)	Individual Large Gels 16 x 16 cm or larger (1 L)
Fixing	Ethanol	100 ml	400 ml
	Glacial acetic acid	25 ml	100 ml
	Water	To 250 ml	To 1 L
Sensitizing	SC (10×)	25 ml	100 ml
	BRC (100×)	2.5 ml	10.0 ml
	Ethanol	75 ml	300 ml
	Water	To 250 ml	To 1 L
Staining	SRC (50×)	5 ml	20 ml
	Water	To 250 ml	To 1 L
Developing	DBC (10×)	25 ml	100 ml
	IDC (5,000×)	50.0 µl	200.0 µl
	BRC (20,000×)	12.5 µl	50.0 µl
	Water	To 250 ml	To 1 L
Stopping	Glacial acetic acid	12.5 ml	50.0 ml
	Water	To 250 ml	To 1 L

Section 4
Polyacrylamide Gel Staining Procedure

Before starting this procedure, prepare the working solutions as described on pages 5–8.

Step	Description	Small Gels (Mini or Criterion Gels)	Large Gels (16 × 16 cm or larger)
1	**Fixing.** Place gel(s) in fixing solution and shake for indicated period of time.	>30 min (overnight acceptable)	>1 hr (overnight acceptable)
2	**Sensitizing.** Remove the fixing solution and shake gel(s) in the sensitizing solution.	30 min	30 min
3	**Washing.** Remove sensitizing solution and wash 3 times with one working volume of water.	3 × 5 min	3 × 10 min
4	**Staining.** Remove water and shake in staining solution.	20 min	30 min
5	**Rinsing.** Remove staining solution and rinse the gel(s) in one working volume of water.	1 × 1 min, (2 × 1 min)†	1 × 1 min, (2 × 1 min)†
6	**Developing.** Remove water and shake gel(s) in developing solution.	10–30 min	10–30 min
7	**Stopping.** Remove developing solution and shake gel(s) in stopping solution.	10 min	10 min
8	**Washing.** Wash gels in one working volume of water. Gel(s) may be dried, imaged, or used to excise visualized proteins.	10 min	10 min

†When using the Dodeca stainer, pay special attention to minimize the solution draining and filling time between steps 5 and 6, and between steps 6 and 7. However, gels stained individually should be rinsed in step 6 with **two** 1 min water washes.

Section 5
Troubleshooting Guide

Problem	Cause	Solution
Background dark or uneven	Agitation too gentle.	Increase agitation speed during gel incubation and ensure gel is completely submerged.
	Dodeca stainer or staining containers contain residue from prior cleaning or staining runs.	Thoroughly clean Dodeca stainer or containers with soap and water. Rinse **only glass** containers with 50% nitric acid, followed by thorough water rinse to remove the acid.
	Fingerprints or powder from gloves cause staining artifacts.	Use powder-free laboratory gloves.
	Interfering components (glycine, SDS, etc.) not completely removed from gel.	Increase fixing time or use multiple fixing cycles to completely wash interfering compounds from gel.
	Sensitizing working solution improperly prepared.	Prepare new sensitizing solution with BRC.

Problem	Cause	Solution
Background dark or uneven (cont.)	Contaminants in water.	Prepare all solutions with double-distilled water with a resistance of at least 18 MΩ-cm.
Low protein staining sensitivity or no staining	Excessive silver ions washed out of gel.	Verify that each post-staining water wash is exactly 1 min long.
	pH of developing working solution too low.	Verify the working solution pH = 11.6 ± 0.5, and prepare new developing solution with development buffer concentrate, if necessary.
	Reducer not present in developing working solution.	Prepare new developing solution with image developer concentrate.
	Insufficient fixing due to variable fixing characteristics of different proteins.	Extend the time of the fixing step.

Problem	Cause	Solution
Low protein staining sensitivity or no staining (cont.)	Development terminated prematurely.	Increase the development time. Protein stain may be developed up to 30 min with a minimal increase in background.
Negative protein staining (light bands or spots on a darker background)	Protein sample overloaded.	Reduce the amount of protein loaded.

Section 6
Product Information

Catalog #	Description
161-0480	Dodeca Silver Stain Kit, large (10 L), solutions provided in volumes optimized for the large Dodeca stainer
161-0481	Dodeca Silver Stain Kit, small (7 L), solutions provided in volumes optimized for the small Dodeca stainer
161-0472	Image Developer Concentrate, 2.5 ml
161-0473	Sensitizer Concentrate, 700 ml
161-0474	Sensitizer Concentrate, 1 L
161-0475	Background Reducer Concentrate, 75 ml
161-0476	Background Reducer Concentrate, 125 ml
161-0477	Silver Reagent Concentrate, 140 ml
161-0478	Silver Reagent Concentrate, 200 ml
161-0479	Development Buffer Concentrate, 700 ml
161-0482	Development Buffer Concentrate, 1 L
165-3400	Dodeca Stainer, large
165-3401	Dodeca Stainer, small
165-3403	Dodeca Stainer and Dodeca Silver Stain Kit, large
165-3404	Dodeca Stainer and Dodeca Silver Stain Kit, small
345-9920	Criterion Staining/Blotting Trays, 10 trays

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161-0481

Bio-Rad Laboratories, Inc.

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