



**Zinc Stain & Destain Kit
for Electrophoresis
Instruction Manual**

**Catalog Number
161-0440**

BIO-RAD

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Section 1

Introduction

1.1 Introduction and Principle

The Zinc Stain & Destain Kit for electrophoresis provides rapid, reversible visualization of protein bands on Laemmli SDS-PAGE gels. The Zinc Stain & Destain Kit is modified from the staining method developed by Fernandez-Patron *et al.*¹ It enables the proteins to be quantitatively eluted from the gel and subsequently used for blotting, amino acid sequencing, or other analyses.

The Zinc Stain & Destain Kit offers several advantages over the traditional methods of Coomassie Brilliant Blue (CBB) or silver staining. The results are easy to read, the procedure is fast and easy to use, and the stain is flexible in enabling further analysis of the proteins after the stain.

This method is a negative stain which produces an opaque white background in the gel, while the proteins are not stained and remain as a clear spot in the gel. With a black background behind the gel, such as the reverse side of the protocol card included with kit, the protein bands are easily identified against the white gel. The gel can be photographed for permanent storage.

The procedures for this kit are fast and easy. To stain a typical 0.75 mm gel, incubate the gel in the Imidazole (Solution A) for 10 minutes, then in the Zinc Sulfate (Solution B) for 30 seconds. The solutions are all

supplied as 10x concentrates which are easily diluted to 1x and are ready to use for staining and destaining. Since the stain is a negative stain and does not bind to the proteins themselves, after the gel is destained the proteins can be eluted or transferred to a blot membrane for further analysis. The destaining procedure requires only 15 minutes in the Destain Solution.

1.2 Product Description

The Zinc Stain & Destain Kit, catalog number 161-0440, contains:

- 125 ml Imidazole, Solution A, 10x
- 125 ml Zinc Sulfate, Solution B, 10x
- 125 ml Zinc Destain, 10x
- Laminated instruction card
- Complete instruction manual

The Zinc Stain solutions can be ordered separately. The catalog number for the Imidazole (Solution A) is 161-0441, and the Zinc Sulfate (Solution B) is 161-0442. These two solutions must be used together for staining results.

The Zinc Staining Procedure is recommended for mini-gels, especially those 1.0 mm thick or less. The quantities included with this kit can be used to stain 25 mini-gels using 50 ml of both Imidazole Solution and Zinc Solution for each gel, or 2 full-size gels using about 600 ml of both solutions for each gel.

1.3 Materials Required But Not Supplied

- Graduated cylinders
- Distilled, deionized water

Staining and rinsing containers. *To optimize the number of gels stained per kit, small staining containers, such as the lid of a pipet tip box, should be used for staining mini-gels.*

1.4 Safety Considerations

Eye protection and gloves should be worn while handling this product.

1.5 Disposal Methods

Laws governing disposal of laboratory chemicals vary by region. Check local laws for disposal of Zinc Stain.

Section 2 Instructions

2.1 Zinc Stain Protocol

1. In separate containers, dilute one part Imidazole (Solution A) and one part Zinc Sulfate (Solution B) with nine parts water to make the working reagents. Mix the solutions thoroughly.
2. Remove the gel from the electrophoresis cell.

3. Place the gel in a container with the Imidazole Solution. Place the container on an orbital mixing platform set to a low mix speed and incubate at room temperature for 10 minutes.
4. Transfer the gel to the diluted Zinc Sulfate Solution. Completely immerse the gel to insure even staining. (A Mini-PROTEAN® II gel can be completely immersed in as little as 50 ml of solution, provided the staining vessel is small.) Set on the rocking platform as in Step 3. Allow 30 seconds for the gel to develop.
5. Transfer the gel to a container filled with DDI water and rinse for 3 minutes. Discard this water wash and replace it with fresh DDI water. The gel can be stored for weeks in water.
6. To visualize the protein bands, place the gel against a black background. (The reverse side of the laminated protocol card included with this kit is black for this purpose.) The protein bands will be visible as dark bands against an opaque white background. The gel can be photographed to provide a permanent record of the separation. For best results, illuminate the gel at an oblique angle with four high-intensity 150-W flood light bulbs. The optimum angle of exposure is empirical.
7. Alternatively, because the Zinc-Imidazole complex fluoresces under UV light, the gel can be visualized using fluorescent imaging systems. We have successfully imaged Zinc stained gels using the Gel Doc™ 1000 imaging system, and the Glyco Doc imaging system.

2.2 Zinc Destain Protocol

1. The Zinc Destain Solution is 1x Tris/Glycine. If additional Destain Solution is required, use lab Tris/Glycine, or reorder catalog number 161-0734, 10x Tris/Glycine, 1 L.
2. Consult Table 1 for Zinc Destain dilutions and wash times. Note that 1.5 mm gels are not recommended for this procedure; the rinse times are long, thereby increasing the likelihood of band spreading.
3. Completely immerse the stained gel in a 1:10 dilution of Zinc Destain, and gently agitate for 5 minutes. (Mini-gels require 100 ml of destaining solution for each step. Full size gels require considerably more destain solution. Volumes must be determined empirically.)
4. Replace this rinse with fresh, diluted Zinc Destain and gently agitate again for the recommended time. Repeat with 1:20 Zinc Destain, if recommended in Table 1.
5. The gel is now ready for Coomassie® staining, silver staining, blotting, or other analyses.

Table 1. Zinc Destaining Wash Times

The following destaining wash times are recommended. The destaining procedure has been optimized for mini-gels. Slight variations in the protocol may be necessary to optimize results with full-size gels.

<u>Gel Thickness</u>	<u>Destain #1</u>	<u>Destain #2</u>	<u>Destain #3</u>
0.5 mm	5 minutes	3 minutes	—
0.75 mm	5 minutes	5 minutes	5 minutes
1.0 mm	5 minutes	10 minutes	5 minutes

The Zinc Destain solution is diluted 1:10 with water. If necessary, Destain #3 can be further diluted to 1:20, which will help to conserve reagents.

3.2 References

1. Fernandez-Patron, C., L. Castellanos-Serra and P. Rodriguez, *BioTechniques*, **12**, 564 (1992).

Section 3 Additional Information

3.1 Commonly Asked Questions

1. Can I blot the zinc stained gel? Yes, after destaining. The Zinc Stain *reversibly* fixes the proteins in the gel.
2. Am I able to stain the gel later with either CBB or silver? Yes. In fact, only with a silver stain must you first destain the gel. The low pH of the Coomassie staining solution acts as a destainer.
3. Can the Zinc Stain be reused? For optimum results, discard the solutions after each use. Results show that it might work a second time, but with each use the staining is less effective.
4. Can the Zinc Destain be reused? No, this is not recommended.
5. Can I stain DNA in a TBE gel? No, bands do not develop when zinc staining a DNA gel.

6. Can a native gel be stained with Zinc Stain? Yes, but you must add 0.1% SDS to the diluted Imidazole Solution and incubate the gel for 15–20 minutes.
7. How many gels can be stained per kit? This depends on the size of the staining container. A mini-gel can be stained in as little as 50 ml of Zinc Stain, providing that the entire gel is submerged during the staining step. The limiting factor is the volume of the stain, not the concentration of Zinc Stain.

Section 4 Product Information

4.1 Zinc Stain

Catalog Number	Description
161-0440	Zinc Stain & Destain Kit for Electrophoresis , includes 125 ml 10x Imidazole, 125 ml 10x Zinc Sulfate, 125 ml 10x Zinc Destain, laminated protocol, and instruction manual
161-0441	Imidazole, Zinc Stain Solution A , 10x, 125 ml
161-0442	Zinc Sulfate, Zinc Stain Solution B , 10x, 125 ml
161-0734	10x Tris/Glycine (Zinc destain) , 10x, 1 L

4.2 Related Materials

Catalog Number	Description
161-0732	10x Tris/Glycine/SDS , 1 L
161-0755	10x Tris/Glycine/SDS , 6 x 1 L
161-0757	10x Tris/Glycine , 6 x 1 L

For information on the vertical slab cell systems, PROTEAN® II xi cells, Mini-PROTEAN II cells, Precast Ready Gels, and the Ready Gel Cell, consult the catalog or contact your local Bio-Rad representative.

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