



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

**Catalog Number  
161-0470**

**BIO-RAD**



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# Section 1

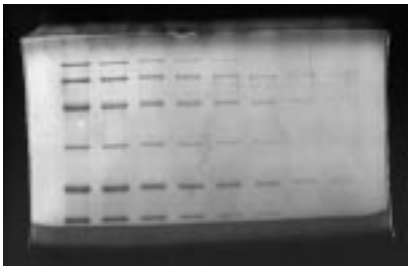
## Introduction

### 1.1 Introduction and Principle

The Copper Stain & Destain Kit for Electrophoresis provides a rapid, reversible visualization of protein bands on a Laemmli SDS-PAGE gel. The Copper Stain & Destain Kit is modified from a new staining method developed by Lee *et al.*<sup>1</sup> It enables the proteins to be quantitatively eluted from the gel<sup>2</sup> and subsequently used for blotting,<sup>3</sup> as antigens in ELISA,<sup>4</sup> for amino acid sequencing,<sup>5</sup> or for other analyses.

The Copper Stain & Destain Kit offers several advantages over the traditional methods of Coomassie Brilliant Blue (CBB) or silver staining. First, the Copper Stain & Destain procedures save time. For a typical 0.75 mm gel, a brief water rinse is followed by a 5 minute incubation with Copper Stain. The gel is destained within 15 minutes, so this entire process can be completed in less than 30 minutes. Second, the Copper Stain & Destain procedures are convenient. Only a simple dilution of the reagents is required. In addition, the proteins are reversibly fixed within the gel, so that the polyacrylamide gel can be destained and the proteins eluted for further study.

The Copper Stain, a negative stain, produces a blue-green opaque background. The protein bands are visualized against a black surface, and can be photographed to provide a permanent record, as shown in Figure 1.



**Fig. 1. Mini-PROTEAN® II 12% ready gel stained with Copper Stain.** Bio-Rad's LMW SDS-PAGE standards were diluted and electrophoresed at 200 V for 30 minutes. Standards were serially diluted from 500 ng of each protein per well to 3.91 ng of each protein per well. The gel was subsequently stained with Copper Stain.

The gel can be stored in water for several weeks without loss of resolution of the protein bands.

## 1.2 Product Description

The Copper Stain & Destain Kit, catalog number 161-0470, contains:

- 125 ml Copper Stain, 10x
- 125 ml Copper Destain, 10x
- Laminated instruction card
- Complete instruction manual

The Copper Stain can be ordered separately, as catalog number 161-0471. The Copper Destain, a 10x Tris/Glycine buffer, can be reordered in a 1 liter bottle as catalog number 161-0734.

The Copper Staining procedure is recommended for mini-gels, especially those of 1.0 mm thickness or less. The quantities included with this kit can be used to stain 25 mini-gels, using 50 ml Copper Stain for each gel, or 2 full-size gels, using about 600 ml stain 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 Copper Stain.

## Section 2

### Instructions

#### 2.1 Copper Stain Protocol

1. Dilute one part Copper Stain with nine parts water to make the working reagent. Alternatively, the entire contents of the bottle can be emptied into a 1 liter bottle containing 900 ml of DDI water. Mix the solution thoroughly.
2. Remove the gel from the electrophoresis cell.
3. Place the gel in a container with distilled, deionized water. See Table 1 for recommended rinse time. Place the container on an orbital mixing platform and set to a low mix speed.
4. Transfer the gel to diluted Copper Stain. *Completely* immerse the gel to insure even staining. (A Mini-PROTEAN II gel can be completely immersed in as little as 50 ml of Copper Stain, provided the staining vessel is small.) Allow 5 minutes 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 instruction card is colored black for this purpose.) The protein bands will be visible as dark bands against an opaque blue-green background. The gel can

now 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.<sup>2</sup>

#### Table 1. Copper Staining Wash Times

The following are recommended water rinse times done prior to copper staining. The optimum time is noted with an asterisk, although any time within the range can be used.

<u>Gel Thickness</u>	<u>Rinse Time</u>
0.5 mm	15*-30 seconds
0.75 mm	30*-60 seconds
1.0 mm	3*-5 minutes

#### 2.2 Copper Destain Protocol

1. Consult Table 2 for Copper 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.
2. Completely immerse the stained gel in a 1:10 dilution of Copper 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.)

3. Replace this rinse with fresh, diluted Copper Destain and gently agitate again for the recommended time. Repeat with 1:20 Copper Destain, if recommended in Table 2.
4. The gel is now ready for Coomassie staining, silver staining, blotting, or other analyses.

**Table 2. Copper Destaining Wash Times**

The following are recommended destaining wash times. 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 Copper 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.

## Section 3 Additional Information

### 3.1 Commonly Asked Questions

1. Can I blot the copper stained gel? Yes, after destaining. The Copper 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 Copper Stain be reused? For optimum results, discard the solution after each use. Results show that it might work a second time, but with each use the staining is less effective.
4. Can the Copper Destain be reused? No, this is not recommended.
5. Can I stain DNA in a TBE gel? Bands do not develop when copper staining a DNA gel.

6. Can a native gel be stained with Copper Stain? Yes, but the staining pattern is reversed. In this case, the protein bands stain an opaque blue-green while the background remains clear. However, the effect of staining native proteins has not been examined.
7. How many gels can be stained per kit? This is dependent on the size of the staining container. A mini-gel can be stained in as little as 50 ml of Copper 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 Copper Stain.

### 3.2 References

1. Lee, C., Levin, A. and Branton, D., *Anal. Biochem.*, **166**, 308 (1987).
2. Dzandu, J. K., Johnson, J. F. and Wise, G. E., *Anal. Biochem.*, **174**, 157 (1988).
3. Wang, D., Dzandu, J. K., Hussain, M. and Johnson, R. M., *Anal. Biochem.*, **180**, 311 (1989).
4. Vos, G. J. and Gardiner, P.R., *Parasitology*, **100**, 93 (1990).
5. Vanfleteren, J. R. and Peeters, K., *J. Biochem. Biophys. Methods*, **20**, 227 (1990).

## Section 4 Product Information

### 4.1 Copper Stain

- 161-0470 **Copper Stain & Destain Kit for Electrophoresis**, includes 125 ml 10x Copper Stain, 125 ml 10x Copper Destain, laminated protocol, and instruction manual
- 161-0471 **Copper Stain**, 10x, 125 ml
- 161-0734 **10x Tris/Glycine (Copper destain)**, 10x, 1L

### 4.2 Related Materials

- 161-0900 **Mini-PROTEAN II Ready Gels**, 7.5% single percentage gels, 0.375 M Tris-HCl, 10
- 161-0901 **Mini-PROTEAN II Ready Gels**, 12% single percentage gels, 0.375 M Tris-HCl, 10
- 161-0902 **Mini-PROTEAN II Ready Gels**, 4 - 15% gradient gels, 0.375 M Tris-HCl, 10
- 161-0903 **Mini-PROTEAN II Ready Gels**, 4 - 20% gradient gels, 0.375 M Tris-HCl, 10
- 161-0732 **10x Tris/Glycine/SDS**, 1L
- 161-0755 **10x Tris/Glycine/SDS**, 6 x 1L
- 161-0757 **10x Tris/Glycine**, 6 x 1L

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

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