

USER GUIDE

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by *life* technologies™

# SimplyBlue™ SafeStain

For fast, sensitive, and safe Coomassie G-250  
staining of proteins

Catalog numbers LC6060, LC6065

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**For Research Use Only. Not intended for any animal or human  
therapeutic or diagnostic use.**

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# Kit Contents and Storage

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## Contents and Storage

SimplyBlue™ SafeStain is available in 2 sizes:

- 1 L (Cat. no. LC6060)

Sufficient stain is provided in 1 L to stain 50 mini-gels

- 3.5 L with a pump dispenser (Cat. no. LC6065)

Upon receipt, store SimplyBlue™ SafeStain at room temperature, 15°C to 30°C. Be sure to keep the bottle capped when not in use. The stain is stable for 6 months when stored at room temperature.

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## Product Use

**For research use only.** Not intended for any animal or human therapeutic or diagnostic use.

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# Introduction

## Overview

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### **Product Description**

SimplyBlue™ SafeStain is a ready-to-use, proprietary Coomassie G-250 stain that is specially formulated for fast, sensitive detection and safe, non-hazardous disposal. This stain does not require the use of methanol or acetic acid which must be disposed of as hazardous waste.

SimplyBlue™ SafeStain has been tested for environmental hazards (see page 12).

Proteins stained using the SimplyBlue™ SafeStain are compatible with mass spectrometry (MS) analysis.

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# Methods

## Using SimplyBlue™ SafeStain

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### Important

An alcohol/acetic acid fixing step prior to staining with SimplyBlue™ SafeStain is **NOT** required or recommended.

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### Basic Protocol

For general use with 1.0-mm and 1.5-mm Tris-Glycine gels, 1.0-mm NuPAGE® Novex® Gels, and 1.0 mm Tricine mini-gels (8 × 8 cm). To achieve maximum sensitivity, refer to the protocol on page 7.

After electrophoresis follow the instructions below. Be sure the gel moves freely in water or stain to facilitate diffusion during all steps. **Note:** For large format gels, see page 4 for recommended volumes of water and stain.

1. **Rinse** the mini-gel 3 times for 5 minutes with 100 mL deionized water to remove SDS and buffer salts, which interfere with binding of the dye to the protein. Discard each rinse.
2. **Stain** the mini-gel with enough SimplyBlue™ SafeStain (~20 mL) to cover the gel. Stain for 1 hour at room temperature with gentle shaking. Bands begin to develop within minutes. After incubation, discard the stain. The stain cannot be re-used.

**Note:** The gel can be stained for up to 3 hours, but after 3 hours, sensitivity decreases. If you need to leave the gel overnight in the stain, add 2 mL of 20% NaCl (w/v) in water for every 20 mL of stain. This procedure will not affect sensitivity.

3. **Wash** the mini-gel with 100 mL of water for 1–3 hours. The gel can be left in the water for several days without loss of sensitivity. There is a small amount of dye in the water that is in equilibrium with the dye bound to the protein, so proteins remain blue.
4. To obtain the clearest background for photography, perform a second 1 hour wash with 100 mL water.

**Note: Sensitivity will now decrease if the gel is allowed to stay in the water for more than 1 day.** Decrease in the amount of free dye in water favors dissociation of the dye from the protein. If you need to store the gel in water for a few days, add 20 mL of 20% NaCl.

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# Using SimplyBlue™ SafeStain, Continued

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## Maximum Sensitivity

To achieve maximum sensitivity, follow the procedure below. Adding salt results in significant improvement in sensitivity. Values are given for 1.0 mm gels. For 1.5 mm mini-gels, use the value in parentheses.

**Important:** Sensitivity is primarily a function of the dye-protein interaction, which is unique for each protein.

1. Prepare a 20% NaCl (w/v) solution in deionized water. You need 20 (30) mL per gel.
  2. Perform steps 1 and 2, page 6.
  3. After staining the gel, wash the gel with 100 (150) mL of water for 1 hour.
  4. Add 20 (30) mL 20% NaCl to the water in step 3 of this procedure and continue to wash for an additional 2 hours or overnight (if desired).
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## Large Format Gels

For large format gels, increase the volume of water for each rinse and wash step, and the volume of stain to achieve optimal results. Refer to the following table to determine the volume of water or stain required and follow the **Basic Protocol** for staining.

Gel Size	Water	Stain
15 × 15 cm, 1 mm	300 mL	60 mL
15 × 15 cm, 1.5 mm	500 mL	100 mL
20 × 20 cm, 1 mm	600 mL	120 mL

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# Using SimplyBlue™ SafeStain, Continued

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## Other Applications

Information for staining IEF Gels, 1.5 mm NuPAGE® Novex® Gels, and polyvinylidene difluoride (PVDF) membranes is provided in the following table. Follow the **Basic Protocol** (page 2) using the indicated changes.

**Note:** Staining nitrocellulose and wet PVDF membranes results in high background and is **not** recommended.

Stain the Zymogram gel with SimplyBlue™ SafeStain after renaturing and developing the gel for enzyme activity.

Gel or Membrane	Fix	Rinse	Stain	Wash
IEF Gels	100 mL 12% TCA for 15 minutes	Basic Protocol, step 1	100 mL stain for 1 hour	Basic Protocol, step 3
1.5 mm NuPAGE® Gels	N/A	150 mL water 2 × 5 minutes 1 × 10 minutes	Basic Protocol, step 2	Basic Protocol, step 3
Dry PVDF Membrane	N/A	N/A	10–20 mL stain for 1–2 minutes*	10–20 mL water 3 × 1 minute
Zymogram Gels	N/A	Basic Protocol, step 1	100 mL stain for 1 hour	Basic Protocol, step 3

\*Incubating dry PVDF membranes in stain for >2 minutes results in high background.

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# Using SimplyBlue™ SafeStain, Continued

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## Microwave Procedure

The microwave procedure is fast, takes just 12 minutes, and yields results with sensitivity as low as 5 ng with an additional incubation with a salt solution. The procedure is for 1.0 mm mini-gels. For 1.5 mm mini-gels, use the values in parentheses.

**Caution:** Use caution while using the stain in a microwave oven. Do not overheat the staining solutions.

1. After electrophoresis, place the gel in 100 mL of ultrapure water in a loosely covered container and microwave on High (950 to 1,100 watts) for 1 minute until the solution almost boils.
  2. Shake the gel on an orbital shaker for 1 minute (2 minutes). Discard the water.
  3. Repeat steps 1 and 2 of this procedure 2 more times.
  4. After the last wash, add 20 mL (30 mL) of SimplyBlue™ SafeStain and microwave on High for 45 seconds to 1 minute (1.5 minutes) until the solution almost boils.
  5. Shake the gel on an orbital shaker for 5 minutes (10 minutes). **Detection limit: 20 ng BSA.**
  6. Wash the gel in 100 mL of ultrapure water for 10 minutes on a shaker. **Detection limit: 10 ng BSA.**
  7. Add 20 mL of 20% NaCl for at least 5 minutes. **Detection limit: 5 ng BSA.** The gel can be stored for several weeks in the salt solution.
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## Dry the Gel

Incubating any Coomassie-stained gel in an alcohol solution will eventually result in destaining of the bands. If you use the Gel-Dry™ Drying Solution from Life Technologies, incubate the gel in the solution for 5 minutes or less.

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## Destain Protein Bands for MS Analysis

Use the following general guidelines for destaining the protein bands prior to MS analysis. Contact your MS facility or the protein core facility for detailed protocols.

- Excise the protein band of interest from the gel using a clean scalpel and destain with 10–30% ethanol or 20–30% acetonitrile for 10–15 minutes or until clear.
  - Rinse the gel piece in ultrapure water and proceed for MS analysis.
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# Appendix

## Technical Support

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### Obtaining support

For the latest services and support information for all locations, go to [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
  - Search through frequently asked questions (FAQs)
  - Submit a question directly to Technical Support ([techsupport@lifetech.com](mailto:techsupport@lifetech.com))
  - Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
  - Obtain information about customer training
  - Download software updates and patches
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### Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

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### Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) and search for the Certificate of Analysis by product lot number, which is printed on the box.

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### Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.lifetechnologies.com/termsandconditions](http://www.lifetechnologies.com/termsandconditions). If you have any questions, please contact Life Technologies at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

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# Purchaser Notification

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Research  
Use Only**

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial services of any kind, including, without limitation, reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact [outlicensing@lifetech.com](mailto:outlicensing@lifetech.com) or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

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# Safety Information

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**Environmental Testing** SimplyBlue™ SafeStain has been tested by an independent and certified laboratory for environmental hazards. The results are summarized in the following table:

Analysis	Method	Results
Ignitability	EPA 1010	Non-flammable (>144°F)
Corrosivity	EPA 150.1	Non-corrosive (pH ≥2.1)
Corrosivity (by Corrositex®)	DOT-E 10904	Non-corrosive
Reactivity (Cyanide and Sulfide)	EPA 9010B/9014 EPA 9030A	No Reactivity Detected
Aquatic Toxicity (fathead minnow; definitive, CAC, Title 22)	CA Fish and Game	Non-toxic

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## Disposal Information

For disposal requirements in your area, consult your local environmental safety officer or regional waste water authority to determine pH levels that can be discharged into the sink.

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## SDS Requests

Safety Data Sheets are available at [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

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# Notes

# Notes





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