BacLight™ Bacterial Stains

B-35000 *Bac*Light™ Green bacterial stain
B-35001 *Bac*Light™ Red bacterial stain

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Desiccate
- Protect from light

Abs/Em: See Table 1

Solvent for Stock: DMSO

Introduction

The *Bac*Light™ Green and *Bac*Light™ Red bacterial stains are fluorescent, non–nucleic acid labeling reagents for detecting and monitoring bacteria (Table 1). Bacteria stained with the *Bac*Light Green and *Bac*Light Red bacterial stains exhibit bright green and red fluorescence, respectively, and can be resolved using the appropriate flow cytometric channels. Although these dyes were specifically chosen for flow cytometry applications, bacteria stained with these *Bac*Light reagents can also be visualized by fluorescence microscopy with only minor, if any, adjustments in the staining concentrations. Furthermore, the *Bac*Light bacterial stains are compatible with formaldehyde or alcohol fixation methods.

These *Bac*Light bacterial stains efficiently label a variety of different bacteria species. The intensity of the staining appears to depend on several factors, including gram character, outer membrane composition and overall membrane integrity. In the species tested, gram-positive bacteria generally exhibited brighter fluorescence than gram-negative bacteria, and cells with compromised membranes accumulated more dye than intact cells (Figure 1). Because the stains are compatible with various labeling schemes, the *Bac*Light bacterial stains can also be combined with other fluorescent cell probes — including nucleic acid stains, lectin conjugates and antibody conjugates — for multiparameter analyses.

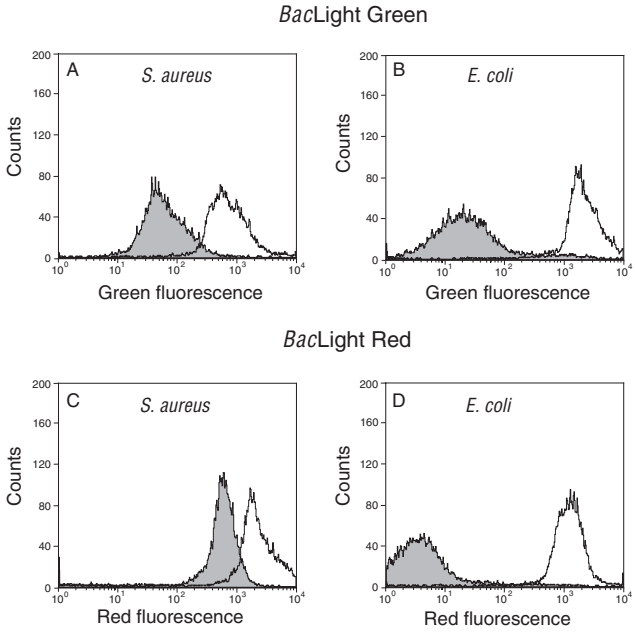

Figure 1. Flow cytometry histograms from a BD FACScan (BD Biosciences, San Jose, CA) showing fluorescence of live and dead gram-positive and gram-negative bacteria stained with the *Bac*Light bacterial stains. Untreated and alcohol-treated gram-positive (*Staphylococcus aureus*, (A and C)) and gram-negative (*Escherichia coli*, (B and D)) bacteria were each stained separately with 100 nM of either the *Bac*Light Green (A and B) or the *Bac*Light Red (C and D) bacterial stains in 0.85% NaCl buffer and then analyzed by flow cytometry. The histograms for the untreated (shaded histogram curve) and alcohol-treated (unshaded histogram curve) bacteria samples were overlaid for each species and *Bac*Light bacterial stain.

Storage and Handling

The *Bac*Light bacterial stains are provided in specially packaged sets of 20 separate vials, each containing 50 μg of solid for reconstitution as required. Upon receipt, the vials of dye should be stored desiccated at ≤–20°C, and protected from light.

Note: Before opening a vial containing a solid or DMSO solution, allow the product to warm to room temperature. To avoid multiple freeze-thaw cycles, divide any unused DMSO stock solution into single-use aliquots before storing desiccated at ≤–20°C. When stored properly, the *Bac*Light bacterial stains are stable for at least six months.

Table 1. Spectral characteristics of *Bac*Light bacterial stains.

Catalog #	*Bac*Light Bacterial Stain	Abs *	Em *	Recommended Optical Filters
B-35000	*Bac*Light Green	480	516	Filters suitable for fluorescein
B-35001	*Bac*Light Red	581	644	Filters suitable for tetramethylrhodamine or Texas Red dye.

* Fluorescence absorption and emission maxima, in nm, determined in methanol; values may vary somewhat in cellular environments.

Caution: No data are available addressing the mutagenicity or toxicity of these reagents. The DMSO stock solutions should be handled with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues. Please dispose of the stains in compliance with all pertaining local regulations.

Experimental Guidelines

The following protocols are provided as examples to guide researchers in the development of their own staining procedures. Researchers at Molecular Probes have used these procedures and found them to be simple and reliable for both gram-positive and gram-negative bacteria, including *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus* and *Staphylococcus aureus*. To thoroughly optimize the staining of a particular bacteria population, experimenting with a range of concentrations of *Bac*Light bacterial stains and then evaluating the staining pattern using the desired instrumentation is recommended.

Reagent Preparation

1.1 Prepare a stock solution of the *Bac*Light bacterial stain. Allow a vial of the *Bac*Light bacterial stain to warm to room temperature before opening. Prepare a 1 mM stock solution of dye by dissolving the vial contents in DMSO — 74 µL for the *Bac*Light Green bacterial stain or 69 µL for the *Bac*Light Red bacterial stain.

1.2 Prepare a working solution of the *Bac*Light bacterial stain. Prepare a 100 µM working solution of the *Bac*Light bacterial stain by adding 2 µL of the 1 mM stock solution prepared in step 1.1 to 18 µL of DMSO in a microcentrifuge tube and mix well. Note: For most experimental samples, a 100 µM working concentration for a 1:1000 final dilution is generally adequate, however, the optimal staining concentration may depend on the specific application and species. Adjust the working solution concentration and volume accordingly to achieve the desired final staining concentration.

Staining Protocol

2.1 Prepare the bacteria sample. Grow a bacteria culture or collect a bacteria sample in appropriate medium or buffer. If comparisons among samples are being performed, normalize the cell number in each sample. Perform dilutions in phosphate-buffered saline (PBS), 0.85% NaCl or other appropriate buffer.

2.2 Add *Bac*Light bacterial stain to the bacteria sample. Stain bacteria by adding 1 µL of the working dye solution prepared in step 1.2 to 1 mL of the bacteria sample prepared in step 2.1.

2.3 Incubate the bacteria sample with the stain. Incubate the sample for 15 minutes at room temperature.

2.4 (Optional) Wash the bacteria sample. Because the *Bac*Light bacterial stain is nonfluorescent when not associated with cells, wash and centrifugation steps are not necessary. However, the cells can be washed with buffer to remove excess dye if required for subsequent labeling steps or analysis methods.

2.5 (Optional) Fix the bacteria sample. If fixation is desired, centrifuge the bacteria sample and resuspend the pellet in 1–4% formaldehyde or 70% alcohol. Alternatively, prepare a 2X formaldehyde solution and then mix equal volumes of the bacteria sample in the staining solution and the concentrated formaldehyde solution. Incubate the bacteria in the formaldehyde solution for 10–15 minutes at room temperature. Analyze the fixed bacteria sample directly by flow cytometry or fluorescence microscopy. Alternatively, centrifuge the sample, resuspend the pellet in PBS, 0.85% NaCl or other appropriate buffer, and then proceed with the analysis.

Analyzing the Stained Bacteria by Flow Cytometry

Instrument capabilities may vary considerably, but the techniques and parameters established here should aid considerably in setting up similar analyses in the majority of flow cytometers now in use, both in research and clinical environments. Stained bacteria can be assayed in a flow cytometer equipped with a laser emitting at 488 nm. Fluorescence is collected in the green- and red-fluorescence channels for the *Bac*Light Green and *Bac*Light Red bacterial stains, respectively. Filters used for detecting fluorescein are generally suitable for analyzing bacteria stained with the *Bac*Light Green bacterial stain, whereas filters suitable for detecting tetramethylrhodamine or the Texas Red dye are generally suitable for analyzing bacteria stained with the *Bac*Light Red bacterial stain. The forward scatter, side scatter and fluorescence should be collected with logarithmic signal amplification.

3.1 Adjust the flow cytometer. Instrument adjustments are especially critical for detecting relatively small particles such as bacteria. To avoid contamination of the data by electronic noise, the following procedure for instrument setup is recommended. The appropriate controls — including unstained bacteria, known gram-positive and gram-negative bacteria or untreated and treated bacteria — can be used to locate bacteria populations and determine optimal compensation settings. Using an unstained bacteria sample, acquire signals with the amplifiers set to logarithmic amplification. Use the side scatter as the parameter for setting the acquisition trigger. Set the amplification of the signals from forward and side scatter such that the bacteria are in the middle of the data space. Adjust the trigger level (also named "threshold level" on some instruments) to minimize electronic noise appearing on the monitor. Gate on the bacteria in forward versus side scatter, and set the amplification of the green- or red-fluorescence channel such that the signals from the unstained bacteria appear in the lowest quadrant of the logarithmic scale. Briefly run selected stained bacteria samples to ensure that the fluorescence

signal is on scale. If necessary, adjust the fluorescence amplification to bring the stained samples on scale.

3.2 Analyze a sample by flow cytometry. After adjusting the flow cytometer as described in step 3.1, apply an experimental sample containing stained bacteria. Gate on the bacteria in forward versus side scatter, and collect stained populations in the appropriate fluorescence channel.

Visualizing the Stained Bacteria by Fluorescence Microscopy

Although the *Bac*Light bacterial stains were selected based on their utility for staining bacteria analyzed by flow cytometry, the reagent preparation and staining protocol described above may also be useful for visualizing bacteria by fluorescence microscopy. However, further optimization the staining conditions to enhance differences in gram character, membrane permeability or other attributes may be necessary.

4.1 Prepare a sample for fluorescence microscopy. Transfer 5-10 µl of the stained bacteria to a clean, glass microscope slide, and place a clean coverslip over the sample. If appropriate, seal the edges of the coverslip.

4.2 Visualize a sample by fluorescence microscopy. The fluorescence from the *Bac*Light Green and *Bac*Light Red bacterial stains may be viewed separately using bandpass filter sets appropriate for fluorescein and tetramethylrhodamine (or the Texas Red dye), respectively (Table 1). Alternatively, a mixed population of bacteria stained with both of these bacterial stains may be viewed simultaneously using a longpass filter set appropriate for fluorescein.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
B-35000	*Bac*Light™ Green bacterial stain *special packaging*	20 x 50 µg
B-35001	*Bac*Light™ Red bacterial stain *special packaging*	20 x 50 µg

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information.

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