**BacLight™ Bacterial Stains**

**B-35000**  BacLight™ Green bacterial stain  
**B-35001**  BacLight™ Red bacterial stain  

**Quick Facts**

**Storage upon receipt:**
- ≤–20°C  
- Desiccate  
- Protect from light  

**Abs/Em:** See Table 1  

**Solvent for Stock:** DMSO  

**Introduction**

The BacLight™ Green and BacLight™ Red bacterial stains are fluorescent, non–nucleic acid labeling reagents for detecting and monitoring bacteria (Table 1). Bacteria stained with the BacLight Green and BacLight 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 BacLight reagents can also be visualized by fluorescence microscopy with only minor, if any, adjustments in the staining concentrations. Furthermore, the BacLight bacterial stains are compatible with formaldehyde or alcohol fixation methods.

These BacLight 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 BacLight bacterial stains can also be combined with other fluorescent cell probes — including nucleic acid stains, lectin conjugates and antibody conjugates — for multiparameter analyses.

**Storage and Handling**

The BacLight 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 BacLight bacterial stains are stable for at least six months.
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 BacLight 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 BacLight 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.

Experimentation 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 BacLight bacterial stains and then evaluating the staining pattern using the desired instrumentation is recommended.

Reagent Preparation

1.1 Prepare a stock solution of the BacLight bacterial stain. Allow a vial of the BacLight 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 BacLight Green bacterial stain or 69 µL for the BacLight Red bacterial stain.

1.2 Prepare a working solution of the BacLight bacterial stain. Prepare a 100 µM working solution of the BacLight 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.

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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 BacLight Green and BacLight Red bacterial stains, respectively. Filters used for detecting fluorescein are generally suitable for analyzing bacteria stained with the BacLight Green bacterial stain, whereas filters suitable for detecting tetramethylrhodamine or the Texas Red dye are generally suitable for analyzing bacteria stained with the BacLight 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 BacLight 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 BacLight Green and BacLight 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.

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