Bac*Light™ RedoxSensor™ Green Vitality Kit*

Quick Facts

Storage upon receipt:

- ≤-20°C
- Desiccate
- Protect from light

Ex/Em:

- 490/520 nm (RedoxSensor™ Green reagent, reduced form)
- 490/635 nm (propidium iodide)

Introduction

Bacterial oxidation-reduction activity is an informative parameter for measuring cell vitality. Bacterial oxidases and reductases engage in important functions involving the electron transport chain, catabolic and anabolic pathways, and xenobiotic compound metabolism.[1] RedoxSensor™ Green reagent is an indicator of bacterial reductase activity; this reductase activity is, in turn, a reliable marker for changes in electron transport chain function and for changes in vitality that occur following antibiotic treatment. RedoxSensor™ Green reagent penetrates both gram-positive and gram-negative bacteria, although differences in signal intensity may be observed based upon cell wall characteristics. Following reduction, the RedoxSensor™ Green reagent will produce a stable green-fluorescent signal in 10 minutes that is compatible with formaldehyde fixation techniques. Stain intensity of cells stained with RedoxSensor™ Green reagent is altered when cells are treated with reagents that disrupt electron transport, such as sodium azide (Component C), or carbonyl cyanide 3-chlorophenylhydrazone (CCCP, Component D). Species differences in responses may be observed. For instance, sodium azide disrupts the fluorescence response in *Escherichia coli*, and CCCP disrupts fluorescence response in *Staphylococcus aureus* (Figure 1). Propidium iodide is also provided as an indicator of membrane integrity. *Bac*Light™ RedoxSensor™ Green Vitality Kit has been developed for flow cytometric analysis, but may be appropriate for other analysis platforms.

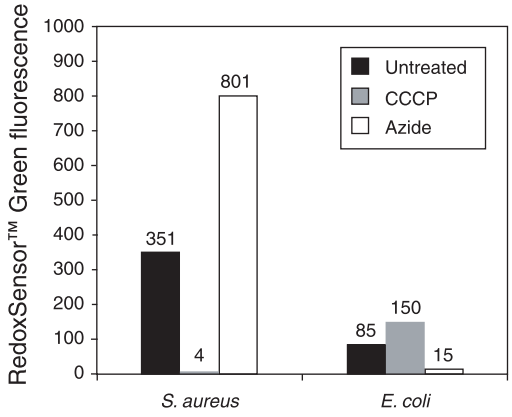

Figure 1. Cultures of *S. aureus* and *E. coli* were either untreated, treated with CCCP (10 µM), or treated with sodium azide (10 mM) and then stained with RedoxSensor™ Green reagent. GeoMean fluorescence values for each sample are shown for each treatment.

Materials

Kit Contents

- RedoxSensor™ Green reagent (Component A), 200 µL of a 1 mM solution in DMSO
- Propidium iodide (Component B), 300 µL of a 20 mM solution in DMSO
- Sodium azide (Component C), 1 mL of a 2 M solution in water
- CCCP (Component D), 400 µL of a 5 mM solution in DMSO

Number of Tests Possible

At the recommended reagent dilutions and volumes, the kit contains sufficient material to perform ~200 tests by flow cytometry.

Storage and Handling

Upon receipt the kit should be stored frozen at ≤–20°C, desiccated and protected from light. Stored properly, the kit components should remain stable for at least one year. Allow the compounds to warm to room temperature before opening the vials. DMSO solutions absorb water, which can cause a loss of dye activity. DMSO stock solutions of RedoxSensor™ Green reagent should be stored desiccated and used within a short period of time.

Caution: Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) and sodium azide are toxic materials. Use safety equipment and practices appropriate for the hazards posed by these chemicals. DMSO stock solutions should be handled with particular care, as DMSO is known to facilitate the entry of organic molecules into tissues. Please dispose of the reagents in compliance with all pertaining local regulations.

Experimental Protocols

General Considerations

The *Bac*Light™ RedoxSensor™ Green Vitality Kit has been tested at Molecular Probes on logarithmically growing cultures of the following bacterial species: *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus cereus*, *B. subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella cholerasuis*. Most gram-positive bacteria stain more efficiently than many gram-negative bacteria. The response of each bacterial system should be investigated and optimized. The experimental protocols below are provided as examples to guide researchers in the development of their own bacterial staining procedures.

Advance Preparation

1.1 RedoxSensor™ Green reagent use guidelines.
For gram-negative organisms, such as *E. coli*, 1 µL of Component A in a tube containing ~10^6 cells in 1 mL is an appropriate staining concentration. For gram-positive organisms, such as *S. aureus*, 1 µL of a 1:10 dilution of Component A in a tube containing ~10^6 cells in 1 mL is an appropriate staining concentration. We recommend that the optimal amount of reagent be determined empirically for each organism. Any dilutions of RedoxSensor™ Green reagent should be made using high-quality, anhydrous DMSO.

Note: Scientists at Molecular Probes have observed that DMSO at concentrations above 1% may be detrimental to bacterial redox activity.

1.2 Sodium azide and CCCP use guidelines.
The electron transport chain uncouplers CCCP (10 µM) and sodium azide (10 mM) were found to be effective negative control reagents for *S. aureus* and *E. coli* responses, respectively (Figure 1). The effectiveness, if any, of either of these two negative control reagents will have to be determined empirically for your strain.

1.3 Preparation of sterile buffers.
Phosphate-buffered saline (PBS) or Hanks' Balanced Salt Solution (HBSS) are excellent staining buffers for both gram-positive and gram-negative bacteria. Additives such as 1 mM EDTA, 0.1% Pluronic® F127 (P3000MP), or 0.01% Tween® 20 may assist dye entry into bacteria, but may also interfere with dye activity or cell vitality. Sterilize staining buffers using 0.2 µm filtration.

Staining the Bacteria

2.1 Grow cultures according to necessary growth conditions. Cells in log-phase with optimal vitality will give the best signal intensities.

2.2 Dilute cells either washed in buffer (PBS or HBSS are suitable) or directly from the culture into prewarmed buffer (37°C) to ~10^6 cells/mL. Aliquot ~1 mL to flow cytometry tubes.

2.3 Add negative control reagent (5 µL Component C: azide or 2 µL Component D: CCCP), depending on the strain used) to appropriate tubes, and allow reagent to react with cells (1–5 minutes). Prepare unstained controls for each treatment to assist with analysis based upon scatter properties or cell morphology.

2.4 Add 1 µL RedoxSensor™ Green reagent (see step 1.1 for dilution guidelines). Vortex gently to mix.

2.5 OPTIONAL: To assess membrane integrity of the cells, add 1 µL of Component B (propidium iodide) per ~1 mL of bacterial culture (~10^6 cells/mL) and vortex gently to mix.

2.6 Incubate in the dark at room temperature or 37°C for 10 minutes. Samples can be analyzed by flow cytometry without washing or can be fixed by adding methanol-free formaldehyde to a final concentration of 1–4% before flow cytometric analysis.

Adjusting the Flow Cytometer and Analyzing the Samples

Note: In the flow cytometer, bacteria are identified solely on the basis of their size and staining. *It is best to inspect each sample by fluorescence microscopy to confirm that the particles detected are indeed bacteria cells* (see *Analyzing the Stained Bacteria by Fluorescence Microscopy*). In addition, with the long data-acquisition times required for very dilute bacteria samples, the number of noise events acquired in the bacterial frame may become significant.

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. The unstained and single-color controls can be used to locate cell populations and determine compensation settings, if necessary.

3.1 Instrument configuration. Stained bacteria can be assayed in a flow cytometer equipped with a 488 nm argon-ion laser. Fluorescence from samples stained with RedoxSensor™ Green reagent may be collected using a 530/30 bandpass filter. Fluorescence from propidium iodide–stained controls may be collected with a ≥ 610 longpass filter.

3.2 Forward and side-scatter amplification settings.

3.2.1 Set amplifiers to logarithmic amplification. Use forward or side scatter as the acquisition trigger parameter.

3.2.2 With an unstained sample of bacteria cells, set the amplification of the signals from forward and side scatter so that the bacteria are in the middle of the data space (Figure 2).

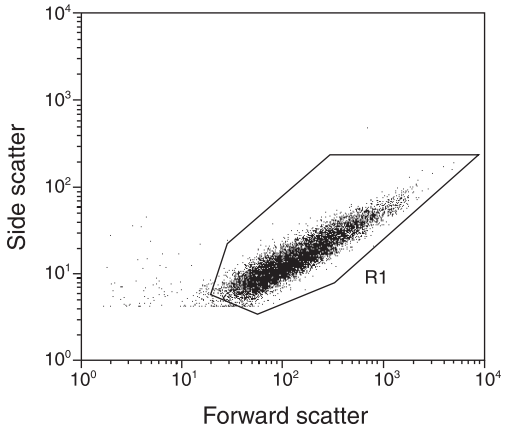

Figure 2. Dot plot of forward scatter vs. side scatter obtained on a sample of unstained *S. aureus* analyzed using a BD FACSCalibur™ flow cytometry system with trigger set on side scatter. The region R1 contains particles of the appropriate size for bacterial cells and is used to set the instrument to exclude debris in the sample.

3.2.3 Adjust the acquisition trigger level (also named "threshold level" on some instruments) to minimize noise appearing on the monitor. To avoid coincidence error, maintain flow rate at ≤1000 events/second.

3.3 Fluorescence amplification settings. Set amplifiers to logarithmic amplification. NOTE: Compensation is not necessary, but may be useful in resolving intermediate populations

3.3.1 Set the amplification of the green-fluorescence channel so that the signals from the live (untreated) bacteria stained with the RedoxSensor™ Green reagent appear in the middle to top range of the signal axis (Figure 3). If necessary, adjust the compensation settings so that the signal is in the lower range of the opposite axis.

3.3.2 Set the amplification of the orange or red-fluorescence channel so that the signals from the killed bacteria stained with propidium iodide appear in the top range of the signal axis (Figure 3). If necessary, adjust the compensation settings so that the signal is in the lower range of the opposite axis.

3.4 After adjusting the flow cytometer as described above, apply experimental samples containing stained bacteria.

3.5 Process data by gating on bacteria using forward versus side scatter, and analyze gated populations a green histogram (Figure 4), side scatter vs. green fluorescence dot plot, or red vs. green dot plot (Figure 3). Bacteria with damaged cell membranes may be excluded by gating on the propidium iodide–negative population.

Analyzing the Stained Bacteria by Fluorescence Microscopy

Bacteria stained using *Bac*Light™ RedoxSensor™ Green Vitality Kit may be viewed using most standard epifluorescence microscopes with the appropriate filters. Fluorescence from both live and dead bacteria may be viewed simultaneously with any standard fluorescein longpass filter set. Alternatively, the live (green fluorescent) and dead (red fluorescent) cells may be viewed separately with fluorescein and Texas Red® bandpass filter sets. A summary of the fluorescence microscope filter sets recommended for use with the *Bac*Light™ RedoxSensor™ Green Vitality Kit shown in Table 1.

To analyze any of the samples using fluorescence microscopy, trap 5 µL of the stained bacteria suspension between a slide and an 18 mm square coverslip and observe in a fluorescence microscope equipped with any of the filter sets listed in Table 1.

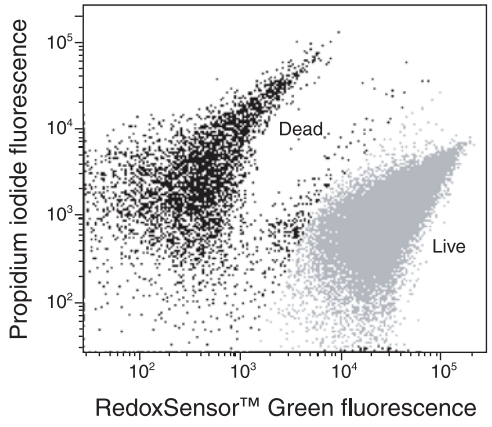

Figure 3. A mixture of healthy and killed *S. aureus* cells were stained with 100 nM RedoxSensor™ Green reagent and propidium iodide. Dual-color dot plot analysis of the sample using the BD™ LSR II system (gated as in Figure 2 with appropriate compensation) shows both the healthy and the membrane-compromised cell populations.

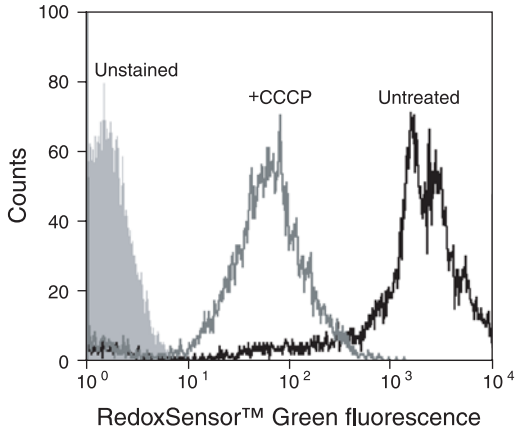

Figure 4. *S. aureus* cells were treated with 100 nM RedoxSensor™ Green reagent in the absence or presence of 10 µM CCCP. Untreated cells show more intense green fluorescence than CCCP-treated cells. For comparison, the histogram corresponding to unstained *S. aureus* cells is shown on the far left. Data was obtained on the BD FACSCalibur™ system, gating on live bacteria by scatter (Figure 2) and by fluorescence (Figure 3).

Table 1. Characteristics of common filters suitable for use with the *Bac*Light™ RedoxSensor™ Green Vitality Kit.

Omega® Filters*	Chroma® Filters*	Notes
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual-emission filters useful for simultaneous viewing of RedoxSensor™ Green reagent and propidium iodide
XF22, XF23	31001, 41001	Bandpass filters for viewing RedoxSensor™ Green reagent alone
XF32, XF43, XF102, XF108	31002, 31004, 41002, 41004	Bandpass filters for viewing propidium iodide alone

* Catalog numbers for recommended bandpass filter sets for fluorescence microscopy. Omega® filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma® filters are supplied by Chroma Technology Corp. (www.chroma.com).

Reference

1. J Microbiol Methods 29, 161 (1997).

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

Cat #	Product Name	Unit Size
B34954	*Bac*Light™ RedoxSensor™ Green Vitality Kit *for flow cytometry*	1 kit

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 Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — **probes.invitrogen.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Service: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License
For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300. Fax: (541) 335-0504.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.