

Product Information

BactoView™ Viability Kits

Product List

Component	32019 BactoView™ Viability Kit (Green/Red)	32020 BactoView™ Viability Kit (Green/Far-Red)
Dead Stain (1000X in DMSO)	40109: BactoView™ Dead 570/585, 100 uL	40111: BactoView™ Dead 655/670, 100 uL
Viability Counterstain (500X in DMSO)	99877-100uL: BactoView™ Viability Green Counterstain, 100 uL	99877-100uL: BactoView™ Viability Green Counterstain, 100 uL

Unit Size: 100 uL each of concentrated stain. Number of assays varies depending on your specific application.

Detection Settings: BactoView™ Viability Kit (Green/Red, Cat. No. 32019)

Component	Ex/Em (nm) (with DNA)	Detection Settings
BactoView™ Dead 570/585 (40109)	572/583	Rhodamine, PI, PE
BactoView™ Viability Green Counterstain (99877-100uL)	498/522	FITC

Detection Settings: BactoView™ Viability Kit (Green/Far-Red, Cat. No. 32020)

Component	Ex/Em (nm) (with DNA)	Detection Settings
BactoView™ Dead 655/670 (40111)	653/671	Cy@5, APC
BactoView™ Viability Green Counterstain (99877-100uL)	498/522	FITC

Storage and Handling

Store at -20°C, protect from light. Product is stable for at least 12 months from date of receipt when stored as recommended.

Spectral Properties: See Kit Content tables, or Figure 1 (page 2) for dye spectra.

Product Description

These kits include Biotium's novel BactoView™ Stains for two-color staining of live and dead bacteria. BactoView™ Dead Stains are membrane-impermeant DNA-binding dyes that selectively stain dead bacteria with compromised membranes. BactoView™ Viability Green Counterstain is designed for two-color staining with BactoView™ Dead and labels both live and dead bacteria with green fluorescence. Kits are available with dye combinations for green/visible red or green/far-red fluorescence. The stains are fluorogenic for no-wash staining and can be used directly in culture medium or buffer.

Traditional vital dyes like propidium iodide or ethidium homodimer are excluded from live gram-negative bacteria due to their protective outer membrane, but often stain live gram-positive bacteria, leading to high background and poor discrimination. BactoView™ Dead Stains have novel structures that are excluded from both gram-positive and gram-negative strains, enabling highly selective live/dead discrimination.

See Related Products for our full selection of BactoView™ Dead Stains in colors from green to near-infrared. We also offer BactoView™ Live Stains with green or red fluorescence.

BactoView™ Stains cannot distinguish bacteria from eukaryotic cells, as they will stain other cell types. For mammalian cells, see our NucSpot® Nuclear Stains for live/dead discrimination or counterstaining of fixed cells. For live nuclear staining, see our NucSpot® Live Stains (see Related Products).

Considerations

When combining BactoView™ Stains with other stains, particularly other DNA dyes, we recommend titrating the concentration of each stain separately and together to find the optimal concentration and staining protocol (for example, co-incubation of the stains vs. sequential staining).

Note: The BactoView™ Viability Counterstain will stain dead cells more intensely than live cells, with live gram-positive strains staining more efficiently than live gram-negative cells.

Bacteria Staining Protocol

This protocol has been developed for staining laboratory bacterial strains in liquid culture. Optimization may be needed for other sample types.

1. Grow cells in the appropriate growth medium and growth conditions. We typically grow bacteria overnight at 37°C.
2. If desired, collect the cells by centrifugation and resuspend in a buffer for staining. BactoView™ Dead Stains can be used to stain cells in growth medium, as well as in buffers like PBS, or in 150 mM NaCl.

3. Add each BactoView™ Dead Stain to the bacterial sample at a final concentration of 1X. For example, if the sample volume is 1 mL, add 1 µL of the BactoView™ Dead Stain, mix gently, and then add 2 µL of Bactoview™ Viability Green Counterstain and mix again.

Notes:

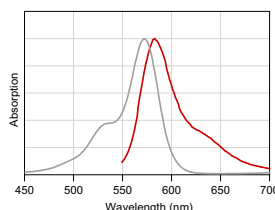
- a. We recommend adding the two stains sequentially, rather than mixing them together before adding to the sample. Dye concentration may be optimized for different cell or sample types.
 - b. For smaller sample volumes, you may prepare an intermediate dilution of each stain. For example, add 1 µL of BactoView™ Dead stain to 19 µL buffer and mix well. In a separate tube, add 1 µL of BactoView™ Viability Green Counterstain to 9 µL of buffer and mix well. Add 1 µL of each intermediate dilution to 50 µL of sample. Prepare the intermediate dilutions just before use, and do not store the diluted dyes.
 - c. We recommend performing single stain controls in addition to double staining, to control emission crosstalk between detection channels.
4. Incubate samples at room temperature or 37°C for 30 minutes, in the dark.
 5. Optional: Collect cells by centrifugation and resuspend them in a fresh buffer of your choice.
 6. For fluorescence microscopy, mount 5 µL of the sample on a slide with an 18 mm coverslip. Alternatively, you may pipet 100 µL of sample into a 96-well optical bottom plate. Image cells in the appropriate detection channels (see Spectral Properties). Live cells will be stained with green fluorescence, while dead cells will be co-stained with green/red or green/far-red fluorescence, depending on which kit is used. Dead cells may appear yellow-orange with co-localized green/red fluorescence, or pink-white with co-localized green/far-red fluorescence.
- Note:** Coating the slide or wells with CellTak™ adhesive to immobilize the cells can facilitate imaging of bacteria by microscopy.
7. For flow cytometry, dilute the sample in FACS wash buffer (PBS + 1% serum) or similar buffer. You may need to dilute the sample 10-fold or more to achieve the desired flow rate. Detect cells in the appropriate detection channels (see Spectral Properties). Live cells will be positive for green fluorescence, while dead cells will be positive for both green/red or green/far-red fluorescence, depending on which kit is used.

Please visit our website at www.biotium.com for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, fluorescent CF® Dye antibody conjugates and reactive dyes, apoptosis reagents, fluorescent probes, and kits for cell biology or microbiology research.

CellTak is a trademark of Corning; Cy Dye is a registered trademark of Cytiva.

Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

BactoView™ Dead 570/585



BactoView™ Dead 655/670

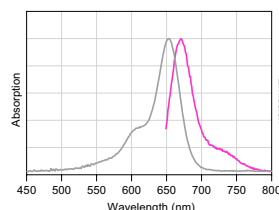


Figure 1. BactoView™ Dead absorbance and emission spectra.

BactoView™ Viability Green Counterstain

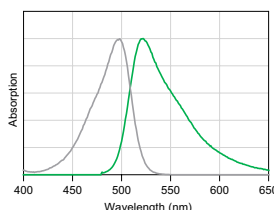


Figure 2. BactoView™ Viability Green Counterstain absorbance and emission spectra.

Related Products

Cat. No.	Product
40107-40113	BactoView™ Dead Stains
40101	BactoView Live™ Red
40102	BactoView Live™ Green
40083... 41038	NucSpot® Nuclear Stains for Live/Dead Discrimination or Fixed Cell Counterstaining
40081, 40082	NucSpot® Live Nuclear Stains
29021... 29077	CF® Dye Wheat Germ Agglutinin (WGA)
30027	Viability/Cytotoxicity Assay for Bacteria Live & Dead Cells
32000	Live Bacteria Gram Stain Kit
40069	PMAxx™ Dye for Viability PCR
40013, 40019	PMA Dye for Viability PCR
E90007	Glo-Plate™ 2.0 Blue LED Illuminator
E90006	PMA-Lite™ 2.0 LED Photolysis Device
31033-31037; 31050, 31051, 31053	Real-Time PCR Bacterial Viability Kits
32002... 32018	Live-or-Dye™ Fixable Viability Staining Kits
70020	SynaptoGreen™ C4 Membrane Stain
70021	SynaptoRed™ C2 Membrane Stain
31062	Yeast Vitality Staining Kit
31063	Yeast Viability Staining Kit
31064	Yeast Fixable Live/Dead Staining Kit
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells