

Product Information

Ethidium Homodimer III (EthD-III)

Catalog Number

40050: 1 mg solid
 40051: 1 mM in DMSO

Storage and Handling

The solid is soluble in DMSO, methanol, or water. Store the solid and the solution at 4°C, protected from light. Product is stable for at least 5 years from date of receipt when stored as recommended. Ethidium Homodimer III dye binds to nucleic acids. The mutagenicity or toxicity of EthD-III is currently unknown. It should be handled using universal laboratory safety precautions.

Spectral Properties

Ex/Em: 532/625 nm* (with DNA)

*Ethidium Homodimer III also has a strong UV absorbance peak at 279 nm

Product Description

Ethidium Homodimer III, also known as EthD-III, is a red fluorescent dead cell stain for bacteria and mammalian cells. It is a cell membrane-impermeant nucleic acid dye that only stains dead cells with damaged cell membranes. Ethidium Homodimer III was developed by Biotium as a superior alternative to Ethidium Homodimer I. The absorption and emission spectra are similar, but EthD-III is 45% brighter.

Ethidium Homodimer III is useful for detecting dead bacteria or mammalian cells by fluorescence microscopy or flow cytometry. It can be used in combination with cell permeable stains (such as: Hoechst, DMAO, or BactoView™ Live Green for bacteria; and Hoechst, DAPI, Calcein AM, or ViaFluor® SE for mammalian cells) to simultaneously label live and dead cells.

Ethidium Homodimer III is not fixable. If a mixture of live and stained dead cells is fixed, dye will leak from the dead cells into the live cells. If you are looking for a fixable, nuclear dead cell stain, try Live-or-Dye NucFix™ Red (32010).

We also offer Ethidium Homodimer III in several combination kits for added convenience, including the Viability/Cytotoxicity Assay Kit for Bacteria (30027) and the Viability/Cytotoxicity Assay Kit for Animal Cells (30002). For more information visit our website.

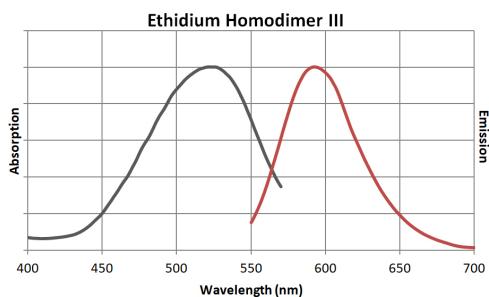


Figure 1. Normalized absorption and emission of Ethidium Homodimer III

General Staining Protocol

This protocol is a general guideline for staining mammalian tissue culture cells and laboratory bacteria strains. Optimization may be needed for other sample types.

Note: EthD-III selectively stains dead cells based on membrane integrity, and must be used on unfixed cells for live/dead discrimination. If used on cells after fixation, it will stain nuclei and cytoplasm of all cells.

1. Grow cells in the appropriate growth medium and growth conditions.
2. Optional positive control (dead) cells preparation: For bacteria, incubate cells at 90°C for 5 minutes and allow to cool to room temperature. For adherent mammalian cells, to kill a subset of cells incubate with 15% ethanol in buffer or medium for 10 minutes at room temperature then wash. To prepare a uniformly killed mammalian cell population, incubate at 56°C for 45 minutes and then cool.
3. If desired, collect the cells by centrifugation (or wash adherent cells) and resuspend in a buffer for staining. EthD-III can stain cells in growth medium, as well as PBS, HBSS, and 150 mM NaCl.
4. Add dye to the cells at a final concentration of 2.5-5 μM (for mammalian cells) or 5 μM (bacterial cells) and mix well. Dye concentration may need to be optimized for different cell or sample types. Other stains may be added simultaneously.
5. Incubate at room temperature or 37°C for 15-30 minutes, in the dark.
6. Optional wash: Collect cells by centrifugation (or wash adherent cells) and resuspend in fresh buffer of your choice.

Note: Ethidium Homodimer III is not fixable. If a mixture of live and dead cells are fixed after staining, dye will leak from the dead cells and into the live cells.
7. For fluorescence microscopy, image cells with Cy@3 or Texas Red® band-pass filter sets. For flow cytometry, detect cells in the PE channel.

Related Products

Catalog number	Product
40101	BactoView™ Live Red
40102	BactoView™ Live Green
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live and Dead Cells
32001	Bacterial Viability and Gram Stain Kit
32010	Live-or-Dye NucFix™ Red
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits
40069	PMAxx™ Dye for Viability PCR, 20 mM in Water
30068	ViaFluor® 405 SE Cell Proliferation Kit
30086	ViaFluor® 488 SE Cell Proliferation Kit

Please visit our website at www.biotium.com for information on our life science research products, including kits for cell biology and microbiology research.

Texas Red is a registered trademark of Thermo Fisher. Cy is a registered trademark of GE Healthcare.

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