

Ethidium Homodimer III

Cat. No. EthD-III-005 **Lot. No.** (See product label)

SPECIFICATION

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 stains only 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.

Form Red solid

Molecular Mass ~1000

Solubility The solid is soluble in DMSO, methanol, or water.

Absorption/Emission 532/625 nm* (with DNA)
*Ethidium Homodimer III also has a strong UV absorbance peak at 279 nm

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 centigrade 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

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medium for 10 minutes at room temperature then wash. To prepare a uniformly killed mammalian cell population, incubate at 56 centigrade 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 μ M (for mammalian cells) or 5 μ M (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 centigrade 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 Cy3 or Texas Red band pass filter sets. For flow cytometry, detect cells in the PE channel.

Probe cellular localization	Nucleus & cytoplasm
Assay type/options	Real-time imaging
Cell permeability	Membrane impermeant
Apoptosis/viability marker	Dead cell stain
Colors	Red
Applications	Can be used in mammalian, yeast, and bacteria cells
Stability	Product is stable for at least 5 years from date of receipt when stored as

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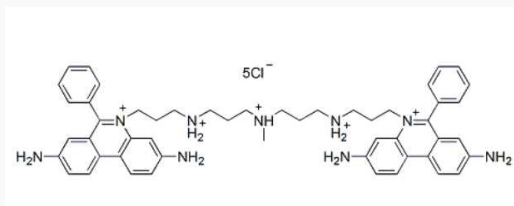
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recommended.

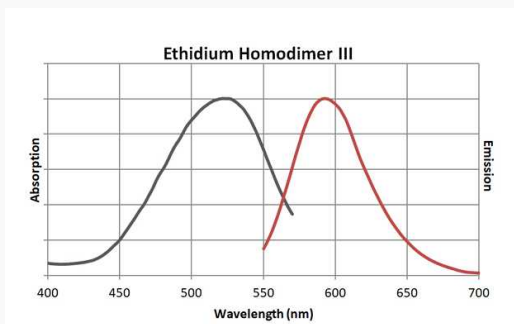
Storage

Store the solid and the solution at 4 centigrade, protected from light.

Structure of Ethidium Homodimer III



Normalized absorption and emission of Ethidium Homodimer III.



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