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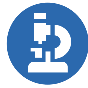
Produktinformation

Forschungsprodukte & Biochemikalien

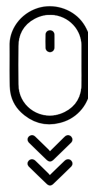
Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik



Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells

Catalog Number: 30027

Unit Size: 1000 assays

Kit Contents

Component	Cat. No.	Size
DMAO, 5 mM in DMSO	30027A	2 x 100 uL
Ethidium Homodimer III, 2 mM in DMSO/H_2O	99905	2 x 150 uL

Storage and Handling

Store at 4°C, protected from light. Product is stable for at least 2 years from date of receipt when stored as recommended. DMAO and EthD-III dyes bind to nucleic acids. The mutagenicity or toxicity of these dyes is currently unknown. Both reagents should be handled using universal laboratory safety precautions.

Spectral Properties

DMAO: Ex/Em: 497/528 nm (with DNA)
Ethidium Homodimer III (EthD-III) Ex/Em: 532/625 nm* (with DNA)
*Ethidium Homodimer III also has a strong UV absorbance peak at 279 nm

Product Description

The Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells provides a convenient assay for detecting dead bacteria (red) as well as all bacteria (green) within the same cell population by flow cytometry or fluorescence microscopy.

DMAO is a bright green fluorescent nucleic acid dye that stains both live and dead bacteria. Ethidium Homodimer III (EthD-III) is a red fluorescent nucleic acid dye that stains only dead bacteria with damaged cell membranes. Staining can be analyzed by fluorescence microscopy or flow cytometry. The assay principles are general and applicable to most bacteria types.

This kit generally yields results that correlate with growth assays in liquid or solid media. Under certain conditions, however, bacteria having damaged membranes may be able to recover and reproduce in nutrient medium, even though such bacteria could be scored as dead in this assay. Conversely, some bacteria with intact membranes may be unable to reproduce in nutrient medium, even though these bacteria would be scored as alive in this assay. The accuracy of your measurements should be established prior to use and these possibilities should be considered if a discrepancy exists between this assay and bacterial growth assays.

General Considerations

- Growth medium should be removed before staining bacteria. The nucleic acids and other media components can bind DMAO and EthD-III dyes, resulting in decreased staining.
- We suggest staining in 150 mM NaCl or 10 mM Tris, pH 7.5. We have observed lower staining efficiency for DMAO in PBS and HBSS.
- The optimal dye concentrations may vary depending on application and bacteria strain. In general, it is best to use the lowest dye concentration that gives sufficient signal. The following protocol was optimized for *E. coli*.
- We recommend using live and dead cell controls for your bacteria of interest in each staining experiment. Dead cell controls can be prepared by heating the bacteria to 90°C for 5 minutes, then allowing to cool.

Experimental Protocols

Staining protocol for fluorescence microscopy

1. Warm the dye stock solutions to room temperature and vortex to mix.
2. Prepare 11 uL of 100X staining solution by combining 1 uL DMAO, 2 uL EthD-III, and 8 uL 150 mM NaCl (see General Considerations bullet #2). Vortex to ensure thorough mixing.

   Note: Volumes may be scaled proportionally as needed.
3. Harvest bacterial cells by centrifugation at 10,000 x g for 5 minutes in microcentrifuge tubes and remove the supernatant.
4. Optional: Wash cells once in 150 mM NaCl by pipetting up and down several times to disperse the pellet.
5. Pellet cells by centrifugation at 10,000 x g for 5 minutes and remove the supernatant from the tube.
6. Resuspend cells in 100 uL of 150 mM NaCl.
7. Add 1 uL of the dye mixture to 100 uL of bacterial suspension.
8. Mix gently and incubate at room temperature in the dark for 15 minutes.
9. Transfer 5 uL of the sample to a slide, apply a glass coverslip, and seal with CoverGrip™ Coverslip Sealant (Cat. No. 23005) or nail polish.
10. Image the labeled cells by fluorescence microscopy. DMAO can be imaged using a FITC filter set, and EthD-III can be imaged using Texas Red® or Cy®3 filter sets.

Staining protocol for flow cytometry

1. Follow steps 1-8 in the staining protocol for fluorescence microscopy, above.

   Note: We suggest also preparing single-stain bacterial samples of dead cells stained with DMAO alone and with EthD-III alone.
2. Add 0.5 mL of your preferred buffer for flow cytometry analysis.
3. Perform flow cytometry analysis, detecting DMAO in the FITC channel, and EthD-III in the PE or PE-Texas Red® channel.

Related Products

Cat. No.	Product
32001	Bacterial Viability and Gram Stain Kit
32000	Live Bacteria Gram Stain Kit
40101	BactoView Live™ Red
40102	BactoView Live™ Green
40069	PMAxx™ Dye for viability PCR, 20 mM in water
40013	PMA Dye for viability PCR
40019	PMA Dye for viability PCR, 20 mM in water
E90006	PMA-Lite™ 2.0 LED Photolysis Device
31033-31037; 31050, 31051, 31053	Real-Time PCR Bacterial Viability Kits (Salmonella enterica, Mycobacterium tuberculosis, Staphylococcus aureus, MRSA, E. coli O157:H7, E. coli, Listeria monocytogenes, Legionella pneumophila)
32002... 32018	Live-or-Dye™ Fixable Viability Staining Kits
29021-29029; 29059; 29064	CF® Dye Wheat Germ Agglutinin (WGA)
70020	SynaptoGreen™ C4 membrane stain
70021	SynaptoRed™ C2 membrane stain
10063	CTC, bacterial respiration dye
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

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