Product Information

Live & Dead™ Viability/Cytotoxicity Assay Kit for Bacteria Cells

Catalog Number: L6060S, L6060L

Product Size: 20T, 100T

Contents:

Component	L6060S (20T)	L6060S (100T)
A. NucGreen	20 μL	100 μL
B. EthD-III	40 μL	200 μL

Parameters

NucGreen: Ex/Em: 503/530 nm (with DNA)

EthD-III: Ex/Em: 530/620 nm (with DNA)

Storage

Store at -20℃ and protect from light. Expiration date marked on the outer packing.

Description

Live & Dead™ Viability/Cytotoxicity Assay Kit for Bacteria catians two fluorescent dyes, NucGreen and EthD-III. NucGreen is a green nucleic acid fluorescent dye, which can stain living and dead bacteria. EthD-III is a red nucleic acid fluorescent dye, which can only stain dead bacteria with damaged cell membrane. When NucGreen and EthD-III are properly mixed, the bacteria with intact cell membrane has green fluorescence, while the bacteria with damaged cell membrane has both green and red fluorescence. The principle of detection is conventional and applicable to most bacterial types.

The common standard of bacterial activity is the ability of bacteria to reproduce in a suitable nutrient medium, which is called growth assay. The results of this kit are in good agreement with those of growth measurement in liquid or solid medium. However, under certain conditions, bacteria with membrane damage may recover and reproduce in a nutrient medium, and such bacteria may be considered dead in this assay. In contrast, some bacteria with intact membranes may not be able to reproduce in nutrient media, but these bacteria may be considered alive in this assay. Therefore, if there is a considerable difference between this test and bacterial growth test, the above possibility should be take into consideration.

Protocol

The following protocol is optimized for *Escherichia coli*

1. Culture bacteria in liquid medium until the late logarithmic phase.

2. Add 1 mL bacterial suspension into EP tube and centrifuge at 5000 ×g for 15 min.

3. Remove the supernatant, add 1mL of 0.85% NaCl solution to resuspend the bacteria.

4. Centrifuge at 5000 ×g for 15 min, remove the supernatant, add 1mL of 0.85% NaCl solution to resuspend the bacteria. Repeat it.

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5. Adjust density of bacterial suspension to 10^8 /mL (OD670 ≈ 0.03).

6. Preparation of dyeing working solution: Take 1 μL NucGreen and 2 μL EthD-III, mix them fully, and then add 8 μL 0.85% NaCl solution.

7. Take 1 mL bacterial suspension and add 10 μL dyeing working solution. Mix fully and incubated in dark for 15 min at room temperature.

8. After dyeing, add 10 μL bacterial suspension to slide with 18 mm square cover glass, and imaging as soon as possible to avoid slides dry.

9. Imaging with fluorescence microscope. Green fluorescence can be observed with FITC applicable filter, red fluorescence can be observed with PI applicable filter.

Notes

1. If 96-well plates is used for detection, the bacterial suspension can be left standing for 10 min, leaving a small amount of bacterial liquid for imaging, which can effectively reduce the background.

2. In order to get closer to the real results, it is recommended to keep the red fluorescence and green fluorescence brightness consistent when merging images.

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