Viability/Cytotoxicity Assay Kits for Bacteria Cells

Catalog No.: RA20073

Basic Information

Product name	Viability/Cytotoxicity Assay Kits for Bacteria Cells
Sizes	20T/100T
Storage	-20℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	NucGreen：503/530 nm（combine DNA); EthD-III:　530/620 nm（combine DNA）

Product Introduction

The bacterial viability and toxicity detection kit contains two fluorescent dyes: NucGreen (a green nucleic acid dye that stains both live and dead bacteria) and EthD-III (a red nucleic acid dye that specifically stains dead bacteria with damaged cell membranes). When mixed appropriately, bacteria with intact cell membranes appear green, while those with damaged membranes show distinct green and red fluorescence signals under different detection channels. This kit is suitable for staining most bacterial types and can be analyzed using either fluorescence microscopy or flow cytometry. The excitation and emission spectra of NucGreen and EthD-III are detailed in the product specifications. A common standard for bacterial viability assessment is growth measurement, which evaluates a bacterium's ability to multiply in nutrient-rich culture media. The kit's results demonstrate excellent consistency with growth measurements obtained from both liquid and solid culture media.

Product features

Wide application scope: suitable for most bacteria;

good stability: strong fluorescence brightness and good anti-quenching, stable product, easy to store and transport.

Product Components

components	Specification (20 T)	Specification (100 T)
A. NucGreen	20 μL	100 μL
B. EthD-III	40 μL	200 μL

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Materials Required (Not Supplied)

1.Consumables centrifuge tube

2. Reagents

(1) 0.85% NaCl

(2) 0.7 mL isopropanol

3. Instruments

(1) spectrophotometer

(2) fluorescence microscope

(3) flow cytometry

Perimental procedure

1.Preparation of Live and Dead Bacterial Sample Controls (Optional)

(1) Cultivate 4 mL of bacteria in liquid medium until the late logarithmic phase.

(2) Prepare two 1 mL bacterial suspensions in EP tubes and centrifuge at 5,000-10,000 g for 10-15 minutes.

(3) Discard the supernatant. In one EP tube, add 0.3 mL of 0.85% NaCl to resuspend the bacteria, and in the other tube, add 1 mL of 0.85% NaCl suspension.

(4) Add 0.7 mL isopropyl alcohol to the 0.3 mL NaCl tube, mix thoroughly (resulting in a 70% isopropyl alcohol concentration) to prepare the dead bacterial sample.

(5) Incubate both samples at room temperature for 1 hour, mixing every 15 minutes.

(6) Centrifuge both samples at 5,000-10,000 g for 10-15 minutes.

(7) Discard the supernatant. In both samples, add 1 mL of 0.85% NaCl suspension and centrifuge again as described in step (6).

(8) Measure the absorbance (OD 670) of both bacterial suspensions at 670 nm using a spectrophotometer.

(9) Adjust the density of both bacterial suspensions (live and dead) to 10^8 bacteria/mL (OD670 ≈ 0.3), then dilute with 0.85% NaCl at a 1:100 ratio to achieve a final density of 10^6 bacteria/mL.

(10) Mix the two bacterial suspensions as shown in Table 1 below to obtain the desired live-to-dead cell ratio.

Table 1 Live and dead bacterial suspensions are mixed in a certain volume to achieve the desired ratio of live cells and dead cells

Living cells: Dead cells	Volume of live bacterial suspension (mL)	Volume of the suspension of dead bacteria (mL)
0：00	0	1.0
10：90	0.1	0.9
20：80	0.2	0.8
30：70	0.3	0.7
50：50	0.5	0.5
100：0	1.0	0

2.Fluorescence Microscopy Staining Protocol

(1) Mix 1 volume of component A (NucGreen) with 2 volumes of component B (EthD-III) in a microcentrifuge tube. After thorough mixing, add 8 volumes of 0.85% NaCl solution to prepare a 100 × staining solution.

(2) Add 1 μL of the 100× staining solution per 100 μL bacterial suspension.

(3) Mix thoroughly and incubate at room temperature in darkness for 15 minutes.

(4) Perform rinsing after incubation to remove residual dye: First centrifuge at 8000 rpm and discard the supernatant, then rinse with 0.85% NaCl 2-3 times, and finally resuspend in 0.85% NaCl.

(5) Drop 5 μL of the stained bacterial suspension onto a coverslip with an 18 mm square cover slip.

(6) Observe under fluorescence microscopy. Both live and dead bacteria exhibit distinguishable fluorescence under any standard FITC long-pass filter. Alternatively, live (green fluorescence) and dead (red fluorescence) bacteria can be observed separately using FITC and Cy3 (or Texas Red) channels.

Note:

1) Ensure complete removal of residual growth medium before staining. Nucleic acids and other culture components may bind to NucGreen and EthD-III dyes, causing unacceptable staining interference. Simple washing procedures are generally sufficient to remove interfering medium components from bacterial suspensions. Phosphate buffer is not recommended as it reduces staining efficiency.

2) Before initiating formal experiments, adjust dye concentrations to enable NucGreen labeling of live bacteria and EthD-III labeling of dead bacteria for differentiation. Optimal concentrations may vary by bacterial strain. Generally, the minimum dye concentration that provides sufficient signal is preferred. These conditions are optimized for live/dead Escherichia coli cell staining.

3) When observing dual-color live bacteria staining, fix the field of view and avoid moving the

sample or adjusting the microscope's fine focus screw, as this may cause field misalignment and affect results.

3. Flow Cytometry Staining Protocol

Before starting experiments, review precautions under Fluorescence Microscope Staining Procedures.

(1) According to Table 1, add 11 different ratios of live and dead bacteria into EP tubes (1 mL volume per sample).

(2)Mix 12 μL component A (NucGreen) with 24 μL component B (EthD-III) in microcentrifuge tubes. Add 3 μL of the mixed dye to each of the 11 samples and thoroughly blend by shaking up and down several times.

Note: Prepare separate control bacterial samples for NucGreen and EthD-III staining alone.

(3) Incubate at room temperature in darkness for 15 minutes.

(4) After incubation, washing is required to remove residual dye. First centrifuge at 8000 rpm to discard the supernatant, then rinse with 0.85% NaCl 2-3 times, and finally resuspend in 0.85% NaCl.

(5) Analyze each sample using a flow cytometer: detect NucGreen-positive cells via FITC channel, and EthD-III-positive cells using PI or PE channels.

Notes

1.Before use, centrifuge the product to the tube bottom immediately before proceeding with subsequent experiments.

2. Remove the bacterial culture medium before staining. Components or free nucleic acids in the medium may be labeled by the dye, causing background interference.

3. The dye should be diluted with 0.85-0.9% physiological saline. Using PBS or HBSS for dilution may affect staining efficacy.

4. Optimize dye concentration and staining time based on specific bacterial samples through condition optimization.

5. For plate-based detection, allow a 10-minute incubation period with residual bacterial liquid for imaging to effectively reduce background noise.

6. Fluorescent dyes are prone to quenching. Ensure proper light protection during operation to minimize quenching effects.

7. For safety, wear lab coats and disposable gloves during handling.

8. This product is strictly for scientific research purposes only and must not be used for clinical diagnosis or treatment.

This product is for research use only!