AIEgen Probe for Bacteria (Dead) Detection

Introduction

- AIE™ Bacterial (Dead) is a low toxicity and high stability staining probe for dead bacteria.
- This product can differentiate dead and living bacteria and serve as a highly fluorescent and photostable probe for long-term viability assay.
- Bacteria with compromised membrane open the access for AIE™ Bacterial (Dead) to reach DNA and giving off strong emission. This probe is a cell-impermeable DNA stain that binds to the groove of double-stranded DNA. The fluorescence intensity could be obtained in following optical condition:

  $$Excitation/Emission = 320 \pm 20 / 450 \pm 40 \text{ nm}$$

- This product has been applied to *E. coli*, *S. epidermidis*, and *B. subtilis*.

Stock Solution Preparation

1. **Bacteria sample preparation:** Select your bacteria of interest and measure its optical density at 600 nm (OD600) and make sure to incubate them to OD600 = 1.
2. **AIE™ Bacterial (Dead) stock solution**: The stock solution with a concentration of 5-mM can be prepared by dissolving 10 μmol of AIE™ Bacterial (Dead) in 2 mL DMSO. The solution was stored in dark before use.
3. **PBS** is prepared by dissolving NaCl (8 g), KCl (0.2 g), Na2HPO4 (1.44 g), and KH2PO4 (0.24 g) in 800 mL distilled water, adjusting pH to 7.4 with HCl, and calibrating to 1 L by adding H_2O. PBS can be sterilized by autoclaving for 20 min at 15 Psi (1.05 kg cm-2) on liquid cycle and stored at room temperature.

Before Your Experiment, You might NEED

1 PBS buffer pH 7.4 and pH 10	2 DMSO	3 75% alcohol
4 Fluorescence Cuvette	5 Suitable culture media	6 Milli-Q Water (or DI water)

Protocol (Recommended)

Bacteria staining	1. A single colony of bacteria on solid culture medium [Luria broth (LB) for E. coli and S. epidermidis, nutrition broth (NB) for B. subtilis] is transferred to 5 mL of liquid culture medium and grown at 37 °C for 10 h. 2. The concentrations of bacteria are determined by measuring optical density at 600 nm (OD600) and then 109 CFU of bacteria was transferred to a 1.5 mL microcentrifuge tube. 3. Bacteria can be harvested by centrifuging at 11700 *g* for 3 min. 4. After removal of supernatant, bacteria are killed by 200 μL 75% alcohol. 5. 1 mL dye solution in PBS at appropriate concentration is added into the microcentrifuge tube to make up the dye concentration between 50 mM to 200 mM. ❖ *Upon imaging any bacteria of your interest, this probe concentration for staining should be kept between 50 – 200 μM.* 6. After dispersing with vortex, the bacteria are incubated in a shaking incubator at 30 °C for 30 minutes. 7. Wash the bacteria with pH 10 buffer three times[NOTE1]. ❖ *If you obtain the imaging with high background and you are recommended to do the dye cleansing step here by centrifuging.*
Fluorescent Imaging	1. **Fluorescent Imaging:** 2 μL of stained bacteria solution is transferred to glass slide and then covered by a coverslip. 2. The image can be collected using 100 × objectives. Recommended condition for imaging[NOTE2]. a. Excitation filter = 330–385 nm; emission filter = 420 nm long pass b. Dichroic mirror = 400 nm 3. **Steady-state fluorescence measurement using photoluminescent instrument (PL)**, 30 μL of the stained bacteria solution is first transferred to quartz cuvette. 4. Then 2.7 mL buffer solution (pH 10) is added to dissolve the free dye to lower the background. 5. PL spectra are collected immediately after mixing the solution by pipetting for 1 min.
Note	1. Cleansing with pH 10 buffer is not recommended over 1 minute (suitable time range is 30 second – 1 minute) 2. Fluorescent imaging condition for your selected bacteria should be carefully study and chosen at researcher's discretion. Imaging quality will be affected by excitation wavelength, excitation power, and staining time.

Reference

1. Zhao, E.; Tang, B. Z. et al. "Highly Fluorescent and Photostable Probe for Long-Term Bacterial Viability Assay Based on Aggregation-Induced Emission" Adv. Healthcare Mater. 2014, 3, pp 88–96.

2. Optical information and suggested storage conditions:

Item	Ex/Em	Qty	Storage Condition*
AIE™ Bacterial (Dead)	320/450 nm	10 μmol	• ≤–20 ℃ (Upon receive this product) • Avoid Light • Keep Dry
* Remember to warm up to room temperature upon opening the vial			