Data Sheet

BioTracker™ BacGO Gram-positive Bacteria Dye

Live Cell Probe

SCT071

Pack Size: 1 mg

Store at –20 °C

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Gram staining has been the gold standard technique for bacterial classification based on characteristic physical properties of the bacterial cell-wall structure. However, Gram staining requires several steps that include fixation of the bacterial cell, two staining steps with two different dyes, followed by decolorization and washing. Notably, the decolorization step often generates false Gram determination, and the washing step can cause significant cell loss. An alternative to the Gram staining method is a fluorescence-based dye approach for flow cytometry or fluorescence microscopy.

The BioTracker™ BacGO Gram-positive Bacteria Dye is an orange-colored dye that can be used to identify Gram-positive bacteria by flow cytometry and fluorescence imaging. BacGO dye has been tested using real samples of colony-forming bacteria, activated bacterial sludge, and a bacterial eye infection model. To fully demonstrate the universal selectivity of BacGO dye, it was tested on 16 bacterial strains (9 Gram-positive and 7 Gram-negative strains) and the results compared with Gram staining. BacGO stained all of the known Gram-positive bacteria, and the result was validated with Gram staining. The universal selectivity of BacGO was also confirmed by flow cytometry using DAPI to stain the nuclei of all the bacteria.

Source

The BioTracker™ BacGO Gram-positive Bacteria Dye (SCT071) does not contain genetically modified organisms.

Spectral Properties

Excitation: 520-570 nm

Emission: 580 nm

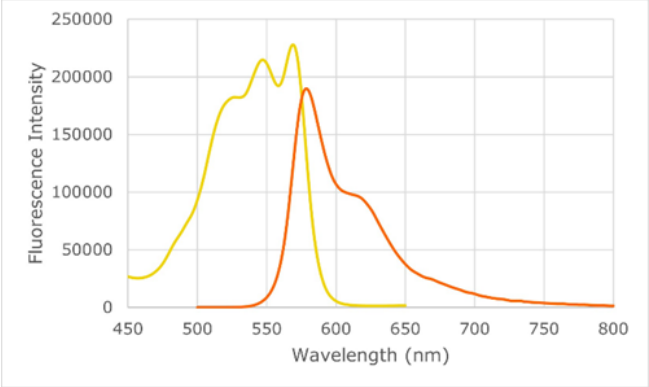

Figure 1. Probe excitation and emission data. 7 µL of probe at stock concentration (10 mM) was diluted in 1 mL of DMSO before undergoing excitation and emission scans. Spectral scans were conducted using a PerkinElmer FL8500 Fluorescence Spectrophotometer.

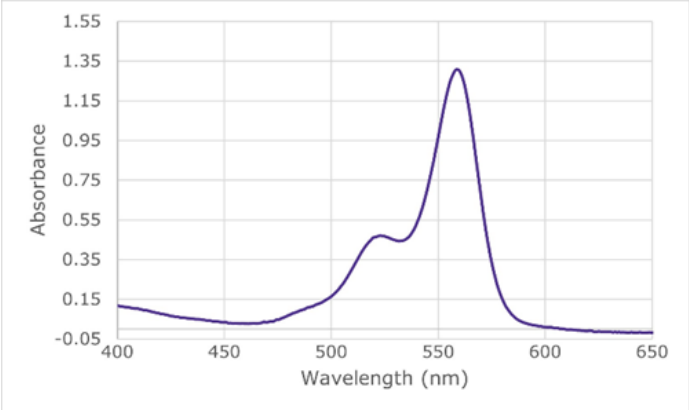

Figure 2. Probe absorbance data. 7 µL of probe at stock concentration (10 mM) was diluted in 1 mL of DMSO before undergoing an absorbance scan. Spectral scans were conducted using a PerkinElmer FL8500 Fluorescence Spectrophotometer.

Quality Control Testing

Purity: ≥ 98% confirmed by HPLC. Structure confirmed by HNMR, FNMR, LC-MS and elemental analysis.

Molar Mass: 485.10 g/mol

Storage and Handling

Store BioTracker™ BacGO Gram-positive Bacteria Dye at –20 °C, desiccated and protected from light.

Note: Centrifuge vial briefly to collect contents at bottom of vial before opening.

Presentation

Lyophilized

Representative Data

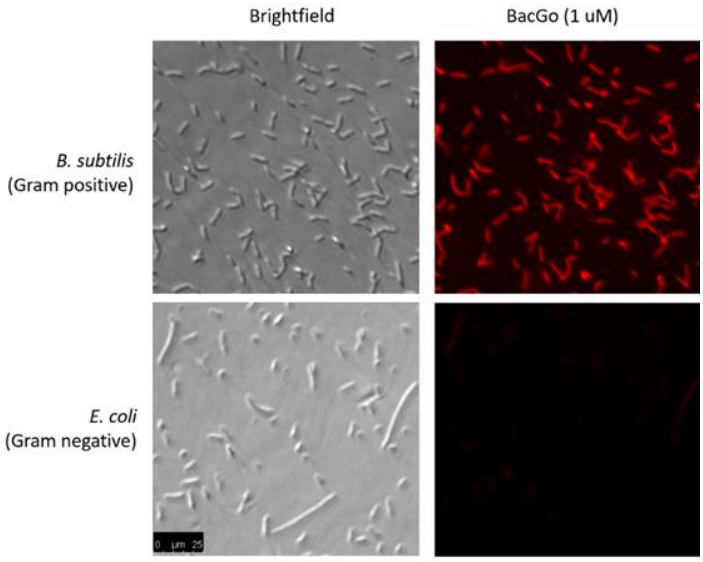

Figure 3. Brightfield and fluorescence imaging of gram-positive *B. subtilis* (Top row) and gram-negative *E. coli* (Bottom row) bacteria using BioTracker™ BacGO Gram-positive Bacteria Dye.

Protocols

Bacterial Culture Preparation

1. Grow bacterial cultures overnight in liquid nutrient medium (for example, LB Broth) with shaking at appropriate temperature.
2. Add 1 mL of culture to a 6-well imaging slide treated with poly-D-lysine hydrobromide.
3. Allow culture to settle on coated surface for 2 hours at room temperature.
4. Remove liquid and wash very gently 3 times with PBS.
5. Fix cells in 4% paraformaldehyde solution for 20 minutes at room temperature.

   Note: Fixation step is not necessary for staining but recommended for obtaining clear microscope images.
6. Wash cells with PBS. Cells are ready for staining.

Reagent Preparation

1. Before opening the vial, spin down the solid to the bottom by a microcentrifuge or by a desktop centrifuge.
2. Warm the vial to the room temperature and add DMSO to make a 1000X stock solution of 1 mM (freeze aliquots at –20 °C).
3. Dilute in cell culture media at a final concentration of 1 µM and add to plated bacterial cells. Incubate at 37 °C for 5 minutes.

   Note: incubation time should be less than 10 minutes. Extended incubation time may result in nonspecific staining of Gram-negative bacteria.
4. Wash cells gently with PBS buffer before imaging.

   Note: Optimal concentration must be determined by end user.

References

1. Kwon H-Y, Liu X, Choi EG, Lee JY, Choi S-Y, Kim J-Y, Wang L, Park S-J, Kim B, Lee Y-A, et al. 2019. Development of a Universal Fluorescent Probe for Gram-Positive Bacteria. Angewandte Chemie International Edition. 58(25):8426–8431. doi:https://doi.org/10.1002/anie.201902537.

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Document Template 20306518 Ver 6.0

20714847 Ver 2.0, Rev 25JUN2024, DP