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Using the Confocal Microscope with *Enterococcus faecalis* and NAO dye.

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[1]In-house protocol

1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

Using the Confocal Microscope with *Enterococcus faecalis* and NAO dye.

PROTOCOL CITATION

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ABSTRACT

Using the Confocal Microscope with *Enterococcus faecalis* and NAO dye.

Steps

1 Prepare an Enterococcus faecalis overnight culture.
- In 10mL of Brain Heart Infusion (BHI) broth put in 1 colony.

2 Next morning, dilute overnight E. faecalis to an OD600 of 0.01 in fresh BHI.

- Supplement as needed.

3 Check OD600 after 3 hours.
- Looking for an OD ~0.4.

4 Isolate 4mL of log phase culture and place in BD Facs tube.

5 Centrifuge 3500 RPM 5 to10 minutes.

6 Suspend pellet in 1mL of sterilized 1X PBS.

7 Stain with NAO dye for 30 minutes in the dark at room temp.

Use 7.5uL of a 10-3diluted NAO stock to give stain with 1.5uM NAO.
- The initial concentration of NAO is 100mg in 1mL of DMSO or 212mM in 1mL of DMSO.
- Make a 1000 fold dilution in DMSO 1uL → 999uL DMSO
- This dilution is stable if kept wrapped in tin foil at room temp.

8 After 30 minutes, flood stained cells with 3mL of 1X PBS.

9 Centrifuge 3500 RPM 5 to10 minutes.

10 Suspend pellet in 4 mL of 1X PBS.

11 Centrifuge 3500 RPM 5 to10 minutes.

12 Suspend pellet in 15uL of 1X PBS.

13 Prepare slides with a poly-L-lysine fixative

14 Add 9uL of stained cells to the fixative.

15 Add a drop of Anti-Fade reagent

16 Place a coverslip.
- Use Kim wipes on both sides of the slide and apply pressure to reduce the amount of floating bacteria.

17 Seal coverslip with VALAP. Smear it around the edges.
- Heat up Vaseline, Lanolin, and Parafin

18 Observe on Confocal.