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

Elizabeth Fozo¹¹In-house protocol

1 Works for me

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ABSTRACT

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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 to 10 minutes.

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

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

Use 7.5uL of a 10-3 diluted 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 to 10 minutes.

- 10 Suspend pellet in 4 mL of 1X PBS.

- 11 Centrifuge 3500 RPM 5 to 10 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.