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NAO staining

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[1]In-house protocol

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

Eadewunm

ABSTRACT

NAO staining protocol - Harp - 2014

PROTOCOL CITATION

Elizabeth Fozo 2021. NAO staining. **protocols.io** https://protocols.io/view/nao-staining-brgfm3tn

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ABSTRACT

NAO staining protocol - Harp - 2014

Steps

1 Incubate *Enterococcus faecalis at 37°C* overnight in 10mL of Brain Heart Infusion (BHI) broth.
- Started with an isolated colony.

2 The next morning check OD_{600} of overnight culture. Should be around ~1.5.

3 Diluted overnight culture to 0.01 using fresh 10mL BHI broth.

4 Add supplements as necessary.

5 Incubate at 37^{o}C without shaking.

6 After ~3 hours check OD of cultures.
- Looking for mid-long phase OD_{600} ~0.4.
- Bile will need its own dilution blank.
- Supplements may need more than 3 hours of incubation.

7 Once the cultures have hit OD_{600} ~0.4, centrifuge cells at 4^{o}C for 5 minutes at 3500 RPM.

8 Re-suspend in equal volumes of 1x PBS.
- (if 10mL culture, add 10mL of PBS).

9 Take a 1mL aliquot of re-suspended culture and stain with 12.5ug of 10^{-2} NAO (100mg in mL) = 12.5uL.
- NAO is diluted with DMSO.

10 Incubate at RT for ~30 minutes in the dark.

11 Flood 1mL of cells/NAO with ~4mL of 1xPBS. Centrifuge at 4^{o}C for 5 minutes at 3500 RPM.

12 Pour off supernatant - flood with 1x PBS - centrifuge.

13 Re-suspend pellet with 20uL of 1x PBS.

14 Take 8uL of stained cells and put on slide - take to microscope.