



Jan 14, 2021

# NAO staining

Elizabeth Fozo<sup>1</sup><sup>1</sup>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>

## LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

## CREATED

Jan 14, 2021

## LAST MODIFIED

Jan 14, 2021

## PROTOCOL INTEGER ID

46311

## DISCLAIMER:

DISCLAIMER: THIS WORK IS IN PROGRESS. IT IS FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](https://protocols.io) is not peer-reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](https://protocols.io), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

## 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<sub>600</sub> 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°C without shaking.
- 6 After ~3 hours check OD of cultures.
  - Looking for mid-long phase OD<sub>600</sub> ~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<sub>600</sub> ~0.4, centrifuge cells at 4°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<sup>-2</sup> 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°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.