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Approximating silica uptake in diatoms using PDMPO and flow cytometry

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Protocol status: Working

We use this protocol and it's working

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Abstract

A protocol to quantify relative change in uptake of silica by diatoms using the proxy stain PDMPO.

This protocol is based on methods developed in:

McNair, H. M., Brzezinski, M. A., and Krause, J. W. (2015). Quantifying diatom silicification with the fluorescent dye, PDMPO. *Limnol. Oceanogr. Methods* 13, 587–599. doi: 10.1002/lom3.10049

McNair, H. M., Brzezinski, M. A., and Krause, J. W. (2015). Quantifying diatom silicification with the fluorescent dye, PDMPO. *Limnol. Oceanogr. Methods* 13, 587–599. doi: 10.1002/lom3.10049

Leblanc, K., and Hutchins, D. A. (2005). New applications of a biogenic silica deposition fluorophore in the study of oceanic diatoms. *Limnol. Oceanogr. Methods* 3, 462–476. doi: 10.4319/lom.2005.3.462

Baker, K. G., Robinson, C. M., Radford, D. T., McInnes, A. S., Evenhuis, C., and Doblin, M. A. (2016). Thermal performance curves of functional traits aid understanding of thermally induced changes in diatom-mediated biogeochemical fluxes. *Front. Mar. Sci.* 3:44. doi: 10.3389/fmars.2016.00044

This protocol is contained in:

Argyle, P. A., Hinnert, J., Walworth, N. G., Collins, S., Levine, N. M., & Doblin, M. A. (2021). A high-throughput assay for quantifying phenotypic traits of microalgae. *Frontiers in microbiology*, 12, 706235.

Image Attribution

P. Argyle

Materials

 LysoSensor™ Yellow/Blue DND-160
 Thermo Fisher Scientific Catalog #L7545

Equipment

CytoFLEX LX

NAME

Flow cytometer

TYPE

Beckman Coulter

BRAND

C40312

SKU

<https://www.beckman.com>

LINK

CytoFLEX LX N3-V5-B3-Y5-R3-I2 Flow Cytometer (21 Detectors, 6 Lasers)

SPECIFICATIONS

Equipment

48 well Clear TC-treated Multiple Well Plates

NAME

Tissue culture plate

TYPE

Costar

BRAND

3548

SKU

<https://ecatalog.corning.com/>

LINK

Equipment

96 Well TC-Treated Microplates

NAME

Microplate

TYPE

Corning®

BRAND

CLS3799-1EA

SKU

<https://www.sigmaaldrich.com>

LINK

Equipment

Breathe-Easy® sealing membrane

NAME

Plate seal

TYPE

Breathe-Easy®

BRAND

Z380059-1PAK

SKU

<https://www.sigmaaldrich.com>

LINK

Protocol materials

 LysoSensor™ Yellow/Blue DND-160 Thermo Fisher Scientific Catalog #L7545



Stock solution of PDMPO

- 1 Create a stock solution of PDMPO (2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl)methoxy)phenyl)oxazole), also sold as LysoSensor™ Yellow/Blue DND-160, of 12.5 micromolar (μM) by diluting the 1 millimolar (mM) purchased solution in Milli-Q water.

 LysoSensor™ Yellow/Blue DND-160 Thermo Fisher Scientific Catalog #L7545

Sample culture and fix for cell counts

- 2 Take a 200 μL aliquot of each experimental culture/well (if growing in well-plates) and transfer into a 96 well round-bottomed plate to count via flow cytometry (see step 6).

Equipment

96 Well TC-Treated Microplates

NAME

Microplate

TYPE

Corning®

BRAND

CLS3799-1EA

SKU

<https://www.sigmaaldrich.com>

LINK

Initiate the assay

- 3 Transfer 2 500 μL aliquots of culture into a 48-well tissue culture plate. One to act as a control, the other to be treated with PDMPO.



- 4 Add  5 μL of the PDMPO stock made in step 1 to the treatment wells. This results in a final concentration of **[M] 0.125 micromolar (μM)**
- 5 Seal the plates with a sealing membrane and agitate gently to ensure even mixing of the stain.

Equipment

Breathe-Easy® sealing membrane

NAME

Plate seal

TYPE

Breathe-Easy®

BRAND

Z380059-1PAK

SKU

<https://www.sigmaaldrich.com>

LINK

Initial cell counts and background fluorescence

- 6 Using a flow cytometer, count the concentration of the culture at T0 from the fixed aliquot using the protocol outlined below.

Protocol

NAME

Measuring growth rates of diatom cells in culture

CREATED BY

Phoebe Argyle

Preview

End the assay



- 7 24 hours after the initiation of the assay, remove plates from the incubator and aliquot  200 µL of each culture (stained and unstained) into a round-bottomed 96 well plate for flow cytometry. Use a scalpel blade to slit the plate seal over each well.

Equipment

96 Well TC-Treated Microplates

NAME

Microplate

TYPE

Corning®

BRAND

CLS3799-1EA

SKU

<https://www.sigmaaldrich.com>

LINK

- 8 Measure the PDMPO fluorescence of the stained and unstained cells using the flow cytometer, measuring at least 200 cells. PDMPO fluorescence is quantified using the near UV channel (275 nm excitation/490-530 nm emission).

Also record the cell concentration.

Calculations

- 9 For the control samples:

Measure the median PDMPO fluorescence of at least 200 cells for each sample using the same method as in step 8.

Calculate the growth rate of the unstained cells over 24 hours using the equation:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

where N is the number of cells per mL at time 2 (24 hours) and time 1 (initiation) and t2 and t1 are 1 and 0 (time in days).

10 For the stained samples:

Measure the median PDMPO fluorescence of at least 200 cells for each sample using the same method as in step 8.

Calculate the difference in median fluorescence between the stained and unstained cells after 24 hours.

To calculate the metric, use the following equation:

$$\text{Silicification metric} = \frac{\Delta \text{ median PDMPO fluorescence over 24 hours}}{\text{growth rate over 24 hours}}$$

Note

Note, sometimes a double peak is observed during the flow cytometry measurements as cells divide (see image). This is accounted for by using the median fluorescence measurement.

