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Argyle, P.A., Hinners, 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(2910).

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., Hinners, 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

Stock solution of PDMPO

1 Create a stock solution of PDMPO (2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl)methoxy)phenyl)oxazole), also sold as LysoSensorTM Yellow/Blue DND-160, of [M] 12.5 micromolar (µM) by diluting the [M] 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

Initiatial 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 intiation 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.