PDMPO protocol and optional fixation steps for tracing newly deposited silica in plagiogrammacean auxospores and their progeny

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Abstract

This protocol describes the tracing of newly deposited silica using PDMPO (2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl)methoxy)phenyl)oxazole) in reproductive studies of diatoms.

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Protocol

PDMPO source

Step 1.

PDMPO (2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl)methoxy)phenyl)oxazole) from Thermo Fisher Scientific, Waltham, MA, contains 20 vials of 50 µL aliquots of 1 mM PDMPO (store in dark, -20^{0}C). A working 100 µM stock is obtained by diluting the 50 µL aliquot of 1 mM PDMPO in 450 µL MilliQ water immediately before use, as per manufacturer's recommendations.

PDMPO dilutions

Step 2.

A final working concentration in the solution containing the sample of 1 µM is recommended by the manufacturer, but a concentration as low as 0.125 µM was still effective and lasting (as in Leblanc & Hutchins 2005). This step is likely species/sample specific and needs to be determined for the purpose of the experiment.

Incubation of samples

Step 3.

Sample with the probe may have to be incubated for 24 hours under the same growth conditions as before PDMPO treatment, when completed, strongly silicified frustules elements are of interest (Znachor and Nedoma 2008). Lightly silicified sequentially deposited elements of the wall are best observed just after a few hours of incubation; the exact time is best determined by testing for individual species and wall element.

Preparation of microscope slides

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Step 4.

Cells were allowed to settle before harvesting and excess of media and PDMPO siphoned off to a volume convenient for microscopical preparation. Both live or fixed (2.5% glutaraldehyde, see annotation below) cells may be examined and imaged. We found that even gentle vacuum suction filtration may damage the cells sufficiently to decrease the usefulness of the preparation.

NOTE: the amount of prepared material may be sufficient for more than one slide – use your judgement. Take care not to damage the cells due to weight of the cover slip. Supporting the cover slip with a Vaseline dike reduces the chance of cell damage and slows down drying of the specimen.

Specimen storage

Step 5.

If necessary, glass slides containing fixed specimens may be preserved in -20^{0}C in darkness until use. We have stored specimens up to several days without noticeable deterioration of PDMPO fluorescence.

Epifluorescence parameters

Step 6.

Cells can be examined with settings typical for DAPI epifluorescence, but fluorescence cubes employing a long pass emission filter gave better results. For example we have used a Zeiss HBO 100 fluorescence illuminator and Filter Set 01 for reconnaissance work (Zeiss Axioskop 2 plus), and a Colibri LED fluorescence illuminator (365 nm LED) and Filter Sets 62HE and 49 was used for in-depth investigation (Zeiss AxioImager.Z2). Simultaneous DAPI and PDMPO epifluorescence is very useful and the color differences (blue for DAPI, green for PDMPO) are easily distinguishable by eye and most color cameras used for epifluorescence work

NOTES

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References

Leblanc K and Hutchins DA 2005 New applications of a biogenic silica deposition fluorophore in the study of oceanic diatoms. Limnology and Oceanography: Methods 3, 462-476

Znachor P and Nedoma J 2008 Application of the PDMPO technique in stainng silica deposition in natural populations of *Fragilaria corotonenis* (Bacillariophyceae) at different depths in a eutrophic reservoir. Journal of Phycology 44: 518-525.

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Glutaraldehyde fixation

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1. Prepare 2.5% glutaraldehyde in seawater from e.g. 25% EM grade glutaraldehyde (Electron Microscopy Sciences, Hatfield PA). Both glutaraldehyde and seawater should be at 4 °C before mixing, a kept at that temperature until use.
2. Allow the sample to be fixed to settle and then concentrate it to the smallest volume practical (~ 1-2 mL).
3. Add the sample to a 10x volume of 2.5% glutaraldehyde, mix well and store at 4 °C for a few hours. If the sample is rich in biological material, re-fix after 24 hours. This requires siphoning off the old fixative and replacing with the same volume of fresh 2.5% glutaraldehyde. Storing at 4 °C and darkness keep such samples useable for DAPI staining for several years, up to 5 years in our experience.

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