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Detection of bacteria in antibiotic-treated diatom cultures and cell harvesting

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1 Works for me dx.doi.org/10.17504/protocols.io.btt5nnq6

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ABSTRACT

We illustrate a simple and rapid method for detecting the presence/absence of bacteria in diatom cultures using DAPI and fluorescence microscopy.

We also describe how we harvest cells for genomic DNA extraction once we have assessed that bacteria are absent from the culture.

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KEYWORDS

DAPI, AXENIC, DIATOMS, MICROSCOPY

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- 1 For the antibiotic treatment to obtain axenic cultures, see the published protocol:

Axenic Diatoms cultures protocol
dx.doi.org/10.17504/protocols.io.bgudjws6

DMI6000 b
Fully Automated Inverted Research Microscope

L
E [https://www.leica-](https://www.leica-microsystems.com/products/light-)
I [microsystems.com/products/light-](https://www.leica-microsystems.com/products/light-)
C
A



Transfer 1 mL of Culture in a new 2 mL tube.



Add 1,6% of neutralized formaldehyde to fix cells and mix gently by inversion


- 3 Add 1uL of DAPI 1 mg/mL and mix gently by inversion.

Keep away from direct light

[4,6-Diamidino-2-Phenylindole, Dihydrochloride \(DAPI\) Thermo Fisher Scientific Catalog #D1306](#)

- 4 Transfer cells in a 35mm glass bottom dish.

µ-Dish 35 mm, high Glass Bottom
A 35 mm imaging dish with a glass bottom for
use in TIRF, single molecule and super-
resolution microscopy applications

i
b [https://ibidi.com/dishes/176--dish-35-mm-](https://ibidi.com/dishes/176--dish-35-mm-high-glass-bottom) 
i high-glas
d
i



Incubate for 10 min away from direct light to permit cells sedimentation

5 

Use an inverted microscope with fluorescence excitation.

* We use a Leica DMI 6000 B microscope.

Set light sources:

- **bright-field**
- **Fluorescence for chlorophyll (580 / 604 nm)**
- **Fluorescence for DAPI (364 / 454 nm)**

6 Assess bacteria absence-presence in the cultures.

Move across the plate, focusing on the cells and on the bottom of the dish.

7 Capture different photos

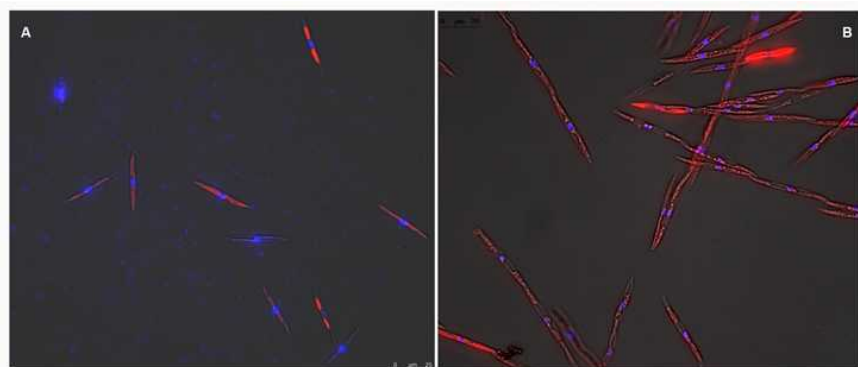


Fig.3A. Normal culture with bacteria (blue spots)

Fig.3B. Axenic culture without bacteria.

Cells filtration

- 8 Filter cells on MF-Millipore™ Membrane Filter, 1.2 µm pore size, gridded 47 mm.



- 9 Move the filter in a new 50 mL tube using tweezers



- 10 Rinse the filter with 1 mL of clean medium using a pasteur pipette

pipet gently, to avoid cell lysis



- 11 Collect the resuspended cells in a new 15 mL tube



- 12 Pellet cells by centrifugation
15 min. 2000 g 18°C and discard supernatant



- 13 Freeze pellet immediately in liquid nitrogen

14 Store at -80°C