Fluorescence microscopy with the marine heterotrophic flagellate Cafeteria roenbergensis

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1 Works for me

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Protist Research to Optimize Tools in Genetics (PROT-G)

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ABSTRACT

Fluorescence microscopy is essential to understand, between others, the cell organization and the life cycle of viruses that infect them. This protocol describes the steps neccesary to fix and permeabilize *Cafeteria roenbergensis* cells allowing its staining with antibodies or chemical reactions as Click-IT reactions.

MATERIALS

NAME	CATALOG #	VENDOR
1.5 mL Eppendorf tubes		
PBS		
Sterile glass slides		
Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS)		
Centrifuge		
Coverslip		
0.5% Triton in PBS1x		
ELGA water		
0.45 µm 25 mm diameter Millipore HA filter	View	
0.2 µm 25 mm Whatman Anodisc filter	View	
Glycerol		
Multi-hole vacuum system		

Remove bacteria from *Cafeteria roenbergensis* culture

1 Follow the protocol: "*Lysozyme-based removal of bacteria from cultures of the marine heterotrophic flagellate Cafeteria roenbergensis*"

If the culture is to be treated with other reagents, for example L-Azidohomoalanine, dilute the cells without bacteria to the required density and apply the treatment.

2 Aliquot 5 x 10^6 *Cafeteria* cells per 1.5 ml microfuge tube for each microscopy sample

Fixation and permeabilization

3 Centrifuge for 5 min at 5,000 g, 20ºC

4 Resuspend each pellet in 500 µL of 4% paraformaldehyde (PFA) in 1X PBS

5 Incubate for 20 min at 20ºC

6 Centrifuge for 5 min at 7,000 g, 20ºC

You can keep the sample overnight at 4 ºC by resuspending the pellet with 250 µL 1X PBS
On the following day, centrifuge for 5 min at 5,000 g, 20ºC and continue with the next step

7 Resuspend the pellets in 500 µL of 0.5% Triton in 1X PBS

8 Incubate for 15 min at 20ºC

Filters preparation to Cafeteria immobilization

9 Clean the filter-support grids of a vacuum manifold system with pure water (e.g. ELGA, milliQ)

10 Add a drop of ELGA water on each grid of the vacuum system that you want to use and place a 0.45 µm Millipore support filter on top of it

11 Apply vaccum to remove the drop of water

12 Add another drop of ELGA water on top of the Millipore filter

13 Place a 0.2 µm, 25 mm Anodisc Whatman filter on top of the ELGA water drop

14 Apply vacuum with a pressure between 5-10 Hg to remove the water

15 If neccesary, block or wash the filter with the required solution.
Remove the solution by applying vacuum

16 Place the *Cafeteria* sample from step 8 on top of the Whatman filter

17 Apply vacuum with a pressure between 5-10 Hg to remove the permeabilization buffer and to immobilize the cells on the filter

Perform all the neccesary blocking and washing steps following the same instructions

Sample staining

18 Place the filter with the *Cafeteria* sample on a piece of Parafilm. Add the staining solution of interest on top of the filter and incubate following the manufacturer's instructions

19 Place the filter on the vacuum system again

20 Apply vacuum with a pressure between 5-10 Hg to remove the remaining staining buffer

21 If required, wash the filter again with the buffer of interest

22 If desired, stain DNA by incubating with an appropriate reagent (DAPI, SYBR, Hoechst)

23 Apply vacuum with a pressure between 5-10 Hg to remove the remaining liquid

Sample mounting

24 Add 10 µL of 70% glycerol on a glass slide and place the filter on top of the glycerol

The filter must be placed with the Cafeteria cells in the top!

25 Add 10 µL of 70% glycerol on the coverslip and place it on top of the filter

26 Apply pressure on the sample during 20 min at room temperature

27 Seal the coverslip with nail polish

Samples can be kept at 4 °C or analyzed with the microscope immediatly