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Starvation & washing protocol for anaerobic ciliates

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1 Works for me [dx.doi.org/10.17504/protocols.io.868hzhw](https://doi.org/10.17504/protocols.io.868hzhw)

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MATERIALS

NAME ▾

DNA/RNA Shield

CATALOG # ▾

R1100-50

VENDOR ▾

Zymo Research

Cell picking

- 1 To pick ciliate cells, use a sterile drawn glass Pasteur pipette, a sterile depression slide with multiple wells, and inverted microscope.

Transfer several hundred live cells into clean media (0.22 µm filtered).

Cell washing

- 2 Then, pass the cells through 3 wells of 0.22 µm filtered clean media.
- 3 Finally, pass the remaining healthy looking and active cells into a sterile 1.5 ml eppendorf tube with 0.5 ml of clean, 0.22 µm filtered and hypoxic media.

Starvation

- 4 Let the cells starve for 4 - 12 hours, if the species or group allows this.
 - 4.1 Using clean glass micropipette, isolate one cell after each hour, place it onto a microscope slide and observe under a light microscope. Depending on the conditions of the cell, presence of full food vacuoles and overall health, extend or decrease the starvation time.
 - 4.2 Starvation time has to be specifically adapted to each ciliate taxa, depending on oxygen tolerance, general endurance, and sensitivity to other conditions.
 - 4.3 If any bacterial growth appears during starvation, transfer the cells into clean filtered media, proceed to wash three times again, and place cells into a new eppendorf tube with clean filtered hypoxic media.
- 5 As soon as you determine absence of food vacuoles in sufficient amount of ciliate cells and at the same time, no bacterial growth is present in the media, end the starvation and proceed to wash.

Washing

- 6 Carefully wash starved cells in a drop of sterile hypoxic filtered media.
- 7 Transfer cells through four to six drops of sterile hypoxic filtered media, depending on the cell conditions, reduce number of washings if the cells tend to burst and increase if you see free-living prokaryotes.
- 8 Transfer 50 - 100 cleaned starved cells into a sterile eppendorf tube with 200 μ l of DNA Shield product (Zymo Research) and proceed to DNA isolation.



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