

Version 1

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# Isolation of clean single-cell samples for physiological or molecular experiments (Radiolaria, Acantharia) V.1

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1 Works for me

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Symbiosis Model Systems

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## ABSTRACT

Many marine protists are not culturable and therefore challenging to study, nonetheless, they are essential in all marine ecosystems. The development of single-cell techniques is allowing for more marine protists to be studied. Thereby Cultivation-independent studies are essential for the study of organisms sensitive to the sampling procedure, such as Radiolaria.

This method describes the means to acquire clean isolated single-cells from plankton nets for physiological and/or transcriptomic analysis. The isolation of cells in this protocol is followed by preparing the cells for RNA extraction (protocol: Single-cell total RNA extraction from marine protists (e.g. Acantharia, *Strombidium cf. basimorphum*, and *Prymnesium parvum*)).

## DOI

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## KEYWORDS

RNA-seq, Radiolaria, cell isolation, Acantharia, protist, plankton, sampling, Single-cell, RNA, protist, plankton

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## GUIDELINES

This work is conducted under field conditions, where facilities are often not as good as in terrestrial-bound laboratories. Thus, sample preparations are typically not conducted working in a controlled air-stream, under a hood, nor has the work area necessarily been treated with RNase inhibitors (or similar). Aseptic techniques and gloves, to minimize further contamination, have been used though.

## MATERIALS TEXT

- [Petri](#)
- [Dish P212121 Catalog #LI-PD01100](#) In 2 steps (glass Petri dishes or 6 well plates can serve the same function, though at least one larger Petri dish is recommended)
- [PCR Tubes & Caps, RNase-free, 0.2 mL \(8-strip format\) Thermo](#)
- [Fisher Catalog #AM12230](#) (x  
#samples)
- Micropipette and 100 µl filter pipet-tips
- [Ice Contributed by users](#)
- [Liquid nitrogen in dewar Contributed by users](#) (optional, but preferred)
- [Borosilicate Glass Disposable Pasteur Pipettes, Plain End, 145mm Thermo](#)
- [Fisher Catalog #CP2555402](#)
- [Bunsen burner Contributed by users](#) (a portable Spirit burner can also do the job)
- Tubing to fit the glass Pasteur pipet
- [Filter mouthpiece](#)
- [Stericup Quick Release-GP Sterile Vacuum Filtration System Millipore](#)
- [Sigma Catalog #S2GPU02RE](#) (or other  
0.2 µm-pore-size filtering method)
- [Ethanol Contributed by users](#) for cleaning
- [RNAqueous&trade;-Micro Total RNA Isolation Kit Thermo](#)
- Lysis buffer from [Fisher Catalog #AM1931](#)

Stereo microscope EZ4, Leica 10447197  
Leica EZ4 educational stereomicroscope.



(or another preferred stereo microscope that allows for micro handling in a Petri dish under it).

## SAFETY WARNINGS

Great care must be taken in the sampling method, for Radiolaria are fragile.  
For endosymbiotic uncultivable plankton, sampling using slow horizontal plankton net tows are proven to be the best compromise between ease of sampling and retaining cell quality.

Clean single-cells can be acquired by this protocol for any plankton. The sample preparation for RNA extraction is though only verified for Acantharia, *Strombidium cf basimorphum*, and *Prymnesium parvum*.

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Many marine protists are not culturable and therefore challenging to study, nonetheless, they are essential in all marine ecosystems. The development of single-cell techniques is allowing for more marine protists to be studied. Thereby Cultivation-independent studies are essential for the study of organisms sensitive to the sampling procedure, such as Radiolaria.

This method describes the means to acquire clean isolated single-cells from plankton nets for physiological and/or transcriptomic analysis. The isolation of cells in this protocol is followed by preparing the cells for RNA extraction (protocol: Single-cell total RNA extraction from marine protists (e.g. Acantharia, *Strombidium cf basimorphum*, and *Prymnesium parvum*)).

#### BEFORE STARTING

Collect your favorite marine protists with a plankton net of choice.  
We use a 150 µm-mesh-size plankton net for Acantharia.

#### Preparations

30m

- 1 Prepare filter-sterilised seawater (FSW, 0.2 µm-pore-size)
- 2 Prepare your Pasteur pipet isolation tool by elongating the pipet tip under a flame. Carefully break the tip of the pipet to the desired length (and width, longer is thinner).  
Attach the tubing and optionally a filter on the other end.



Bend and elongated Pasteur pipet with tubing and mouthpiece filter

#### Single-cell isolation

4h

- 3 Acquire a sub-sample from a concentrated plankton net sample in a

 Petri

Dish P212121 Catalog #LI-PD01100

#### 3.1 Fill another Petri dish with FSW

- 4 Working under a

Stereo microscope EZ4, Leica 10447197  
Leica EZ4 educational stereomicroscope.



Isolate healthy cells from the sub-samples into the [Dish P212121 Catalog #LI-PD01100](#) with clean FSW using a micropipette or modified Pasteur pipette.

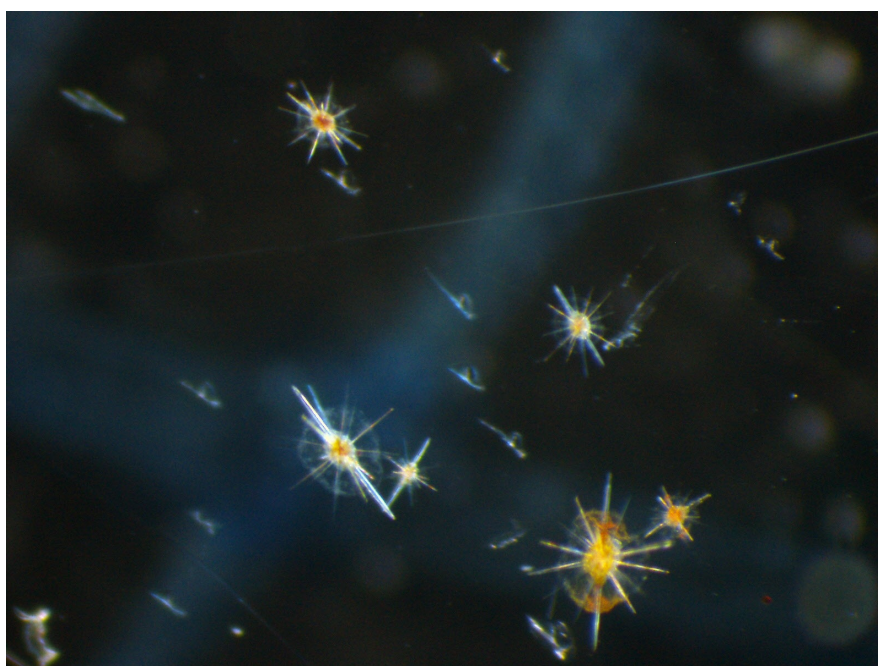
Dedicate no more than 20 Acantharia cells to a single Petri dish.

5 Incubate cells in FSW for [🕒01:00:00](#) . 1h

5.1 Repeat the isolating procedure with a transfer to fresh FSW and [🕒01:00:00](#) incubation a total of <sup>2h</sup>  
**three times.**

This procedure allows for self-cleaning of particles attached to the cells and dilution to achieve effective extinction of any contaminating organisms accidentally taken with during isolation.

6 Clean cells can be used for physiological experiments before proceeding to the next step.



Some isolated Acantharia

Isolation for RNA extraction 30m

7 Prepare small plastic zip-locked bags for the samples along with a sample label (pencil on water-proof paper).

8 Prepare 100 µL lysis buffer (from RNAqueous kit) in 0.2 mL PCR tubes ⚠ **On ice**

Where possible continue to work on ice

9 Transfer one cell per lysis buffer-filled tube and put it in a dedicated small plastic bag.  
It is preferred to use a micropipette and 100 µl filter tips. Setting the volume to the minimum allows isolation of the cells with very little residue and a consistent amount of FSW.

**Immediately** freeze in liquid nitrogen.

Disregarding freezing in liquid nitrogen, and proceeding immediately to storage at ⚠ **-80 °C** has proven to suffice. Though it is still preferred to freeze in liquid nitrogen when possible, especially for physiological experiments.

10 Store quickly at ⚠ **-80 °C**

RNA extraction

11 Follow the protocol for RNA extraction.

Single-cell total RNA extraction from marine protists (e.g. Acantharia, *Strombidium cf. basimorphum*, and *Prymnesium parvum*).

<https://www.protocols.io/private/EDB0E27A323211EB952C0A58A9FEAC2A>