



Bocas ARTS



Protocols for Preserving Sponges

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These protocols are based on those used by the Porifera Tree of Life (PorToL) project and can be found at www.portol.org/protocols

Basic Preservation for DNA Extractions and Limited Morphological Work

- A) Each individual specimen should be placed into a separate container. We find that 50 ml tubes or WhirlPaks work well.
- B) Be sure to label the outside of the container with your specimen ID number. If you do not already have a system in place, you could use your initials followed by a number (e.g., RT53; or in conjunction with a collection event, e.g., Bali2010-RT53).
- C) Appropriately label a small piece of solvent-resistant paper with pencil and place inside each specimen container.
- D) Before fixing each specimen, carefully remove all dirt and contaminating macroorganisms, if possible.
- E) Place specimen into the container and fill with 95% ethanol. It is best to fix a portion of the sponge that includes ectosome and choanosome. If a specimen is very large, these may need to be separate samples. We find that a 1:2 ratio of specimen:ethanol works well. For thick specimens, partially dissecting the specimen can enhance preservation. Note that this concentration will dilute, since the specimen contains water itself.
- F) After 12-24 hours, change the fixative, replacing it with fresh 95% ethanol. If pigments continue to be extracted from the specimen, an additional change can improve preservation. Be sure to note the color of any extracted pigments.
- G) Store at 5°C or room temperature.

Preservation for Histology using 4% paraformaldehyde (PFA) and 70% ethanol

For many morphological features, especially those observed with histological techniques, it is useful to fix specimens in 4% PFA, followed by storage in 70% ethanol. However, specimens processed in this manner yield little to no successful DNA extraction, PCR, and sequencing. The following procedure works well to prepare specimens for histological sections used in confocal microscopy and FISH staining.

- A) Prepare fixative by first filter-sterilizing seawater; then add PFA for a final concentration of 4%.
- B) Incubate specimen in 4% PFA overnight.
- C) Transfer specimen to 70% ethanol.
- D) Store at 5°C or -20°C.



Preservation for RNA (including microRNAs) using a 75% ethanol series at -20°C

To adequately preserve RNA, and particularly ensuring the preservation of microRNAs, multiple changes of ice-cold 75% ethanol can be used.

- A) Keep materials on ice or ice-cold at all times.
- B) It is best to preserve 2 “thumb-size” pieces (or 4 1-cubic-cm pieces) in a 50 ml tube of ethanol. The larger volume ratio of ethanol:sponge, the better.
- C) Put fresh tissue in 75% ethanol.
- D) Change to fresh 75% ethanol after 15 minutes.
- E) Change to fresh 75% ethanol after 1 hour.
- F) Change to fresh 75% ethanol the next day.
- G) Store at -20°C.

Preservation for RNA/DNA using RNAlater (Ambion, Inc.)

This commercially available fixative is often used with success; other labs find it problematic. It is apparently not a good means for preserving microRNAs. Best practices are as follows:

- A) Maintain a fixative:tissue ratio of 5:1 at least. More fixative is better.
- B) Cut the tissue into very small pieces, e.g., 1-2 mm cubes.
- C) Incubate overnight at room temperature.
- D) Store at -20°C or -80°C.
- E) Some labs report better nucleic acid extraction if pieces are rinsed in sterile water prior to extraction.

This protocol is adapted from previous work by the Porifera Tree of Life project (www.portol.org).