

## Fixing and Embedding Protocol for Octocorals and Their Sclerites

Elizabeth Bray, SCE&G Central Laboratory  
bbray@sc.rr.com

Many organisms are mostly soft tissue, with hard bits embedded within them. This includes many invertebrates, and vertebrates with calcified areas in their tissues. This procedure works well for soft-bodied corals which have hard pieces of calcium carbonate (sclerites) embedded in their tissues. It should also work for similar soft-tissue/calcified bits in other animals.

### Reagent Preparation:

Buffer: 0.1 M NaCacodylate (CH<sub>3</sub>)<sub>2</sub>AsO<sub>2</sub>Na · 3H<sub>2</sub>O, MW = 214.02

21.4 g NaCacodylate (NaCaco ) diluted to 1000 mL with DW. Adjust the pH to 7.4 with 4% NaOH or 1:10 HCl.

Fixative: 2% paraformaldehyde (PFA), 0.15% glualdehyde (GA), 0.05 M NaCacodylate in filtered sea water:

6.3 mL of 16% PFA

0.15 mL of 50% GA

25.0 mL of 0.1 M NaCacodylate, pH = 7.4

18.55 mL of Millipore filtered Sea Water

Total Volume of fixative = 50 mL

Embed in LR White acrylic embedding medium.

### Procedure:

1) Fix the specimen in PFA/GA/NaCaco fixative for 1 hour.

2) Rinse in 0.05 M NaCaco Buffer 3 x 10 minutes.

3) Dehydrate in a series of EtOH (ethyl alcohol) washes of ever increasing strength:

50% EtOH 10 minutes.

75% EtOH 10 minutes.

95% EtOH 15 minutes.

100% EtOH 15 minutes x 2, done under vacuum.

4. Three (3) changes of LR White, all done under vacuum. The first two changes for at least 30 minutes, and the last change overnight. The last change should be done in the BEEM capsules in which the specimen will be cured.

NOTE: Be sure that when first filling the capsules with the LR White for the final time, there are no air bubbles in them. This will cause the level of embedding medium to become lower in the capsule as it sits overnight under vacuum, and when they are capped for curing in the oven, the air inside will lead to incomplete curing making it difficult, if not impossible, to properly section the hard coral material. To ensure that there are no bubbles present, allow the capsules to sit under vacuum for 30 minutes to 1 hour, go back and top off any capsules that need it, then reestablish the vacuum and allow them to sit overnight. Before capping, ensure that the level of embedding medium is at the top of the capsule. If it is not, carefully add sufficient medium to restore the proper level and proceed with capping.

5. Cap to ensure the exclusion of air and cure overnight in a 60 degree C oven. ■

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