

Protocol for Processing Sorus Tissue

Ensure your work area is clean and sterilize any surfaces that will be used with a generous amount of ethanol.

1. Gather supplies:

- ☐ Gloves
- ☐ Ethanol spray bottle
- ☐ Absorbent paper towels or kimwipes
- ☐ Permanent marker
- ☐ Razor blades or scissors
- ☐ Small beakers (100–200 mL)
- ☐ Pasteurized seawater
- ☐ 0.15% sodium hypochlorite solution
 - For other cleaning in the nursery, you can use household bleach with no added chemicals, but use sodium hypochlorite instead of household bleach when handling live material.
- ☐ Cotton balls or soft sponge pieces
- ☐ Cutting board
- ☐ Tweezers

2. Ensure all supplies and the working surface are clean.

3. Start by preparing your media and bleach solution.

- You will need about 200 mL of seawater and 75 mL of 0.15% sodium hypochlorite solution per blade you are processing.
- Since the NaOCl solution is volatile, prepare it in small batches and use immediately.

4. On a clean surface outside of the hood, begin cutting out the sorus from the blade with scissors or a razor blade. The washers ensure that the bleach touches every part of your spool. Bleach is only effective through contact.

- Only handle one blade at a time to avoid cross contamination.
- Cut away all pieces of the blade that are not sorus.
- Do not keep any pieces of the sorus that look like they have epiphytes or are otherwise fouled.

5. Dip a cotton ball, clean paper towel, or small piece of soft sponge into pasteurized seawater to dampen it. Squeeze out the excess water.

6. Gentle wipe the surface of sorus with the sponge.

7. Place the wiped sorus into a labeled beaker and move to a chilled area if you are processing many individuals.

8. Once all of your sorus is cut from the blades and wiped clean, you will begin the rinsing process under a hood or on a separate cleaned surface.

- From here on, handle the sorus with clean tweezers, not by hand.

9. Pour enough 0.15% NaOCl solution into the beaker to cover the sorus and swirl frequently.

10. After 1 minute, pour the bleach solution out. Be careful to not dump any sorus material by holding it back with tweezers.

11. Pour pasteurized seawater over the sorus until it is submerged and swirl frequently.

12. After 2 minutes, pour the pasteurized seawater out.

13. Rinse with pasteurized seawater a second time.

14. After 1 minute, pour the pasteurized seawater out.

15. Use the tweezers to transfer the sorus to a paper towel.
16. Use the paper towels to gently dab the sorus until it is dry enough to have a matte appearance.
 - Do not rub the sorus.
17. Move the dabbed sorus to a new paper towel and arrange it in a single layer.
18. Cover the sorus with another paper towel and label the paper towel with the blade number and location.
19. Move the dried sorus wrapped in paper towels to a sealable container or plastic bag.
 - Do not completely seal the bag or container to allow air to vent.
20. Sorus from multiple plants can be stacked on top of one another in the same container as long as there are 2 layers of paper towel between them.
21. Sterilize the tweezers and begin this process for the next blade.
22. Do not store material out of the cold for more than 30 minutes.
23. Once all of the blades are processed, move the container to a cold, dark place for 16 to 24 hours before the release.