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Lemna minor (duckweed) sterilization protocol V.2

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ABSTRACT

This protocol is designed to sterilize fronds of the common duckweed (*Lemna minor*) but should also be applicable to other members of the *Lemnnoideae*. It involves both the surface sterilization of plants in bleach and testing for the presence of bacteria on plants through culturing in enriched media as a test for successful sterilization.

Duckweed Sterilization : Preparation

- 1 In a tea-strainer, strain duckweed plants under running water (doesn't have to be sterile). This step gets rid of some surface algae.
- 2 Move surface-rinsed duckweed into a water bath; remove up to 50 plants from water bath and place in 50 mL Falcon tube approximately half full with autoclaved Phosphate Buffered Saline (PBS).
- 3 Vortex or shake intensely for 5 minutes.

Duckweed Sterilization : Sterilization

- 4 All of the subsequent sterilization steps should take place in a safety cabinet.
- 5 Strain plants into a tea-strainer reserved for bleaching. Plants stuck to the side of the Falcon tube can be dislodge with distilled water.
- 6 Place in (1% sodium hypochlorite) bleach for 60 seconds.
- 7 Remove from bleach; rinse tea-strainer thoroughly by moving around in jar of autoclaved distilled water.
- 8 Soak in autoclaved distilled water for 5 minutes or more. Repeat this step 3 times, in a new jar of fresh autoclaved distilled water each time.
- 9 Open tea-strainer; pour autoclaved medium (e.g. Hoagland's, Krajncic medium) over tea-strainer to dislodge plants. This should be done over a clean, autoclaved jar (I use 500 mL Mason jars). Seal with lid and move to environmental chamber.

Checking Duckweed Sterility

- 10 Wait at least 3 days to give plants a chance to recover from the bleaching.
- 11 In the safety cabinet (or other sterile location), transfer plants to autoclaved Mason jar approximately half full of your ENRICHED media of choice. I add 1 g yeast extract and 10 g sucrose per L to make it enriched.
- 12 In as little as 2-3 days, you will know if your duckweed remains contaminated with bacteria – in this case, the media will become opaque as microorganisms replicate. If the media remains clear, congratulations!!! You have successfully sterilized your duckweed plants.
- 13 If the media is cloudy, wash plants and transfer to Mason jar with autoclaved, unenriched media (in safety cabinet). After plants have recovered (ie. look green and healthy, after a week or more) you can re-sterilize them until all bacteria are removed.



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