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Whole colony fixation V.2

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Works for me

dx.doi.org/10.17504/protocols.io.brw6m7he

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ABSTRACT

This protocol has been successfully tested on Botrylloides diegensis and Ciona robusta.

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PROTOCOL CITATION

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KEYWORDS

Botrylloides diegensis, Ciona robusta, colonial ascidians, fixation, colony

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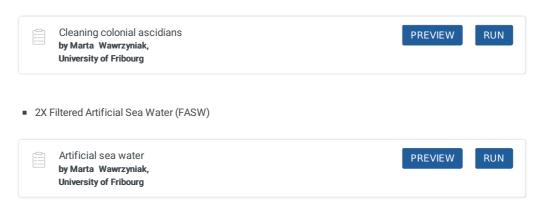
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GUIDELINES

Fixation is done in 50 mL Falcons for small (7.5 x 2.5 cm) microscopy glass slides. Solutions are thus detailed for 50 mL. Larger slides will need larger containers and hence higher volumes of solutions.

Reference for the anaesthesia: Eugenol, another way to anaesthetize ascidians

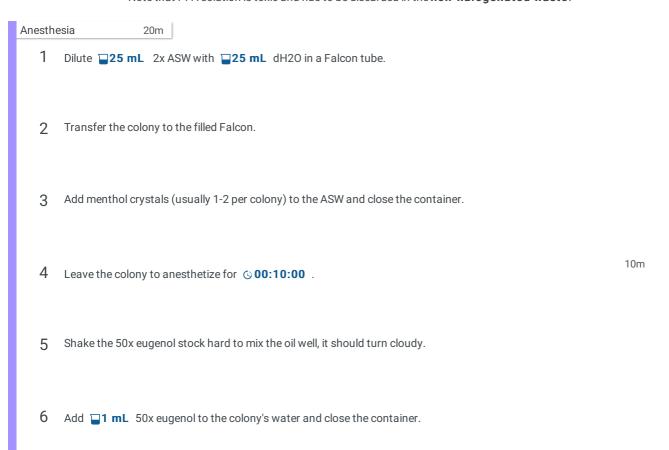
A cleaned Botrylloides colony to be fixed



- Menthol crystals: typically 1-2 1mm crystals per colony
- 50X eugenol in FASW: 50 drops of eugenol (clove oil) in 1 L 1X FASW
- Paraformaldehyde (PFA) powder (30525-89-4)
- 10 M NaOH: 40g NaOH in 100 mL H₂O
- 1 N HCl: 8 mL 12 N (37%) HCl in 92 mL H₂O

SAFETY WARNINGS

Note that PFA solution is toxic and has to be discarded in the **non-halogenated waste**.



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7 Leave until total relaxation of the colony's siphons; around **© 00:10:00**.

Fixative

- 8 Heat **□25 mL** dH20 in a beaker at § 60 °C using a microwave.
- 9 Place the beaker on a stirring hot 8 60 °C plate in a fume hood.
- 10 Add **22** g of PFA (4 % final concentration) powder to the stirring water.
- 11 Wait for the powder to sink in the water (increase stirring if needed).
- Add [M] 10 Molarity (M) NaOH drop by drop until the solution clears.
- Add [M] Molarity (M) HCl drop by drop until solution reaches pH 7.5-8.2.
- 14 Add **25 mL** 2x FASW and cool at § Room temperature.

Fixation 2h 30m

- 15 Mix **350 mL** 4 % PFA with **1 mL** 50X eugenol in a Falcon tube.
- 16 Transfer the anesthetized colony to the fixative.
- 17 Colony should be fully relaxed, if not extend anesthesia or increase eugenol.
- 18 Fix © Overnight over night at & Room temperature

 20 Wash twice © 00:30:00 in 1x FASW at & Room temperature (under rocking if available).

21 Wash three times © 02:00:00 in PBS (under rocking if available).

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Keep in PBS at § 4 °C .