



Version 2

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Whole colony fixation V.2

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Works for me

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ABSTRACT

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

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KEYWORDS

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

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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](#)

MATERIALS TEXT

- A cleaned *Botrylloides* colony to be fixed



Cleaning colonial ascidians
by Marta Wawrzyniak,
University of Fribourg

[PREVIEW](#)[RUN](#)

- 2X Filtered Artificial Sea Water (FASW)



Artificial sea water
by Marta Wawrzyniak,
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[PREVIEW](#)[RUN](#)

- 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**.

Anesthesia

20m

- 1 Dilute 25 mL 2x ASW with 25 mL dH₂O in a Falcon tube.
- 2 Transfer the colony to the filled Falcon.
- 3 Add menthol crystals (usually 1-2 per colony) to the ASW and close the container.
- 4 Leave the colony to anesthetize for 00:10:00 .
- 5 Shake the 50x eugenol stock hard to mix the oil well, it should turn cloudy.
- 6 Add 1 mL 50x eugenol to the colony's water and close the container.

10m

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

Fixative

- 8 Heat 🧊 **25 mL** dH₂O in a beaker at 🌡 **60 °C** using a microwave.
- 9 Place the beaker on a stirring hot 🌡 **60 °C** plate in a fume hood.
- 10 Add 🧊 **2 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).
- 12 Add [M] **10 Molarity (M)** NaOH drop by drop until the solution clears.
- 13 Add [M] **1 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 🧊 **50 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**

- 19 Rinse in 1x FASW.
- 20 Wash twice 🕒 00:30:00 in 1x FASW at 🌡 Room temperature (under rocking if available). 30m
- 21 Wash three times 🕒 02:00:00 in PBS (under rocking if available). 2h
- 22 Keep in PBS at 🌡 4 °C .