



Version 1

Feb 03, 2021

# Whole colony fixation V.1

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1

Works for me

[dx.doi.org/10.17504/protocols.io.bru4m6yw](https://dx.doi.org/10.17504/protocols.io.bru4m6yw)

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DOI

[dx.doi.org/10.17504/protocols.io.bru4m6yw](https://dx.doi.org/10.17504/protocols.io.bru4m6yw)

PROTOCOL CITATION

Simon Blanchoud 2021. Whole colony fixation. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.bru4m6yw>

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CREATED

Jan 27, 2021

LAST MODIFIED

Feb 03, 2021

PROTOCOL INTEGER ID

46716

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.

MATERIALS TEXT



- A cleaned *Botryllodes* colony to be fixed
- 2X Artificial Sea Water (ASW): see specific protocol
- Menthol crystals: typically 6-7 1 mm crystals per colony (< 1 g)
- 50X eugenol in ASW: 50 drops of eugenol (clove oil) in 1 L 1X ASW
- Paraformaldehyde (PFA) powder
- 10 M NaOH: 40g NaOH in 100 mL H<sub>2</sub>O
- 1 N HCl: 8 mL 12 N (37%) HCl in 92 mL H<sub>2</sub>O
- 10X PBS: 90 g NaCl, 5.2 g NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O, 23 g Na<sub>2</sub>HPO<sub>4</sub> in 1 L H<sub>2</sub>O

## 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 to the ASW and close the container.
- 4 Leave the colony to anesthetize for **00:10:00** . 10m
- 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.
- 7 Leave until total relaxation of the colony's siphons; around **00:10:00** . 10m

## 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 **10 Molarity (M)** NaOH drop by drop until the solution clears.
- 13 Add **1 Molarity (M)** HCl drop by drop until solution reaches pH 7.5-8.2.



14 Add  25 mL 2x ASW 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 ASW.

20 Wash twice  00:30:00 in 1x ASW at  Room temperature (under rocking if available). 30m

21 Wash three times  02:00:00 in PBS (under rocking if available). 2h

22 Detach the colony from the slide using a sharp razor blade.

23 Keep in PBS at  4 °C .