



## Whole colony fixation V.1

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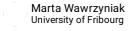




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#### **GUIDELINES**

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.

MATERIALS TEXT

- A cleaned Botrylloides colony to be fixed
- 2X Artificial Sea Water (ASW): see specific protocol
- Menthol crystals: typically 6-7 1 mm crystals per colony (< 1 g)</li>
- 50Xeugenol 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 Dilute **25 mL** 2x ASW with **25 mL** dH20 in a Falcon tube. Transfer the colony to the filled Falcon. Add menthol crystals to the ASW and close the container. 10m Leave the colony to anesthetize for **© 00:10:00** . Shake the 50x eugenol stock hard to mix the oil well, it should turn cloudy. Add 11 mL 50x eugenol to the colony's water and close the container. 10m Leave until total relaxation of the colony's siphons; around $\bigcirc$ **00:10:00** . Fixative Heat **■25 mL** dH20 in a beaker at § 60 °C using a microwave. Place the beaker on a stirring hot § 60 °C plate in a fume hood. 10 Add **22** g of PFA (4 % final concentration) powder to the stirring water. 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. 13 Add [M]1 Molarity (M) HCl drop by drop until solution reaches pH 7.5-8.2.

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Add 25 mL 2x ASW and cool at 8 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 8 Room temperature

19 Rinse in 1x ASW.

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.