Jun 07, 2016 Version 2

Buccopharyngeal morphology of tadpoles in Scanning Electron Micrography V.2

DOI
dx.doi.org/10.17504/protocols.io.e3rbgm6

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.e3rbgm6

Protocol Citation: Diogo Provete 2016. Buccopharyngeal morphology of tadpoles in Scanning Electron Micrography. **protocols.io https://dx.doi.org/10.17504/protocols.io.e3rbgm6**

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Protocol status: Working

Created: June 07, 2016

Last Modified: March 02, 2018

Protocol Integer ID: 2897

Materials

STEP MATERIALS

- Glutaraldehyde EM Grade 25% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G5882-50ML
- Osmium tetroxide solution 2% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**75633
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1 Wash quickly the specimens in tap water and dissect tadpoles following **Wassersug (1976: p. 4-5)**.

Note

Usually tadpoles are fixed and conserved in 10% formalin, buffered or not. I'm here assuming that your samples are already in formalin.

2 Fix the dissections in 4% Glutaraldehyde.

02:30:00

Note

To make this fixing solution, you'd use 200 mL of Millonig's phosphate buffer to 0.5 g of 1% Tannic Acid, and 6 mL of 25% Glutaraldehyde

Glutaraldehyde EM Grade 25% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**G5882-50ML

3 Post fix the dissections in 1% Osmium tetroxide solution.

20 mL

02:30:00

Note

Usually you'd have a 2% solution and you'd have to dilute it in Millonig's phosphate buffer solution until your dissections are fully covered.
Safety check: this solution is highlytoxic and volatile, so this procedure is better done in exhaust hoods.

Expected result

At the end of this step all your specimens would have to be entirely black.

Osmium tetroxide solution 2% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**75633

4 Dehydrate in a ascending series of acetones, starting at 30%

20 mL

00:15:00

5 Put in acetone 50%

20 mL

00:15:00

6 Put in acetone 70%

20 mL

00:15:00

7 Put in acetone 90%

20 mL

00:15:00

8 Put in acetone 95%

20 mL

00:15:00

9 Put in another solution of acetone 95%

20 mL

00:15:00

10 Put in another solution of acetone 95%

20 mL

00:15:00

11 Put in acetone 100%

20 mL

00:15:00

12 Put in another solution of acetone 100%

20 mL

00:15:00

13 Now take your specimens to a Critical Point Dryer
http://www.leica-microsystems.com/products/em-sample-prep/biological-specimens/room-temperature-techniques/drying/details/product/leica-em-cpd300/

Expected result

Specimens are completely dried, rigid, and somewhat whitish

14 Mount the specimens in stubs and attach them with a double-sided tape
https://us.vwr.com/store/catalog/product.jsp?catalog_number=100492-314

15 Take the specimens to the High Vaccum Coater and you're ready to analyze them.
http://www.leica-microsystems.com/pt/produtos/preparacao-de-amostras-para-microscopia-eletronica/especimes-biologicos/tecnicas-em-temperatura-ambiente/revestimento/detalhes/product/leica-em-ace600/