

Buccopharyngeal morphology of tadpoles in Scanning Electron Microscope Version 4

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Abstract

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Protocol

Step 1.

Wash quickly the specimens in tap water and dissect tadpoles following [Wassersug \(1976: p. 4-5\)](#).

NOTES

Diogo Provete 08 Jun 2016

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

Step 2.

Prepare the fixing solution

LINK:

<https://www.protocols.io/view/Fixing-solution-to-SEM-e3wbgpe>

Step 3.

Fix the dissections in 4% Glutaraldehyde diluted in the fixing solution

REAGENTS

Glutaraldehyde EM Grade 25% G5882-50ML by [Sigma Aldrich](#)

DURATION

02:30:00

Step 4.

Post fix the dissections in 1% Osmium tetroxide solution.

AMOUNT

20 ml Additional info:

REAGENTS

Osmium tetroxide solution 2% [75633](#) by [Sigma Aldrich](#)

 **DURATION**

02:30:00

 **EXPECTED RESULTS**

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

 **NOTES**

Diogo Provete 08 Jun 2016

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 highly toxic and volatile, so this procedure is better done under exhaust hoods.

Step 5.

Dehydrate in an ascending series of acetones, starting at 30%

 **AMOUNT**

20 ml Additional info:

 **DURATION**

00:15:00

Step 6.

Transfer to acetone 50%

 **AMOUNT**

20 ml Additional info:

 **DURATION**

00:15:00

Step 7.

Transfer to acetone 70%

 **AMOUNT**

20 ml Additional info:

 **DURATION**

00:15:00

Step 8.

Transfer to acetone 90%

 **AMOUNT**

20 ml Additional info:

 **DURATION**

00:15:00

Step 9.

Transfer to acetone 95%

 [AMOUNT](#)

20 ml Additional info:

 [DURATION](#)

00:15:00

Step 10.

Transfer to another solution of acetone 95%

 [AMOUNT](#)

20 ml Additional info:

 [DURATION](#)

00:15:00

Step 11.

Transfer to another solution of acetone 95%

 [AMOUNT](#)

20 ml Additional info:

 [DURATION](#)

00:15:00

Step 12.

Transfer to acetone 100%

 [AMOUNT](#)

20 ml Additional info:

 [DURATION](#)

00:15:00

Step 13.

Transfer to another solution of acetone 100%

 [AMOUNT](#)

20 ml Additional info:

 [DURATION](#)

00:15:00

Step 14.

Now take your specimens to a Critical Point Dryer

 [LINK:](#)

<http://www.leica-microsystems.com/products/em-sample-prep/biological-specimens/room-temperature-techniques/drying/details/product/leica-em-cpd300/>

 [EXPECTED RESULTS](#)

Specimens are completely dried, rigid, and somewhat whitish

Step 15.

Mount the specimens on stubs and attach them with a double-sided tape

 LINK:

https://us.vwr.com/store/catalog/product.jsp?catalog_number=100492-314

Step 16.

Take the specimens to the High Vacuum Coater and you're ready to analyze them.

 LINK:

<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/>