Buccopharyngeal morphology of tadpoles in Scanning Electron Micrography Version 3

Diogo Provete

Abstract

Citation: Diogo Provete Buccopharyngeal morphology of tadpoles in Scanning Electron Micrography. **protocols.io** dx.doi.org/10.17504/protocols.io.e3xbgpn
Published: 07 Jun 2016

Protocol

Step 1.

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

NOTES
Diogo Provete 07 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/Fiixing-solution-to-SEM-e3wbgpe

Step 3.

Fix the dissections in 4% Glutaraldehyde.

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

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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 07 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 highlytoxic and volatile, so this procedure is better done in exhaust hoods.

Step 5.

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

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 6.

Put in acetone 50%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 7.

Put in acetone 70%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 8.

Put in acetone 90%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 9.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Put in acetone 95%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 10.

Put in another solution of acetone 95%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 11.

Put in another solution of acetone 95%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 12.

Put in acetone 100%

AMOUNT
20 ml Additional info:
DURATION
00:15:00

Step 13.

Put in 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

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Step 15.

Mount the specimens in 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 Vaccum 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/

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited