

Paraffin Embedding of Drosophila Pupal Tissues

Sonia M. Hall

Abstract

Drosophila Tissue Processing for Paraffin Embedding modified by the Baehrecke Lab from published protocols by Ristifo and White, 1991 (Pubmed ID 1936557) and Muro et al., 2006 (Pubmed ID 16887831)

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Before start

Collect white pre-pupae, raised at 25 degrees Celsius, every forty minutes until desired number is obtained. Age for 24 hrs at 25 degrees Celsius and then begin fixation based on the time collected for each individual.

Protocol

Step 1.

Puncture posterior end of staged pupa with dissecting scissors, fine forcepts, or .22 gauge needle.

Place into Eppendorf tube with 500 ul of FAAG and store overnight at 4 degrees Celsius

■ AMOUNT

500 µl Additional info:

▶ PROTOCOL

. Fixation Solution - FAAG

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Step 2.

Remove FAAG in the hood and dispose in "Aldehyde Waste" container.

Dehydrate through ethanol series and clear in Xylenes. Rotate in nutator at RT.

Solution 1: 80% Ethanol 500 ul

■ AMOUNT
500 μl Additional info:
© DURATION
00:15:00

Step 3.

Dehydrate through ethanol series continued Solution 2: 85% Ethanol 500 ul AMOUNT 500 µl Additional info: © DURATION 00:20:00 Step 4. Dehydrate through ethanol series continued Solution 3: 90% Ethanol 500 ul AMOUNT 500 µl Additional info: **O DURATION** 00:25:00 Step 5. Dehydrate through ethanol series continued Solution 4: 100% Ethanol 500 ul **■** AMOUNT 500 µl Additional info: **O DURATION** 00:30:00 Step 6. Repeat Step 5 Dehydrate through ethanol series continued Solution 4: 100% Ethanol 500 ul **O DURATION** 00:30:00 Step 7. Repeat Step 5 Dehydrate through ethanol series continued Solution 4: 100% Ethanol 500 ul © DURATION 00:30:00 Step 8. Repeat Step 5 Dehydrate through ethanol series continued Solution 4: 100% Ethanol 500 ul **O DURATION** 00:30:00 Step 9. Replace 100% Ethanol with 500ul Xylenes for thirty minutes. Dispose of Xylenes in "Aldehyde Waste" container AMOUNT 500 μl Additional info: **O DURATION** 00:30:00

Step 10.

Repeat Step 9: Remove Xylenes and replace with new500ul Xylenes for thirty minutes.

Dispose of Xylenes in "Aldehyde Waste" container

■ AMOUNT

500 µl Additional info:

O DURATION

00:30:00

Step 11.

Repeat Step 9: Remove Xylenes and replace with new500ul Xylenes for thirty minutes.

Dispose of Xylenes in "Aldehyde Waste" container

■ AMOUNT

500 µl Additional info:

© DURATION

00:30:00

Step 12.

Repeat Step 9: Remove Xylenes and replace with new500ul Xylenes for thirty minutes.

Dispose of Xylenes in "Aldehyde Waste" container

■ AMOUNT

500 μl Additional info:

O DURATION

00:30:00

Step 13.

Paraffin Infiltration

Transfer pupae to scintillation vial with 2 ml of Xylenes using designated paintbrush.

Mark the level of Xylenes on tube.

AMOUNT

2 ml Additional info:

Step 14.

Add 20 paraffin chips (paraplast xtra) and melt in 42 degree Celsius incubator. (Volume should double)

Leave overnight.

In 500 ml beaker, add melted paraffin and incubate at 56 degrees celsius for use tomorrow.

Step 15.

Remove Xylenes/Wax and place onto wax waste tray.

Add a small amount of melted paraffin at 56 degrees Celsius to dilute residual Xylene, remove and place onto wax waste tray.

Add melted 56 degree Celsius paraffin incubate for 1-2 hours.

O DURATION

01:30:00

Step 16.

Remove paraffin and replace with fresh melted 56 degree Celsius paraffin incubate for 1-2 hours.

O DURATION

01:30:00

Step 17.

Remove paraffin and replace with fresh melted 56 degree Celsius paraffin and fill to the top of the glass vial.

Incubate overnight.

Step 18.

Make mold by taping the ends and sides of cut index cards and tape ends to prevent leaks.

Pre-heat hot block to 56 degrees Celsius and cover completely with tin foil.

When hot block reaches temp, swirl pupae in the vial and quickly pour wax and pupae into mold on top of hot block. orient the samples quickly before the wax hardens. Fill the remainder of the boat with melted paraffin.

Let sit for 15 minutes to begin to solidify.

O DURATION

00:15:00

Step 19.

Carefully remove boat from hot block and coll overnight at room temp.

Step 20.

Cut wax into individual pyramid shaped blocks for sectioning using a razor blade. Mount blocks onto chuck by melting paraffin with heated flat spatula.

Step 21.

Cection blocks on microtome at 7um and float onto slides with water that are pre-warmed on slide warmer at 42 degrees Celsius. Allow the sections to expand on slide warmer for 2-5 minutes. Remove water by wicking with a kimwipe. Bake slides on 42 degree Celsius slide warmer overnight.

Step 22.

Hydrate samples by placing in Xylenes (container 1) for 3 minutes

O DURATION

00:03:00

Step 23.

Hydrate samples by placing in Xylenes (container 2) for 3 minutes

O DURATION

00:03:00

Step 24.

Hydrate samples by placing in 100% Ethanol (container 1) for 3 minutes

© DURATION

00:03:00

Step 25.

Hydrate samples by placing in 100% Ethanol (container 2) for 3 minutes

O DURATION

00:03:00

Step 26.

Hydrate samples by placing in 95% Ethanol for 3 minutes

O DURATION

00:03:00

Step 27.

Hydrate samples by placing in 70% Ethanol for 3 minutes

O DURATION

00:03:00

Step 28.

Rinse under running tap water for 5 mins.

© DURATION

00:05:00

Step 29.

Stain Samples

Submerge slides in Weigert's Hematoxylin for 5 mins.

O DURATION

00:05:00

Step 30.

Rinse under running tap water for 5 mins.

O DURATION

00:05:00

Step 31.

Submerge in Pollak Trichrome for 7 mins.

O DURATION

00:07:00



Pollak Trichrome Solution

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Step 32.

Rinse in distilled water for approx 10 seconds to remove the bulk of the stain.

O DURATION

00:00:10

Step 33.

Submerge in Acidified distilled water for 10 sec.

O DURATION

00:00:10

PROTOCOL

. Acidified Distilled Water

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Step 34.

Dehydration:

Dip slides into 70% Ethanol 3 times

Step 35.

Dip slides into 95% Ethanol (container 1) 3 times

Step 36.

Submerge slides into 95% Ethanol (container 2) for 3 mins.

O DURATION

00:03:00

Step 37.

Submerge slides into 100% Ethanol (container 1) for 3 mins.

© DURATION

00:03:00

Step 38.

Submerge slides into 100% Ethanol (container 2) for 3 mins.

O DURATION

00:03:00

Step 39.

Submerge slides into Xylenes (container 1) for 3 mins.

O DURATION

00:03:00

Step 40.

Submerge slides into Xylenes (container 2) for 3 mins.

O DURATION

00:03:00

Step 41.

Remove slides from Xylenes and drain for 2 mins on a paper towel

© DURATION

00:02:00

Step 42.

Use 78ul of Permount (for large coverslip) and apply coverslip. Let slides harden overnight.

■ AMOUNT

78 µl Additional info: