

EM PROCESSING.

1. Place tissue in **1% OsO₄** in 0.1M phosphate buffer for **1 Hr**
2. Wash tissue **2 X 2 mins** in cold (**4°C**) **0.1M Sodium acetate**
3. Place in 2% aqueous Uranyl Acetate **45 mins at 4°C**
4. Dehydrate

Do not let the tissue dry out during these solution changes. Keep them wet at all times. This is particularly critical for step F-G because the Propylene Oxide evaporates very quickly.

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| A | 25% alcohol | 5min. |
| B | 50% alcohol | 5min. |
| C | 75% alcohol | 5min. |
| D | 95% alcohol | 5min. |
| E | 100% alcohol | 5min. |
| F | 100% alcohol | 5min. |
| G | Propylene Oxide | 5-15min. |

You can start making the Epon anytime during this stage as it has to be thoroughly mixed. Use following proportions 100ml EPON, 1ml. BDMA (calculate the amount you will need for steps H,I and J.)

You can transfer the tissue to the tin dishes at this stage. It keeps the bottles Epon free so they can be washed and re-used. The best method is to dump tissue and Propylene Oxide into a dish. Suck off the majority of the Propylene Oxide still leaving the tissue wet, then add the Propylene Oxide/Epon mixture.

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| H | 50/50 Propylene Oxide/Epon (i.e. with BDMA) | 30 min. |
| I | Epon | 30min - 2hr |

This step is to remove as much Propylene Oxide/Epon as possible before final embedding. To make the Epon more liquid and to evaporate some of the Prop Oxide off you can place the tissue in the 37°C during this time **but no longer than 30 min. Do NOT wander off for a day or two forgetting you put it in the 37°C oven.**

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| K | Embed on slides or dish |
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You can embed the tissue in **fresh Epon** using foil tins, plastic moulds or acetate strips

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|---|------|--------|
| L | 37°C | 24Hrs |
| M | 60°C | 24 Hrs |

1% OsO₄
5ml 2% OsO₄
5ml 0.2M PO₄ buffer

0.1M Sodium Acetate
6.8 g Sodium Acetate
500 ml H₂O