

# TEM protocol for Drosophila larvi brains

Albert Cardona

Adapted from a protocol originally from the Unitat de Microscopia Electronica dels Serveis Cientifico-Tecnics de la Universitat de Barcelona, by Albert Cardona ( cardona@ucla.edu ).

## 1 General guidelines

- Work under the hood and with gloves whenever possible: that is, all steps except brain dissection.
- Never let the samples dry, rather, leave a significant amount of liquid and do more washes.
- Be sure to have all necessary solutions in the fridge before starting and keep them cold always.
- Do not expose the samples to warm or room temperature liquids. If you have prepared a solution too late, let it go cold before use.
- Be careful not to confuse 0.1 M and 0.2 M phosphate buffers!

## 2 Phosphate Buffer preparation

Prepare 2 different buffers A and B and mix them right before use, following the table below to obtain a 0.2 M buffer at the appropriate pH. These buffers are VERY IMPORTANT to get a perfect fixation.

- Buffer A: 0.2 M  $\text{NaH}_2\text{PO}_4$
- Buffer B: 0.2 M  $\text{Na}_2\text{HPO}_4$

Table of pH:

pH	6.4	6.8	7.0	7.2	7.4	7.6	7.8
A (ml)	73.5	51.0	39	28	19	13	8.5
B (ml)	26.5	49	61	72	81	87	91.5

Buffer storage:

- Buffer A at 4°C.
- Buffer B at RT in a lighth-protected case (in the fridge will be fine too).

### 3 Fixation

All steps on ice except dissection.

- Dissect *Drosophila* brains and put them in cold PBS. Proceed when enough brains have been dissected or a maximum of 30 minutes have transpired.
- Prepare fixative solution: 4% paraformaldehyde, 2.5% glutaraldehyde in phosphate buffer 0.1 M pH 7.3 (0.2 ml of A, 0.8 ml of B, 0.4 ml PFA 20%, 0.2 ml glutaraldehyde 25%, and 0.4 ml of H<sub>2</sub>O<sub>d</sub>). For convenience, 20% paraformaldehyde can be prepared and stored at -20°C, then thawed at 60° for a few minutes and brought to 4° on ice.
- Fix for 24 hours at 4°C. If the samples cannot be processed right away, store them in this fixative solution at 4°C. They will be preserved well for several weeks; tissue contraction is noticeable after about a week in fixative and increases over time to some extent.
- Wash 5 x 10 min in phosphate buffer 0.1 M pH 7.3
- 60 min 1% Osmium tetroxide in phosphate buffer 0.1 M pH 7.3 at 4°C (0.5 ml H<sub>2</sub>O<sub>d</sub>, 0.5 ml 4% OsO<sub>4</sub>, and 1 ml phosphate buffer 0.2 M pH 7.3). Keep samples in the dark.
- 4 x 10 min H<sub>2</sub>O<sub>d</sub>.

### 4 Dehydration

Dehydrate through acetone series at 4°C (keep acetone bottles in the fridge, which should be pre-cooled in any case). Keep glass vial on ice.

- 10 min 50%
- 10 min 70%
- 10 min 96%
- 3 x 10 min 100%.

### 5 Embedding in plastic

Do either or: not both! Either Spurr, or Epon.

#### 5.1 Spurr embedding

##### 5.1.1 Spurr's recipe

Prepare the Spurr's resin by mixing the ingredients in the order and quantities below, with the help of a scale and the tare function. Afterwards, mix well with a magnetic mixer for over 10 min.

- 10g ERL 4206 (epoxy resin)
- 6 g DER 736 (flexibilizer)

- 26 g NSA (hardener)
- 0.4 g DMAE(S-1) (polymerization accelerator)
- 0.8 g DBP (fragility)

### 5.1.2 Dehydration and embedding

Agitation and room temperature required except for polymerization.

- 2h 1:3 spurr:acetone
- 3h 2:2 spurr:acetone
- ON 3:1 spurr:acetone
- 2h spurr
- ON spurr
- 48 h spurr at 60C for polymerization.

## 5.2 Epon embedding

Prepare epon using the lab recipe (whatever works, there are dozens).

### 5.2.1 Epon recipe

In the Hartenstein's lab, we use the araldite/epon recipe made from Polysciences #02595, which includes:

- 7.1g Dodecenylsuccinic Anhydride (DSA)
- 4.8g Epon 812
- 2.5g Araldite
- 0.25 ml DMP-30

Mix the first three components to homogeneity and then add the fourth, and mixe again.

Store epon in 5 ml plastic syringes at -20°C.

### 5.2.2 Dehydration and embedding

Prepare the acetone mixes right before use.

- 2h 1:3 epon:acetone
- 3h 2:2 epon:acetone
- ON 3:1 epon:acetone
- 2h epon

- ON epon
- 16h polymerization at 60°C on flexible plastic molds. Do not polymerize longer, for the plastic will become brittle.

Agitation and room temperature required except for polymerization.

### 5.3 Tips

- After 2 hours into polymerization, epon is dense enough that samples can be reoriented under the scope.
- For best convenience, use a small glass container with a broad opening. Ependorffs are not recommended because it's difficult to see the samples inside.
- Transfer samples from the pure epon step to the molds with the help of a wooden toothpick. Epon is dense enough that pushing and fiddling around the sample is enough to grab it.

## 6 Counterstaining

Handle grids with care!

Prepare three 50 ml recipients with  $H_2O_d$  for washing.

- 40 min. 8% Uranyl acetate in  $H_2O_d$  at RT. One tiny drop (5-10 microliters) per grid, on a piece of parafilm extended inside closed glass petry dish. During the last 10 minutes, prepare tiny drops of  $H_2O_d$  to place the grids after washing.
- Wash the grid by dipping three times into each washing recipient (total 9 times), and place one grid per drop of water. Wait 5 minutes.
- Prepare fresh Reynold's lead citrate: 0.40 g of lead nitrate in 14.4 ml  $H_2O_d$  in a glass vial. Handle with care (very toxic). Place a lid and gently rotate to mix. When dissolved, pour 0.53 g of trisodium citrate  $2xH_2O$ . Finally add 0.4 ml of 4M NaOH to dissolve the citrate. The lead solution stays good for about 20 minutes in a closed recipient (from then on, black precipitate may be left on your grids).
- 2.5 minutes in lead citrate (again each grid on a tiny drop). Place some NaOH lentels inside the glass petry dish as scavengers.
- Wash the grid as above, in new clean water and place on the grid box.