

## EMBEDDING OF EYES IN PLASTIC (FOR THIN SECTIONS)

### Step 1: Dissection

Decapitate fly and then cut one eye off leaving the eye of interest attached to the proboscis.

### Step 2: Fixation

Fix heads for **2 hours** in dissection wells covered with filter paper at room temperature (shake occasionally). FIX: 1% paraformaldehyde/1% glutaraldehyde in PBS.

Rinse w/PBS **2 x 15 minutes** in eppendorf tubes, inverting the tubes often.

### Step 3: Postfixation

Treat with 1% OsO<sub>4</sub> in PBS for **2 hours** at room temperature.

### Step 4: Dehydration

Incubate heads for **5 minutes** in each alcohol soln (50%, 75%, 85%, 95%, 100%, 100%)

Incubate in propylene oxide **2 x 5-10 minutes** at room temp.

### Step 5: Infiltration

Incubate heads in 1:1 propyleneoxide/epon-araldite mixture **over night**.

Transfer heads into pure plastic (fresh) and dessicate **3-4 hours** at room temperature.

### Step 6: Embedding

Pour plastic into flat molds and transfer heads. Orient heads into position with a 22 gauge needle. Let heads settle for **one hour** and then double check their orientations.

### Step 7: Polymerization

Incubate at 60 degrees for **3 to 4 days**.

## Epon 812 / Araldite Mixture:

7.1 g Dodecenylsuccinic Anhydride [Poly #0563]

4.8 g Epon 812 [Poly #8791]

2.5 g Araldite [Pella #18060]

Mix to homogeneity then add .25 ml DMP-30 [Poly #06553]

## EMBEDDING PROTOCOL FOR THICK SECTIONS

- I. Dissect eye
- II. Fix for **1 hour** on ice in 2% gluteraldehyde in PBS.
- III. Rinse in PBS for **10 minutes** on ice.
- IV. Dehydrate in alcohol solutions on ice
  - 50% for **5 minutes**
  - 70% for **5 minutes**
  - 90% for **5 minutes**
  - 100% for **5 minutes**
- V. Clear in 100% propylene oxide for **5 minutes** on ice.
- VI. Infiltrate in 1:1 propylene oxide/plastic mixture for **15 minutes** on ice.
- VII. Dessicate in pure plastic **overnight**.
- VIII. Embed as above.