**Formaldehyde (FA) fixation for ImmunoEM on Drosophila Ovaries/Embryos**

1) Prepare 8% FA fresh…
   • weigh out 8 gms of paraformaldehyde;
   • add 40 mls H$_2$O;
   • heat with stirring to 60$^0$C, and add 4 drops of 1N NaOH; and continue stirring until the solution is clear. Be careful to avoid over heating as FA will rapidly break down to formic acid. Also, DO NOT prepare FA from formalin (37.7% FA in a bottle) as this contains 10% methanol as a redox stabilizer.
   • add 50 mls of 200mM cacodylate buffer; check pH to ~7.4; and bring the volume to 100mls.

2) Add fixative to ovaries/embryos; allow to fix for 15’.

3) Aspirate off medium/fixative mixture; and replace with fresh 100% fixative; continue fixing for 18-24 hours at 4$^0$C.

4) Aspirate off fixative and replace with 2.3M sucrose containing 20% PVP (polyvinylpyrollidone, 10,000 mw) for cryoprotection. Allow ovaries/embryos to infuse for a minimum of 2 hours.

5) Mount ovaries onto cryopins and freeze in liquid N$_2$. 