E63-1 Antibody Staining of \textit{Drosophila} Larval Salivary Glands (Whole Mounts)

**RECIPES:**

**PEM**
- 60mM PIPES
- 5mM EGTA
- 5mM MgCl$_2$
- pH 6.9

**PROTOCOL:**
1. Make fresh reagents each time: PEM (50mls good for 4 wells), 4% paraformaldehyde (0.04g paraformaldehyde into 1mL PEM, heat 70°C until dissolved)

2. Dissect salivary glands in PBS or D-PBS and keep in buffer until ready for fixing (no longer than 15min.)

3. Transfer glands into glass slide with wells containing 200ul of 4% paraformaldehyde fix for no more than 15min. room temp.

4. Transfer fixed glands into Corning Transwell (Corning #3472) insert containing .1ml of PEM in insert and .6ml in well (\textbf{NOTE: this volume will always be used unless otherwise specified}). Wash for 3x5 min., RT, light shaking.

5. Permeabilize with 1ml 0.4% Triton X-100 in PEM 3x10min. RT, light shaking.

6. Block in: 10% Normal Goat Serum/ 0.4% Triton X-100 in PEM [Block] – for 1hr. RT.

7. Incubate w/ Anti-E63-1 in Block (1:10 000) overnight, 4°C on Orbitron.

8. Wash 3x10min PEM/Triton.

9. Incubate 2 hrs. with GAR-FITC in Block (1:200), RT. \**This and the following steps must be done in the dark***

10. Wash 2 x 10 min in PEM/Triton, RT. Then, 2x10min in PEM, RT.

11. Aspirate glands out of insert using P200 and transfer to glass slide with wells.

12. Transfer glands for mounting on slide with a drop of mounting solution (80% 1M Tris pH8.8 + 0.5% PDA and 20% glycerol). Seal slide with wax or nail polish.