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Golgi Immunostaining of *Drosophila* Larval Salivary Glands (Whole Mounts)

PROTOCOL:

1. Make fresh fix solution each time: 4% paraformaldehyde and 1.5% glutaraldehyde in PBS. (0.04g paraformaldehyde and 15ul glutaraldehyde into 1mL PBS, 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 fix solution for no more than 15min. room temp.
4. Transfer fixed glands into Corning Transwell (Corning #3472) insert containing 100ul of PBS in insert and 600ul in well (**NOTE: this volume will always be used unless otherwise specified**). Wash for 3x5 min., RT, light shaking.
5. Permeabilize with 0.3% Triton X-100 in PBS 3x10min. RT, light shaking.
6. Block in: 5% Normal Goat Serum/ 0.3% Triton X-100 in PBS [Block] – for 1hr. RT.
7. Incubate w/ Anti-Golgi (mouse) in Block (1:250) overnight, 4°C on Orbitron.
8. Wash 3x10min 0.3% Triton-X in PBS.
9. Incubate 2 hrs. with GAM-FITC in Block (1:200), RT. * *****This and the following steps must be done in the dark*****
10. Wash 2 x 10 min in Triton/PBS, RT. Then, 2x10min in PBS, 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.