

**ANTIBODY STAINING of OVARIES** Date started:  
AG/AU intensification for electron microscopy

**DAY 1**

1. Dissect 30 OR ovaries in 1X PBS.  
Take rest of larvae up to step 5 ready for preabsorption.
2. Fix for 10' then 20' in PEMS with 0.25% glu.
3. Wash 2X10' in 1XPBS.
4. Permeabilize in 1X PBS, 0.01% saponin, 10% goat serum, 1mg/ml BSA and 2mg/ml glycine for 5X 10'.
5. Incubate O/N at 4°C in permeabilization buffer with 1 in 10 of rat -Star 6,7 primary in nunc plates.  
START at \_\_\_\_\_ STOP at \_\_\_\_\_ = \_\_\_\_\_ hours

**DAY 2**

6. Wash in baskets for 7X10' in 1x PBS, 0.01% saponin. Start preabsorbing secondary.
7. Incubate in preabsorbed rat-HRP secondary at 1 in 400 for 3 hours at RT in 1X PBS, 10% goat serum and 0.01% saponin.
8. Wash 2X 10' in permeabilization buffer.
9. Wash 7X 10' in 1X PBS.
10. Post fix for 20 minutes in 2% gluteraldehyde in 1X PBS.
11. Wash 3X 10 minutes in 1X PBS.
12. Incubate for 20 minutes in 250 DAB and 250 1XPBS w/3 of H<sub>2</sub>O<sub>2</sub>. DO NOT USE Ni
13. Wash 2X 15 minutes in 2% fresh NaAc.
14. Place in 10% thioglycollic acid O/N @ 4°C START:  
STOP:

**DAY 3**

15. Wash 4X 15 minutes in 2% NaAc.
16. Incubate for 10 minutes in silver developer.
17. Wash 5X 10 minutes in 2% NaAc.
18. Wash 8 minutes in AuCl (0.025g in 50ml).
19. Wash 3X 10 minutes in 2% NaAc.
20. Wash 3X 10 minutes in 3% Na thiosulphate.
21. Wash 3X 10 minutes in 2% NaAc.
22. Wash 3X 10 minutes in 1X PBS.
23. Wash for 60 minutes in 2% OsO<sub>4</sub> in 0.8% K

24. Wash 3X 10 minutes in 1X PBS.
25. Dehydrate in 30%, 50%, 70%, 80%, 95%, 3X 100% etOH for 10 minutes each.
26. Transition to propylene oxide for 3X 10 minutes.
27. Then 1:1 propylene oxide and pure plastic O/N.

#### DAY 4

28. Transfer to pure plastic for 3 hours under vacuum.
29. Embed, bake for 3 days at 60°C and section.

#### RESULTS: