

Date: \_\_\_\_\_

# Ovary Staining Protocol

Schüpbach lab

Purpose of experiment:

1. Dissect ovaries from 2-day old flies which were fed yeast as virgins and were incubated with males in 1XPBS (no longer than 30' for any one batch).

<u>Sample #/color</u>	<u>Genotype</u>	<u># discs</u>	<u>1° Ab</u>	<u>dilution</u>	<u>2° Ab</u>	<u>dilution</u>
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2. Fix for 20' in ovary fix, shaking.

3. Wash 3 x 10' in NP40 wash.

4. Block 30' - 2 hours at RT in block solution.

5. Incubate in 1° at 4° C overnight.

6. Wash 4 X 20' in NP40 wash.

7. Incubate in 2° antibody at room temp. for 2 hours.

8. Wash 4 X 20' in NP40 wash.

9. Rinse with PBS

9. Color reaction: put samples in 0.5 mg/ml DAB (diluted 1:2 in PBS from 2X stock) + 5 µl 8% NiCl/ml solution. Add 2 µl of 30% H<sub>2</sub>O<sub>2</sub>/well.

10. Stop reaction in 1XPBS.

11. Mount in GOOD glycerol; seal with nailpolish.

Solutions:

**NP40 Wash:**

50 mM Tris (7.4)

150 mM NaCl

0.5% NP40

1 mg/ml BSA

**Ovary Fix:**

200 of 4% formaldehyde in PEMS containing 0.5% NP40

600 of Heptane

**Block**

Same as wash buffer with 20% NGS

Date: \_\_\_\_\_

0.02% NaAzide