

## Immunostaining of *Drosophila* cells

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(adapted from an unknown author)

1. Pipet 30 $\mu$ l of live cells onto 8-well slides (see company information below – it is very important to pretreat slides with poly-L lysine – see directions below).

Allow cells to settle on slide for 15 minutes (keep slides in a humid chamber to prevent evaporation)

2. Add 30 $\mu$ l of 4% fresh paraformaldehyde and incubate for 15 minutes (4% fresh formaldehyde may also be used if paraformaldehyde is not available).

Paraformaldehyde should be made in 1X PBS (or diluted in PBS if starting with a higher concentration).

3. Pipet off the 60 $\mu$ l of solution (do not remove it to the point where the cells/slides dry out) and wash 3X with 1X PBS by pipeting PBS on each well. Washes can be quick (1-2 minutes)
4. Add primary antibody diluted in 1X PBS with 1% NGS and 0.1% saponin. Add 30 $\mu$ l of antibody/NGS/saponin to each well and incubate overnight at 4°C. (See below for exceptions).
5. Wash each well 3X with 1X PBS
6. Dilute secondary antibody in 1X PBS with 1% NGS and 0.1% saponin - 1-2 hours at room temp.
7. Wash three times with 1X PBS
8. Mount in gelutol with N-propyl gallate (see recipe notes below) with coverslip
  - Cure in open container at room temperature overnight, in the dark – coverslips are permanently mounted after this. The process can be reversed by incubating mounted slides in a humid chamber or by dipping in PBS. Store slides at 4°C.
  - Slides may be viewed for up to 2-3 weeks after mounting



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NGS = normal goat serum  
PBS = phosphate buffered saline (use fly lab protocols to make this)

## DGRC Standard Operating Procedure: Immunostaining

**Slides:** Cel-Lin Assoc. Inc. (Newfield, NJ - USA)  
HTC(R) Autoclavable Teflon 6mm diameter circles  
(catalog #10-10)

Treat with poly-L lysine (1% solution for 20 minutes at RT or overnight at 4 degrees. Shake excess poly L-lysine off and air dry before using. Treated slides may be used for up to 3 months when stored at room temperature)

**Saponin:**

From Sigma - make 10% solution in water and freeze aliquots for long-term storage. Aliquots in use may be kept at 4°C for 2-3 months.

**Gelutol (with N-propyl-gallate):** see separate protocol/recipe

**Primary antibodies:** Monoclonal and very clean polyclonals are typically incubated overnight. In my experience some polyclonals do better if incubated at room temperature for 1-2 hours since longer incubations with certain polyclonals tend to give background.

