Immunostaining of *Drosophila* cells

Kris Klueg
(adapted from an unknown author)

1. Pipet 30µl 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 30µl 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 60µl 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 30µl 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

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