IMMUNOSTAINING OF *Drosophila* NEUROBLASTS

1- Dissect the brains (without imaginal discs) of third instar larvae in 0.7% NaCl.

2- Fix the brains in 4% paraformaldehyde (or 3.7% formaldehyde) in cytoskeleton buffer (or PBS) for 30 min.

3- Incubate 3 min in 45% acetic acid (for γ-tubulin immunostaining immerse brains in methanol for 2 min before fixation in acetic acid).

4- Strongly squash the brains in 60% acetic acid under a coverslip and freeze in liquid nitrogen.

5- Carefully remove the coverslips and immediately immerse the slides for 10 min in ethanol at -20°C (inside a black box in a dry ice container.

6- Incubate for 10 min in PBS + 0.1% Triton (black box).

7- Wash 2x 5 min in PBS (black box).

8- Incubate for 45 min in 1% BSA in PBS for blocking (black box).

9- Incubate with primary antibodies in 1% BSA + 0.1% Triton in PBS for 1 hour.

10- Wash 3x 5 min in PBS + 0.1% Triton (black box).

11- Incubate with appropriate secondary antibodies in 1% BSA + 0.1% Triton in PBS for 1 hour.

12- Wash 3x 5 min in PBS + 0.1% Triton (black box).

13- Wash 1x 5 min in PBS (black box).

14- Mount the brains with DAPI diluted 1:1000 in Vecta-Shield and seal the coverslip.