

### **Imaginal Disc Staining with anti-phospho H3 staining**

1. Dissect discs in PBS. Keep on ice.
2. Fix 30 min in 4% formaldehyde in PBS at RT on shaker.
3. For HRP staining, incubate in 0.3% H<sub>2</sub>O<sub>2</sub> in MeOH for 30 min.  
For fluorescent staining, incubate in 100% MeOH for 30 min.  
(RT on shaker.)
4. Incubate in Antibody Mix for 30 mi at RT on shaker.  
  
Antibody Mix:0.3% BSA  
0.1% Triton X-100  
1 X PBS
5. Incubate discs in 1° antibody O/N at RT on shaker. Use 1:5000 rabbit anti-phospho H3 diluted in Antibody Mix.
6. Wash 6X 10 min in PBT, RT on shaker (0.5 ml).
7. Incubate discs in 2° antibody 2-3 hours at RT on shaker.
8. Wash 6X 10 min in PBT. Last wash contains DAPI or Hoechst.
9. View or do HRP reaction.