

# Fluorescent Immunohistochemistry for Taste buds and Nerve Fibers Protocol

1. Fresh freeze the tongue in OCT (do not fix)
2. Section tissue (10-40 $\mu$ m) on a cryostat and thaw mount onto slides.
3. Dry tissue for 1 hour to overnight and then acetone fix for 10 minutes at -20°C.
4. Let dry 1 hour to overnight.
5. Rinse slides 3 X 5 minutes in PBS
6. Incubate overnight at room temperature in anti-neurofilament 150 (1:1000 rabbit, Chemicon AB1981) and Troma 1 concentrated (1:100 rat, Developmental Hybridoma Bank) diluted in antibody solution. Antibody solution : 9 parts PBS/ 1 part DMSO.
7. Wash slides 3 X 5 minutes in PBS.
8. Incubate slides at room temp for 1.5 hours with biotinylated anti-rat (1:200) and Cy2 anti-rabbit (1:200) prepared in antibody solution.
9. Wash slides 3 X 5 minutes in PBS.
10. Incubate slides at room temp for 1.5 hours using Cy3-steptavidin (1:200) in antibody solution.
11. Wash slides 3 X 5 minutes in PBS.
12. Dry overnight.
13. Clear in clean Xylene or Citrisolv. for 10 minutes.
14. Coverslip with DPX.