

Brdu Staining: Brains and Eye discs (from Zip)

- 1) Dissect in sterile, R/T, Schneider's medium
- 2) Collect brains in Schneider's medium in glass well plates
- 3) Incubate 2hrs in Schneider's (500ul) + Brdu (50ul)
- 4) Rinse 3 times in Schneider's and incubate for 15 minutes
- 5) Rinse 3 times in PBS and incubate for 15 minutes
- 6) Fix with 4% Paraformaldehyde in PBS for 15 minutes
- 7) Wash 3 times for 5 minutes in PBT
- 8) Incubate 30 min in PBT/2NHCl (5 parts PBT, 1 part conc HCl)
- 9) Rinse 4 times with PBT
- 10) Incubate in 10% NGS/PBT for 30 minutes
- 11) Incubate in anti-Brdu 1:100 o/n
- 12) Wash 3 times for 10 minutes in PBT
- 13) Incubate in anti-mouse 1:200
- 14) Wash 1 time with PBT for 10 min, 3xPBS (2nd for 1 hr)
- 15) Incubate in DAB with PBS for 10 minutes
- 16) Transfer to next well containing DAB, PBS, H₂O₂
- 17) Mount in glycerol