

NeuN-BrdU Double Staining

Reagents:

PBS: Phosphate Buffered Saline

TBS: Tris Buffered Saline pH 7.4

4% Paraformaldehyde

1 N Hydrochloric Acid (per 200 ml: 17.2 ml HCl, dH₂O to volume)

Wash: 0.3% Triton-X in PBS or TBS

TBS++: TBS + 5% Normal Goat Serum + 0.3% Triton-X

Primary 1: Mouse anti-NeuN in TBS++ (Chemicon, 1:100)

Secondary 1: Goat anti-Mouse AlexaFluor-488 (=FITC) in TBS++ (Molecular Probes, 1:500)

Primary 2: Rat anti-BrdU in TBS++ (Research Diagnostics, 1:10)

Secondary 2: Goat anti-rat AlexaFluor (=TRITC) in TBS++ (Molecular Probes, 1:500)

TOPRO/DAPI: 1 µg/ml ToPro-3 (Molecular Probes) in TBS, 1 µg/ml DAPI (Sigma) in TBS

DABCO for mounting

Protocol:

1. Rinse 3x with TBS
2. Block for 30 min at RT with TBS++
3. Incubate with Primary 1 at 4° C overnight
4. Rinse 3x with TBS
5. Incubate with Secondary 1 overnight at 4° C
6. Rinse 3x with TBS
7. Wet mount to check for staining
8. Fix sections for 10 min at RT with 4% paraformaldehyde
9. Rinse 3x with TBS
10. Soak in PBS for 15 min at RT
11. Incubate with 1 N HCl (made fresh) for 1 h at 37°C
12. Rinse 3x with TBS
13. Block for 30 min at RT with TBS++
14. Incubate with Primary 2 at 4° C for 3 days
15. Rinse 3x with TBS
16. Incubate with Secondary 2 overnight at 4° C
17. Rinse 3X with TBS
18. Incubate with TOPRO/DAPI for 5 min at RT
19. Rinse 3x with TBS
20. Mount with DABCO