

NeuN Immunohistochemistry

1. Make up 1L of fresh Phosphate Buffered Saline pH 7.4 from powder sachet (Invitrogen)
2. Make up 0.2% Triton X-100 in PBS (stir 100mg Triton in 50mL PBS at RT)
3. Place each series in a glass vial and rinse with PBS 3 times before starting primary reactions
4. Add 1mL of 0.2% Triton with 2% Normal Donkey Serum (20 μ L) to each series and swirl briefly.
5. Add **mouse anti-NeuN** primary at 1:200 (5 μ L) to each series.
6. Shake gently for 48 hours at 4°C. Sections should barely revolve around the vial.
7. Rinse sections with PBS 3 times before starting secondary reactions.
8. Add 1mL of 0.2% Triton with 2% Normal Donkey Serum (20 μ L) to each series and swirl briefly.
9. Add **donkey anti-mouse Alexa fluor 488/594 or AMCA** at 1:250 (4 μ L) to each series.
10. Shake gently for 90 minutes at room temperature, protected from light. Sections should barely revolve around the vial.
11. Rinse sections with PBS 3 times before mounting.
12. Mount sections serially on slides with Prolong Gold Anti-fade mounting media. Protect slides from light, and keep at 4°C after 24 hours drying at room temperature.