**NeuN (Millipore) immunostaining with Fluoro Nissl counter**

**(Floating) XG**

Animals

1. Transduce tdTomato in the rat brain via Cre-lox system or virus vector.

Perfusion, Cryoembedding, Sectioning

2. Transcardially perfuse with ice-cold saline following by 4% paraformaldehyde and 0.2% picric acid with 0.1 M phosphate buffer (pH 7.2–7.3). Postfix overnight with the same fixative.

3. Infiltrate with sucrose gradient (10–30%).

4. Cryoembed in cryomold (Tissue-Tek) with O.C.T. compound (dry ice/acetone).

5. Section the brain via cryostat into 20-µm-thick slices and harvest them in 24 well plates containing anti-freeze solution. PBS wash.

Antibody application (Room temperature, Light shielding)

6. 10% NGS in PBS-X, 30 min on shaker

7. 1st antibody in PBS-XG, overnight on shaker.

8. PBS-X wash (quick × 1, 10 min × 2)

9. 2nd antibody in PBS-XG, 2 hr on shaker

10. PBS-X wash (quick × 1, 10 min × 2)

11. PBS wash

Counterstaining with fluorescent Nissl

12. Diluted NeuroTrace (Blue), 40-60 min.

13. PBS wash (quick × 1, 10 min × 2)

Mounting

14. Mount on gelatin-coated glass slides and Air-dry (30 min)

Coverslipping

15. Coverslip with 50% (v/v) glycerol/2.5% (w/v) DABCO in PBS.

Observation

16. Observe with epifluorescence or confocal microscopy.

**Solutions**

**PBS-X** (total 550 ml)

20% Triton X-100 8.25 ml final 0.3%

in PBS 541.75 ml

**PBS-XG** (150 ml)

normal goat serum 1.5 ml (final 1%)

sodium azide 30 mg (final 0.02%)

in PBS-X 148.5 ml

**10% NGS in PBS-X** (5 ml)

normal goat serum 0.45 ml (final 10%)

in PBS-XG 4.55ml

**1st antibody** ([Torashima et al., 2006](#_ENREF_1); [Zhang et al., 2013](#_ENREF_2))

Ms anti-NeuN (Millipore Cat# MAB377, RRID:AB\_2298772) 1:1k dil. in PBS-XG

**2nd antibody** (1:1000 dilution, 2 µg/ml final)

Goat anti-mouse IgG Alexa Fluor 488 (A-11029)

in PBS-XG

**Diluted NeuroTrace Solution** ( NeuroTrace Blue, N-21479)

Confocal microscopy (1:150 dilution with PBS)

Epifluorescence microscopy (1:300 dilution with PBS)

**Reagents**

MAB377 (Millipore): 500 µg, \71,000

A-11029 (Invitrogen): 0.5 ml, 71,300 HUF

N-21479 (Invitrogen): 1 ml, \34,000

Normal Goat Serum IHR-8136 (ImmunoBioScience): 20 ml, \9,000

**References**

Torashima T, Okoyama S, Nishizaki T, Hirai H (2006) In vivo transduction of murine cerebellar Purkinje cells by HIV-derived lentiviral vectors. Brain Res 1082:11-22.

Zhang Z-W, Peterson M, Liu H (2013) Essential role of postsynaptic NMDA receptors in developmental refinement of excitatory synapses. Proc Natl Acad Sci U S A 110:1095-1100.