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

**(Floating) XD**

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 vibrating microslicer into 40-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-XD, overnight on shaker.

8. PBS-X wash (quick × 1, 10 min × 2)

9. 2nd antibody in PBS-XD, 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-XD** (150 ml)

normal donkey serum 1.5 ml (final 1%)

sodium azide 30 mg (final 0.02%)

in PBS-X 148.5 ml

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

normal donkey serum 0.45 ml (final 10%)

in PBS-XD 4.55ml

**1st antibody** (1:200 dilution, 1 µg/ml final)

Cre (mouse anti-Cre) (Millipore Cat# MAB3120, RRID:AB\_2085748)

in PBS-XD

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

Donkey anti-mouse IgG Alexa 555 (A-31570) (1:1000 dilution, 2 µg/ml final)

Or

Donkey anti-mouse IgG Alexa 594 (A-21203) (1:1000 dilution, 2 µg/ml final)

Or

Donkey anti-mouse IgG Alexa 647 (A-31571): (1:1000 dilution, 2 µg/ml final)

in PBS-XD

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

Confocal microscopy (1:150 dilution with PBS)

Epifluorescence microscopy (1:300 dilution with PBS)

**Reagents**

MAB3120 (Merck Millipore): 100 µg, ￥71,000

A-21203 (Thermo Fisher Scientific): 0.5 ml, 74,200 HUF

A-31570 (Thermo Fisher Scientific) 0.5 ml, 71,900 HUF

A-31571 (Thermo Fisher Scientific) 0.5 ml, 79,400 HUF

N-21479 (Thermo Fisher Scientific): 1 ml, 320 USD

Normal donkey Serum IHR-8135 (ImmunoBioScience): 20 ml, $60,00

**References**

Toby A. Fergusona, Steven S. Scherer (2012) Neuronal cadherin (NCAD) increases sensory neurite formation and outgrowth on astrocytes. Neuroscience letters 522.PubMed ID 22698587.