**Calbindin (Frontier) immunostaining with Fluoro Nissl counter**

**(Floating) XD**

Animals

1. If needed, transduce some reporter proteins (eg. tdTomato) in the rat brain via Cre-lox system or virus vector.

Perfusion, Agarose-embedding, Sectioning

2. Transcardially perfuse with saline followed 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. Remove the dura. If possible, the arachnoid membrane, the pia also.

4. Embed the brain into the 4% electrophoresis quality agarose.

5. Section the brain with vibrating blade microtome into 50-µm-thick slices and harvest them in 12 well plates containing PBS

Antibody application (Room temperature, Light shielding)

6. 10% NDS 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** (Miura et al., 2006) (1:200 dilution, 1 µg/ml final)

Go anti-CB-28k (Frontier Institute Cat# Calbindin-Go, RRID:AB\_2571569)

in PBS-XD

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

Donkey anti-goat IgG Alexa 488 (A-11055)

Or

Donkey anti-goat IgG Alexa 633 (A-21082)

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**

Calbindin-Go-Af1040 (frontier science): 50 µg, USD700.00

A-11055 (Thermo Fisher Scientific): 0.5 ml, 71,300 HUF

A-21082 (Thermo Fisher Scientific): 0.5 ml, USD196.00

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

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

http://www.frontier-institute.com/wp/wp-content/uploads/pdf/Calbindin.pdf

**References**

Miura, E., Fukaya, M., Sato, T., Sugihara, K., Asano, M., Yoshioka, K., Watanabe, M. (2006) Expression and distribution of JNK/SAPK-associated scaffold protein JSAP1 in developing and adult mouse brain. J. Neurochem. 97:1431-1446. PMID: 16606357