**VGluT2 (Frontier) immunostaining with Fluoro Nissl counter**

**(Floating) PBS-XD**

Perfusion and Sectioning

1. Transcardially perfuse with cool 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.

2. Section the brain via vibrating microtome into 40 to 50-µm-thick slices and harvest them in 24-well plates containing PBS.

Antibody application (Room temperature, Light shielding)

3. PBS wash.

4 10% NDS in PBS-X, 30 min on shaker

5. 1st antibody in PBS-XD, ON on shaker

6. PBS-X wash (quick × 1, 10 min × 2)

7. 2nd antibody in PBS-XD, 2 hr on shaker

8. PBS-X wash (quick × 1, 10 min × 2)

9. PBS wash

Counterstaining with fluorescent Nissl

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

11. PBS wash (quick × 1, 10 min × 2)

Mounting

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

Coverslipping

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

Observation

14. 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)

GP anti-VGluT2 IgG (Frontier Institute Cat# VGluT2-GP, RRID:AB\_2571621)

in PBS-XD

**2nd antibody**

Do anti-GP IgG Alexa 647 (AP193SA6) (1:500 dilution, 3 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**

VGluT2-GP-Af810 (Frontier Institute): 50 µg, USD 700.00

AP193SA6 (EMD Millipore): 0.5 mg

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

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

https://www.labome.com/product/Enzo-Life-Sciences/BML-GC3108-0100.html

**References**