**c-Fos (Millipore) immunostaining with Fluoro Nissl counter**

**(Floating) XG**

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% NGS in PBS-X, 30 min on shaker

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

6. PBS-X wash (quick × 1, 10 min × 2)

7. 2nd antibody in PBS-XG for 2 h 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-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** (1:1000 dilution)

Rb anti-cFos (Millipore Cat# ABE457, RRID:AB\_2631318)

in PBS-XG

**2nd antibody** (1:500 dilution, 4 µg/ml final) (stock as 2 mg/ml)

Goat anti-rabbit IgG Alexa 488 (A-11034)

Or

Goat anti-rabbit IgG Alexa 633 (A-21071)

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**

ABE457 (Millipore): 100 µg, 269 USD

A-11034 (Thermo Fisher Scientific): 0.5 ml, 71,900 HUF

A-21071 (Thermo Fisher Scientific): 0.5 ml, 71,900 HUF

N-21479 (Invitrogen): 1 ml, 320 USD

Normal Goat Serum G9023-10ML (Sigma): 10 ml, 29 USD