Fluorescence immunostaining of vibratome sections

Original protocol from Huttner Lab, modified by Zerial lab

1) Dissect the liver and cut into pieces (~5x5x5 mm)
2) Fix the liver pieces with 4% PFA in PBS for 2 hours at room temperature then overnight at 4ºC
3) Wash liver pieces in PBS
4) Embed the liver lobes into 4% agarose in PBS
5) Make vibratome sections (50 ~ 200 µm thickness)
6) Place selected slices in 48-welled plate (1 slice per well).
7) Permeabilize with 0.5% Triton X-100 in PBS for 60 min at room temperature.
8) Quench sections in 10mM NH_4Cl in PBS for 30 min at room temperature.
9) Wash with TxBuffer 3 times for 3 min each.
10) Add primary antibody in TxBuffer (2 overnights at 4˚C).
11) Wash with 0.3% Triton/PBS 5x 5 min.
12) Add secondary antibody in TxBuffer (2 overnights at 4ºC).
13) Wash with 0.3% Triton/PBS 5x 5 min.
14) Wash in PBS 3x 1 min.
15) Clear the samples with glycerol (to reduce background):
    - Incubate in 25% glycerol/PBS until samples settling on the bottom of dish
    - Increase the glycerol concentration to 90% in a step-wise manner (50% → 75% → 90%):
        i. For overnight change to 50% glycerol in PBS
        ii. Next morning change to 75% glycerol in PBS
        iii. For overnight change to 90% glycerol in PBS
16) Mount on glass slide with 90% glycerol/0.2% n-propyl-gallate covered by #1.5 coverslips (thickness 0.17 ± 0.005 mm)
17) Image

TxBuffer (1L):

0.2% gelatin (100mL 2% gelatin in PBS filtered, stored at -20˚C)
300mM NaCl (17.53g)
0.3% Triton X-100 (3mL)
Add PBS to make 1L, aliquot to 50mL falcon tubes, store at -20˚C