Protocol for immunofluorescent staining of mouse frozen sections

Tissue: cryosections adhered to slides from blocks embedded in OCT using the 2-methylbutane (isobutene) method: see cryoprotection and processing of embryonic tissue protocol. This protocol is also suitable for 40μm free floating sections cut on a vibratome (see protocol for free floating immunohistochemistry).

Day 1

1. Allow sections to come to room temperature (~30min) and rinse in PBS for 5 min at RT.
2. Fix for 5 min with 4% paraformaldehyde st RT.
3. Wash 2 x 5 min in PBS, 1 x 5 min in PBT (0.25% TritonX-100/PBS, 10ml 20% Triton X-100 in 800mL PBS).
4. Block 30 min- 1 hour on slide with 300-400µL 10% normal donkey serum/PBT per slide at RT in humid box. Prepare primary antibodies (below) while waiting.
5. Incubate in 300 µl Ab solution o/n at 4 degrees C with a parafilm coverslip in humid chamber to prevent evaporation.

Day 2

1. Remove humid chamber from 4°C and allow slides to come to RT.
2. Float off coverslips with PBS and remove with a forcep.
3. Wash with PBT 5X 10 min in a coplin jar (Prepare secondary antibodies during washes. See procedure below).
4. Apply 300-400µl secondary antibodies under dim light and incubate for 2 hrs at RT in a dark humid chamber.
5. Wash with PBT 5x 10min in coplin jar covered with foil.
6. Counter stain with Hoechst 33342 from molecular probes (H-3750; 6µL/60mL PBS) for 5min at RT in foil covered coplin jar.
7. Wash in PBS 1x, 5 min.
8. Coverslip with 2-3 drops of Biomeda Gel/Mount (M01) in dark.
9. Let dry for one hour. Apply nail polish to seal the edges of the coverslip.

Prepare primary Abs:

anti-b-gal from Biogenesis (4600-1409, goat IgG; 1:500 in 10% donkey serum/PBT)

anti-ChAT Ab (cholinergic neurons) from Chemicon (AB-144P), goat IgG, 1:100 [from 1/10 stock in PBS] in 10% donkey serum/PBT

anti-TH Ab (dopaminergic neurons) from Chemicon (AB152, rabbit IgG; 1:500 in 10% donkey serum/PBT)

anti-5-HT Ab (serotonergic neurons) from Immunostar (5-HT, rabbit IgG, 1:500 [from 1/10 stock in PBS] in 10% donkey serum/PBT)

anti-EGPF rabbit IgG, 1:1000-2000 (very good)

goat IgG, 1:500 (not good for embryonic tissues)

Rat IgG, 1:1000 (expensive, try to use the rabbit antibody for single labeling)

anti--Ror-α rabbit IgG, 1:50-100

anti-calbindin rabbit IgG, 1:2000

anti-P-H3 rabbit IgG, 1:1000

anti-2-di-p-Erk rabbit IgG, 1:200

anti-ki67 mouse IgG, 1:15-20

anti-En-hd rabbit IgG, 1:50

Prepare secondary Abs:

Alexa (red) 555 conjugated Donkey anti-**goat** IgG from Molecular Probes (A-21432; 1:500 in 1% donkey serum/PBT)

Alexa (green) 488 conjugated Donkey anti-**rabbit or Rat** IgG from Molecular Probes; 1:500 in 1% donkey serum/PBT.

Alexa (red) 555 conjugated Donkey anti-**mouse** IgG; 1:500 in 1% donkey serum/PBT.

Alexa (blue) 350 conjugated Donkey anti-**goat** IgG; 1:500 in 1% donkey serum/PBT.