PROTOCOL FOR FLUORESCENCE IMMUNOHISTOCHEMISTRY OF FROZEN SECTIONS

Preparation of 0.2% Sodium Borohydride in 100mM PB (a.k.a. sodium tetrahydroborate, $NaBH_4$)

0.2 g $NaBH_4$
25 ml 0.4 M phosphate buffer pH 7.4
75 ml dd H_2O

WARNING: Sodium borohydride is highly flammable in contact with moisture and is very toxic to the skin. Do not leave the flask uncapped. Close it tightly after weighing, seal with parafilm, wrap it in its non-flammable cloth and return to its original metal canister

1. Take tissue sections out of the freezer and dry them 1h x 37C on a slide warmer.

1b) OPTIONAL: POST-FIXATION (required if tissue was not fixed by perfusion)
Dip the sections 10min in cold fixative If you do this step, take the sections after fixation and let them dry at room temperature x 15-20 minutes. Suggested fixatives:
- Normal fixative: 4% paraformaldehyde (or 10% formalin) in 100 mM phosphate buffer, pH 7.4
- Volatile fixative: 100% methanol or cold 100% acetone (will quench fluorescence from fluorescent proteins in the tissue)
- Strong fixative: 70% ethanol + 10 % formalin + 5% glacial acetic acid in ddH_2O

2. Mark the borders of the sections with clear silicone or hydrophobic pen.

3. Rinse the sections in 10 mM PBS (pH 7.4) 2x10 min (add two extra washes if there was post-fixation).

3b) OPTIONAL: STEAMING FOR ANTIGENIC RECOVERY
- Fill up the steamer with dd H_2O and turn it on.
- When the water starts to steam, open the steamer, place the sections inside and cover each one with a large amount of citrate buffer (10 mM sodium citrate, pH 6-6.5 adjusted with HCl)
- Steam the sections 5-10 minutes

4. Rinse the sections in 10 mM PBS 3 x 5 min.

4b) OPTIONAL: REDUCTION OF AUTOFLUORESCENCE
- Dip the sections in 0.2% sodium borohydride 2 x 1 min each
- Rinse the sections in 10 mM PBS 3 x 5 min

5. *Block and permeabilize the sections:* 10 mM PBS with 5% normal serum from the species donor of the secondary antibody (usually donkey or goat) and 0.1% to 0.3% Triton X100. Alternatively use 5% bovine serum albumin instead of serum. Incubate sections 1h at room temperature.

6. Add primary Ab in blocking buffer, overnight in the cold room, inside a humid chamber. Alternatively, leave the Ab a whole day or two in the cold room if the signal is very weak.

7. Wash primary Ab 3-5 times x 10 min each, in 10 mM PBS with 0.05-0.1% Triton X100

8. Add fluorescent secondary Ab in blocking buffer, 2h x room temp or overnight in the cold room, inside a humid chamber. Keep the slides in the dark. The Ab can be added together with DAPI diluted 1/3000 – 1/5000 (final concentration: ~1 µg/ml).

9. Wash secondary Ab 3-5 times x 10 min each, in 10 mM PBS with 0.05-0.1% Triton X100. Keep the slides covered with aluminum foil.

10. Mount each slide with a large drop of Fluoromount (or similar fluorescence-compatible mounting medium) and add a coverslip.

11. Let the medium cure 1 hour at room temperature or overnight in the cold room before adding nail polish to seal the slides.

12. Seal the coverslips with nail polish. Keep the slides in the dark at 4C.

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