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In situ Hybridization for Tyrosinase

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We use this protocol and it's working

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Abstract

In situ hybridization protocol with DIG-labelled riboprobes

Brain sectioning

- 1 Paraformaldehyde perfused brains are cryoprotected in 30% sucrose and frozen with cold isopentane.
- 2 Frozen brains are cryosectioned at 30 microns in a cryostat
- 3 Cryosections are mounted on superfrost slides and dried over-night at room temperature before storing them at -80°C until processing

In situ hybridization

- 4 Sections are fixed for 10 min in 4% paraformaldehyde
- 5 After washing in PBS, sections are permeabilized with Proteinase K for 5 min (1 µg/mL Proteinase K, 50mM Tris·HCl, and 5 mM EDTA)
- 6 Refix in 4% paraformaldehyde for 10 min
- 7 After rinsing in PBS, proceed with the acetylation step for 10 min with 590 mL of H₂O, 8 mL of triethanolamine, 1 mL of 37% HCl, and 1.5 mL of acetic anhydride
- 8 Incubate for 3 h at room temperature for prehybridization (50% formamide, 5× SSC, 5× Denhardt's solution, 250 µg/mL Baker's yeast RNA, and 500 µg/mL salmon sperm DNA)
- 9 Hybridization overnight at 72 °C in 120 µL prehybridization mix with 2 µL of digoxigenin-labeled sense (control probe not complementary) and antisense (test probe complementary) probes for tyrosinase (Riboprobes are generated by PCR and labelled with the DIG RNA labelling kit from Roche (11175033910))
- 10 Wash in 72 °C heated solutions of 5× SSC and 0.2× SSC, and then equilibrated in B1 buffer [0.1 M Tris·HCl (pH 7.5) and 0.15 M NaCl]



- 11 Preincubation for 1 h in 10% heat-in-activated FCS in B1 buffer
- 12 Incubate overnight with antidigoxigenin-AP Fab fragments (Roche, diluted 1:5,000 in B1 with 1% heat-inactivated FCS)
- 13 Wash in B1 buffer and placed the sections in a solution of 0.1 M Tris-HCl (pH 9.5), 0.1 M NaCl, and 50 mM MgCl₂ (B2 buffer)
- 14 mRNA localization is visualized after overnight incubation with 0.2 mM NBT/BCIP (Roche) and 0.24 µg/mL levamisole in 10% PVA/B2 solution protected from light
- 15 The color reaction is terminated by water and mounted in Aquatex (Merck)
- 16 Sections are analysed and photographed with a microscope coupled to a camera