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In situ Hybridization for Tyrosinase

In 1 collection

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

In situ hybridization protocol with DIG-labelled riboprobes

Brain sectioning

1 Paraformaldehide 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 -80ºC 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 H 2O, 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 MgCl 2 (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