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Tissue processing for immunofluorescence

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Benjamin David Webster Belfort[1], Anthony Insalaco[1]

[1]Baylor College of Medicine

Benjamin David Webster Belfort
Baylor College of Medicine

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We have published data resulting from this protocol.

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Abstract

Protocol for murine olfactory bulb tissue processing, including tissue fixation, cryopreservation, and OMP labeling. This protocol is intended for confocal microscopy applications.

Olfactory bulb harvesting, fixation, and cryopreservation

1 Begin by deeply anesthetizing the mouse using any preferred method (following institutionally approved protocols). Confirm surgical plane of anesthesia by firm toe pinch and proceed with transcardiac perfusion of at least 10 mL of ice-cold 1x PBS followed by 10 mL of 4% paraformaldehyde (16% PFA diluted in PBS; Electron Microscopy Sciences Cat# 15710).

2 Harvest olfactory bulbs (OBs) and fix in 4% PFA at 4°C overnight.

3 Move OBs to 30% sucrose at 4°C for 36h for cryoprotection.

4 Freeze OBs in OCT (Fisher HealthCare Cat# 23-730-571).

Tissue sectioning and OMP labeling

5 Section OBs at 40 µm on a cryostat (Leica Cat# CM1860), collecting every third section for analysis.

6 Place tissue sections in blocking buffer (5% Donkey Serum, 1X PBS, 0.1% Triton) for 1 h at room temperature.

7 Wash sections once in PBST (0.1% Triton).

8 Incubate sections with anti-OMP antibody (1:20,000; FUJIFILM Wako Chemicals U.S.A. Corporation Cat# 544-10001-WAKO) in blocking buffer at 4°C overnight.

9 Wash tissue sections three times with PBST (0.1% Triton).

10 Incubate sections with secondary antibody (1:1,000 anti-goat, Alexa Fluor 488 Invitrogen Cat# A-11055) in blocking buffer for 1 h at room temperature.

11 Wash sections three times with 0.1% PBST.

12 Incubate sections with Hoechst (1:1,000, Thermo Fisher Cat# 62249) in 0.1% PBST for 15 min at room temperature.

13 Wash sections once more with 0.1% PBST.

14 Mount sections on slide with Fluoromount-G (Southern Biotech Cat# 0100-01).

Guidelines and Warnings

15 This protocol requires prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.