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Immunofluorescence labelling and imaging of cholinergic interneurons in post-mortem human brain tissues

In 2 collections

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We use this protocol and it's working

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Abstract

This protocol details immunofluorescence labelling and imaging of cholinergic interneurons along with striatal astrocytes in formalin-fixed paraffin-embedded (FFPE) post-mortem human brain tissues.

Troubleshooting

Collection and fixation of post-mortem human brain tissues

1 Collect 6 µm-thick sections of formalin-fixed paraffin-embedded (FFPE) tissues containing the anterior basal ganglia at the level of nucleus accumbens.

2 Place tissue sections in an oven at 70°C for 30 mins.

3 Dewax tissue sections in xylene (3 × 5 mins).

4 Rehydrate tissue sections through decreasing concentration of industrial denatured alcohol (IDA) (100%, 100%, 90%, 70%; 5 mins each)and subsequently in distilled water (5 mins).

Primary Antibody

5 Perform heat-induced epitope retrieval by placing tissue sections in autoclave (121°C, 20 mins) in 0.01 M sodium citrate buffer (pH 6.0).

6 Rinse in PBS (3 × 5 mins).

7 Incubate samples at 4°C overnight with a mixture of the following primary antibodies:
- anti-ChAT antibodies (1:50, AB144P; Millipore, UK; RRID:AB_2313845)
- anti-GFAP antibodies (1:2000; Z0334; Agilent Dako, Santa Clara, United States; RRID:AB_10013382).

Note

Antibodies were diluted in 0.3% Triton X-100, 2% fetal bovine serum (FBS) and PBS.

Secondary Antibody

8 Rinse samples in PBS (3 × 5 mins).

9 Prepare Secondary Antibody mixture of Alexa Fluor 488-conjugated donkey anti-rabbit secondary antibody (1:200; A-11055; ThermoFisher Scientific, UK) and Alexa Fluor 594-conjugated donkey anti-goat secondary antibody (1:200; ThermoFisher Scientific, UK) diluted in 0.1% Triton-X in PBS.

10 Incubate sections in Secondary Antibody mixture in the dark (covered with foil) for 1 hour at room temperature.

Mounting

11 Incubate sections with TrueBlack® Lipofuscin Autofluorescence Quencher (1:20 with 70% ethanol; Biotium, Fremont, CA, United States) for 30 seconds to block endogenous fluorescence signal.

12 Rinse in PBS (3 × 2 mins).

13 Mount and coverslip with Vectashield antifade mounting medium with DAPI (H1900, Vector Laboratories, Peterborough, UK).

Confocal Imaging and Analysis

14 Visualise immunofluorescent-stained slides using Zeiss 880 Airyscan inverted confocal microscope (Carl Zeiss).

15 Acquire images using 10x objectives (Plan-Apochromat/0.45 NA) and 63x objectives (Plan-Apochromat, oil immersion; DIC M27/1.4 NA) with laser excitation at 405 nm (solid state), 488 nm (Argon), and 561 nm (solid state).

16 Perform image capture and processing using the Zen Black and Zen Blue (Carl Zeiss,Germany) software.