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ChAT Immunofluorescent Staining of medulla oblongata fixed sections

Forked from ChAT Immunofluorescent Staining

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We use this protocol and it's working

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Abstract

This protocol is designed for choline acetyltransferase (ChAT) staining using the Millipore AB144P (RRID:AB_2079751) antibody. Tissue stained with this protocol include 30µm free-floating mouse brain sections. All tissue was from mice perfused with 4% PFA.

ChAT Immunofluorescent Staining of medulla oblongata fixed sections

2d 4h 35m

1 Wash tissue slices in tris-buffer saline (TBS: 0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes. Repeat x3. 30m

2 Incubate in in 10% Methanol + 3% H2O2 in TBS for 20 min at room temperature 20m

3 Wash tissue slices in tris-buffer saline (TBS: 0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes. Repeat x3. 30m

4 Incubate in blocking buffer (5% donkey serum, 2% BSA, 0.5% triton X-100 in TBS) for 1 hour at room temperature. 1h

5 Incubate with anti-ChAT primary antibody (RRID:AB_2079751) at 1:500 dilution in 50% blocking solution for 48 hours at 4 *°C.* 2d

6 Wash tissue slices in TBS-T (TBS+ 0.25% Triton x-100) for 10 minutes. Repeat x3. 30m

7 Incubate in fluorescent secondary antibody at 1:200 dilution in 50% blocking buffer for 1 hour at room temperature. 1h

8 Wash tissue slices in TBS-T(TBS+ 0.25% Triton X-100) for 10 minutes. Repeat x 2. 20m

9 Wash tissue slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes. 10m

10 Mount free-floating sections on SuperFrost+ slides (if staining free-floating tissue) and let dry at room temperature for 15 minutes. 15m

11 Coverslip with fluorescent mounting medium and #1.5 coverslips. Outline coverslip with clear nail polish and store at $4^{O}C$.