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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 3-day choline acetyltransferase (ChAT) staining using the EMD Millipore AB144P (RRID:AB_2079751) antibody. Tissue stained with this protocol include 40µm free-floating mouse brain and spinal cord sections, and 20µm pre-mounted retina sections. All tissue was from mice perfused with 10% buffered formalin.

Troubleshooting

ChAT Immunofluorescent Staining

2d 4h 35m

1 Wash tissue slices in 1X phosphate buffered saline (PBS) for 5 minutes. Repeat x2. 5m

2 Incubate in blocking buffer (10% horse serum, 0.5% gelatin, 0.1% triton X-100 in 1X PBS) for 2 hours at room temperature. 2h

3 Incubate in primary antibody (goat anti-ChAT (RRID:AB_2079751) at 1:200 dilution in blocking buffer) at 4^{o}C for 48 hours. 2d

4 Wash tissue with 1X PBS for 5 minutes. Repeat x4. 5m

5 Incubate in secondary antibody for 2 hours at room temperature. 2h

6 Wash tissue with 1X PBS for 5 minutes. Repeat x4. 5m

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

8 Coverslip with fluorescent mounting medium and #1.5 coverslips. Outline coverslip with clear nail polish and store at 4^{o}C or -20^{o}C depending on length of storage. 5m