



Sep 09, 2024

ChAT Immunofluorescent Staining of medulla oblongata fixed sections



Forked from [ChAT Immunofluorescent Staining](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg32nzpv25/v1>

Pietro La Vitola¹

¹German Center for Neurodegenerative Diseases (DZNE)



Pietro La Vitola

German Center for Neurodegenerative Diseases (DZNE), Alignin...

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.kqdg32nzpv25/v1>

Protocol Citation: Pietro La Vitola 2024. ChAT Immunofluorescent Staining of medulla oblongata fixed sections. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.kqdg32nzpv25/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working



Created: September 06, 2024

Last Modified: September 09, 2024

Protocol Integer ID: 107044

Keywords: choline acetyltransferase, ChAT, immunofluorescence, medulla, mouse brain section, choline acetyltransferase, antibody, floating mouse brain section

Funders Acknowledgements:

Aligning Science Across Parkinson's

Grant ID: ASAP-000420

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°C .