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Immunohistochemistry of rTg4510 mouse brain

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Miguel Da Silva Padilha¹, Irina Dudanova¹, Itika Saha², F. Ulrich Hartl², Mark S. Hipp^{3,4}

¹Molecular Neurodegeneration Group, Max Planck Institute of Neurobiology, 82152 Martinsried, Germany;

²Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany;

³Department of Biomedical Sciences of Cells and Systems, University Medical Center Groningen, University of Groningen, Antonius Deusinglaan, 1, 9713 AV Groningen, The Netherlands;

⁴School of Medicine and Health Sciences, Carl von Ossietzky University Oldenburg, 26129 Oldenburg, Germany



Felix Kraus

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We use this protocol and it's working

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Abstract

This protocol describes immunohistochemical staining of the Tau transgenic mice rTg4510 (Santacruz et al, 2005) brain section for Tau aggregates phosphorylated at Ser202/Thr205 and the AAA+ ATPase Valosin-containing protein (VCP). Experiments involving animal models must be performed in accordance with relevant institutional guidelines and regulations.



1 Deeply anesthetize mice with 1.6% Ketamine/0.08% Xylazine and transcardially perfuse with PBS followed by 4% paraformaldehyde (PFA)(Santa Cruz) in PBS.

2 Dissect brains out of the skull and post-fix in 4% PFA in PBS overnight.

12h

3 Embed fixed tissue in agarose and section into 40 μ m thick sections using a vibratome (VT1000S, Leica).

Note

NOTE: The sections can be stored in PBS 0.05% sodium azide at 4°C.

4 Permeabilize sections with 0.5% Triton X-100 and wash with PBS.

5 Incubate sections in blocking solution consisting of 0.2% BSA (w/v), 5% donkey serum (v/v) (abcam), 0.2% lysine (w/v) (Sigma-Aldrich), 0.2% glycine (w/v) (Sigma-Aldrich) in PBS for 30 min at room temperature (RT).

30m

6 Incubate sections with primary antibodies (anti-phospho-Tau AT-8 (Thermo, Cat# MN1020, 1:300); anti-VCP (Novus Biologicals, Cat# NB 100-1558, 1:500) diluted in 0.3% Triton X-100 (v/v), 2% BSA (w/v) in PBS overnight at 4°C.

12h

7 Wash sections in PBS and incubate with secondary antibodies and Neurotrace 640/660 (ThermoFisher, Cat# N21483, 1:500) diluted in 0.3% Triton X-100, 3% donkey serum (v/v) for 2 h at RT.

2h

8 Stain nuclei with 0.5 μ g/ml DAPI.

9 Mount sections on Menzer glass slides using Prolong Glass fluorescence (Invitrogen) mounting medium.