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Immunohistochemistry of rTg4510 mouse brain

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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.

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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 µm thick sections using a vibratome (VT1000S, Leica).

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) 30m

(abcam), 0.2% lysine (w/v) (Sigma-Aldrich), 0.2% glycine (w/v) (Sigma-Aldrich) in PBS for 30 min at room temperature (RT).

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 µg/ml DAPI.

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

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