



SEP 06, 2022

Immunohistochemistry of rTg4510 mouse brain

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OPEN  ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.x54v9den4g3e/v1

External link:

<https://www.biorxiv.org/content/10.1101/2022.02.18.481043v1.full>

Protocol Citation: Miguel Da Silva Padilha, Irina Dudanova, Itika Saha, F. Ulrich Hartl, Mark S. Hipp 2022.

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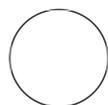
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<https://dx.doi.org/10.17504/protocols.io.x54v9den4g3e/v1>

MANUSCRIPT CITATION:

Itika Saha, Patricia Yuste-Checa, Miguel Da Silva Padilha, Qiang Guo, Roman Körner, Hauke Holthusen, Victoria A. Trinkaus, Irina Dudanova, Rubén Fernández-Busnadiego, Wolfgang Baumeister, David W. Sanders, Saurabh Gautam, Marc I. Diamond, F. Ulrich Hartl, Mark S. Hipp
bioRxiv 2022.02.18.481043;
doi:

<https://doi.org/10.1101/2022.02.18.481043>



Felix Kraus

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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Protocol status: Working
We use this protocol and it's working

Created: Sep 05, 2022

Last Modified: Dec 26, 2022

PROTOCOL integer ID:
69594

Keywords: ASAPCRN

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

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.