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We use this protocol and it's working

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Immunohistochemistry

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ABSTRACT

Immunohistochemistry

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1 Mice were euthanized using isoflurane (VetOne Cat#502017) inhalation followed by cervical dislocation. At autopsy, mouse brains were removed from the calvarium and rapidly placed in a 4% paraformaldehyde (PFA) solution (Thermo Scientific, Cat# J19943-K2) for drop fixation.

2 For immunohistochemistry, 5 mm sagittal tissue sections were stained with antibodies against total a-syn (Synaptic Systems, 1:15,000, Cat #128211) and Ser129P a-syn (Cell Signaling, 1:3000, Cat #23706). Slides were stained using Ventana Discovery Ultra (Ventana Medical Systems, Tucson, AZ, USA).

3 The sections were deparaffinized by three cycles of heating to 72°C in the presence of Discovery Wash (Ventana, Ref# 07311079001) for 4 min with a rinse between each heating step. Subsequently, antigen retrieval was performed using CC1 buffer (Tris-EDTA; pH 8.6 Ventana, Ref# 06414575001) for 40 min (5 cycles of 8 min each with fresh CC1 buffer for each incubation) at 100°C. Endogenous peroxidase was quenched by incubation with inhibitor CM (Ventana, Cat# 760-159) for 8 min. The sections were incubated with primary antibodies for 32 minutes at 37 °C, mixing the samples every 4 min to ensure an even distribution of the antibody.

4 Primary antibodies were detected using the OmniMap system, with an anti-rabbit secondary antibody coupled to an HRP polymer (Roche Cat# 760-4311, RRID:AB_2811043) (Ventana, Ref# 05266548001). Antibody presence was visualized using the Ventana Discovery ChromoMap DAB kit (Ventana, Ref# 05266645001) as a chromagen followed by hematoxylin II (Ventana, Ref#05277965001) for 4 min followed by a 4 min treatment with bluing reagent (Ventana, Ref# 05266769001) as a counterstain.

5 The slides were rinsed, dehydrated with alcohol and xylene, and mounted on coverslips. To confirm the specificity of the pSer129 antibody, the epitope was dephosphorylated using Lambda Protein Phosphatase (New England Biolab, Cat # P0753). Briefly, 2400 units of the Lambda Protein Phosphatase were supplemented with a 10x NEBuffer and 10x $MnCl_2$ and added to the slides for 2h at 37°C. Antigen retrieval and peroxidase quenching were performed on the slides before incubation with the pSer129 antibody.

6 For quantification of Ser129P staining, after background correction, the following ROIs were placed to sample the frontal lobe and hippocampus – ROI measuring ~ 2.5 mm x 1 mm flanking the frontal pole, and an irregular ROI over the CA3 region (which was strongly positive for Ser129P and easy to identify). Average intensities were considered for quantification, and data were analyzed in GraphPad Prism software [(RRID:SCR_002798) http://www.graphpad.com/].