Anti- Phospho-Ser129 Alpha Synuclein Immunohistofluorescence Protocol

Catalog #: p1571-129
Species: rabbit
Tissue: Rat brain

Fixation: Transcardial perfusion, 4% Paraformaldehyde in 1X PBS, post fixed overnight
Antibody incubation: Primary Antibody- 4C, overnight
Secondary Antibody- RT, 90 minutes
Antigen Retrival: None

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde in freshly prepared 1X PBS
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4, pH 7.6
- ✓ **30% sucrose buffer:** 30g of sucrose in 100mls of 1xPBS
- ✓ **cryoprotectant:** 30% glycerol and ethylene glycol in 1xPBS
- ✓ **PBS-T:** 1X PBS with 0.5% Triton X-100
- ✓ **Secondary Antibody:** example used is Donkey-Anti-Rabbit 594 from Invitrogen, cat# A21207 Cat#: 111-165-003
- ✓ **Mounting media:** 80% glycerol in 1xPBS
- ✓ **DNA stain:** Hoechst 33342, Invitrogen, cat# H3570

Before you begin

The tissue used in this protocol was perfused transcardially. Alternatively, tissues can be fixed without perfusion. Immediately after sacrifice cut tissue into 0.5cm sections and place in 4% paraformaldehyde overnight at 4C. Submerge sections into a sufficient volume of fixative, for proper fixation a recommended minimum volume of 20x each in separate containers. For optimal antibody epitope binding, tissues should not stay longer than 24 hours in fixative.

Protocol

1. Transfer tissue section into 30% sucrose in 1X PBS for 72 hours at 4C.
   Tech Tip:
   a. To prevent ice crystals from forming on tissue and destroying antibody epitope binding sites, do not remove the tissue until it has sunk to the bottom of the beaker to ensure complete sucrose infiltration.
2. Freeze tissue sections with dry ice and store at -80C.
3. Mount tissue onto cryostat and cut tissue into 30 mu thick sections at -20C. Place sections into cryoprotectant solution to float freely at -20C.
4. Rinse tissue sections with 1X PBS 3 times, in 5 minute intervals.
5. Permeabilize tissue sections by sequentially washing in 10 minute intervals with PBS-T, then 1x PBS, then PBS-T .

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6. Dilute Anti- Phospho-Ser129 Alpha Synuclein Antibody (Cat. # p1571-129) to 1:200 in PBS-T and incubate primary antibody on tissue sections overnight at 4C.
7. Sequentially wash tissue sections in 10 minute intervals with PBS-T, 1xPBS, PBS-T.
8. Dilute secondary antibody in 1X PBS per manufacturer's recommendation. Incubate tissue sections for 90 minutes at room temperature.

   Tech Tip:

   a. A donkey anti-rabbit- 594 dye diluted 1:100 was used for this protocol.
9. Remove secondary antibody and wash the tissue section with 1X PBS 3 times, in 10 minute intervals.
10. Dilute Hoechst stain 1:500 in 1X PBS and incubate sections for 30 minutes.
11. Remove stain and wash the tissue section with 1X PBS 3 times, in 10 minute intervals.
12. Place tissue sections onto slide and apply mounting medium. Air dry sections for 30 minutes. Gently place glass cover slip before viewing under the microscope.

Reference:

Corwin, C., Nikolopoulou, A., Pan, A.L., Nunez-Santos, M., Vallabhajosula, S., Serrano, P., Babich, J. and Figueiredo-Pereira, M.E., 2018. Prostaglandin D2/J2 signaling pathway in a rat model of neuroinflammation displaying progressive parkinsonian-like pathology: potential novel therapeutic targets. *Journal of neuroinflammation*, 15(1), p.272.

Pierre, S.R., Lemmens, M.A. and Figueiredo-Pereira, M.E., 2009. Subchronic infusion of the product of inflammation prostaglandin J2 models sporadic Parkinson's disease in mice. *Journal of neuroinflammation*, 6(1), p.18.