Anti-Ubiquitin C-terminal Hydrolase 1 (PGP9.5) Immunohistofluorescence Protocol

Catalog #: 2060-UCHL1
Species: mouse
Tissue: Rat spinal cord

Fixation: 4% Paraformaldehyde in 1X PBS for 2 hours
Antibody incubation: Primary Antibody- 4C, overnight
Secondary Antibody- RT, 2 hours or 4C, overnight
Antigen Retrieval: None

Materials Required

- ✓ **Fixative:** 4% Paraformaldehyde in freshly prepared 1X PBS
- ✓ **isopentane:** chilled to -160C
- ✓ **acetone:** chilled to -20C
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **20% /30% sucrose buffer:** 20/30g of sucrose in 100mls of 1xPBS
- ✓ **Blocking buffer:** 1X PBS with 1% goat serum, also use for secondary incubation buffer
- ✓ **Incubation buffer:** 1X PBS with 0.1% goat serum
- ✓ **Secondary Antibody:** example used is Goat-Anti-Mouse Alexa 647 from ThermoFisher
- ✓ **Mounting media:** Vector Laboratories Vectashield with DAPI Cat #: H-1200

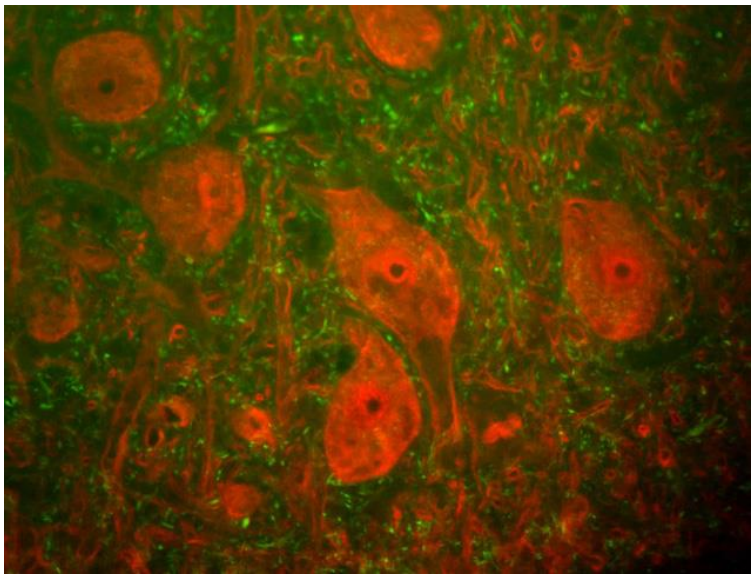
Before you begin

This protocol can be used for tissues fixed with or without perfusion. If tissues are harvested without perfusion, slice tissues into 0.5cm sections and place in 4% paraformaldehyde for 2 hours at RT. Submerge sections into a sufficient volume of fixative, for proper fixation a recommended minimum volume of 20x. Additionally each section of tissue should be in a separate container. If tissues are harvested after perfusion, harvest and place in fixative. For optimal antibody epitope binding, tissues should not stay longer than 24 hours in fixative.

Protocol

1. Place fixed tissue section into 20% sucrose in 1X PBS overnight at 4C.
2. Transfer tissue section into 30% sucrose in 1X PBS for 2-3 days 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.
3. Transfer tissue into isopentane that is at -160C. This can be done by cooling the isopentane in a plastic beaker placed in liquid nitrogen. Once tissue is frozen remove and immediately section with cryostat or store at -70C.

4. Mount tissue onto cryostat and cut tissue into 5-20mm thick sections at -25C and mount onto slides.
   Tech Tip:
   a. Slides can be stored at -70C for long term storage.
5. Wash slides with -20C acetone. Let slides dry.
6. Block slides with blocking buffer for 1 hour at RT.
7. Rinse slides with PBS 3 times, in 5 minute intervals.
8. Dilute Anti-Ubiquitin C-terminal Hydrolase 1 (Cat. # 2060-UCHL1) to 1:500 in incubation buffer. Incubate sections for 2 hours at room temperature or overnight at 4C.
9. Rinse slides with PBS 3 times, in 5 minute intervals.
10. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate sections for 2 hours at room temperature or overnight at 4C.
    Tech Tip:
    a. Alexa Fluor 647 dye diluted 1:2000 was used to produce the image below.
11. Remove secondary antibody and wash with PBS 3 times, in 5 minute intervals.
12. Apply mounting medium onto slide and gently place glass cover slip before viewing under the microscope.
    Tech Tip:
    a. Any mounting media can be used, for this protocol Vector Laboratories Vectashield medium was used. Cat #: H-1200.



Immunofluorescence of a section of rat spinal cord labeled with anti-UCHL1 (cat # 2060-UCHL1, 1:500, red) and anti-Neurofilament H (cat# 1451-NFH, 1:25,000, green). The large cells are alpha-motor neurons and UCHL1 fills the cytoplasm of their perikarya and dendrites.