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

Catalog #: 2060-UCHL1
Species: mouse
Tissue/ Cells: HEK 293 cells

Fixation: 3.7% Neutral buffered formalin for 1 minute
Antibody incubation: Primary Antibody- 4C, overnight
Secondary Antibody- RT, 3 hours or 4C, overnight

Materials Required

- ✓ **Fixative:** 3.7% Neutral buffered formalin (10mls of 37% Fisher formalin, 90mls of 1xPBS)
- ✓ **methanol:** chilled to -20C in a well-sealed container
- ✓ **1X PBS:** 137 mM NaCl, 28 mM Na_2HPO_4, 5.4 mM KCl, 2.9 mM KH_2PO_4 , pH 7.6
- ✓ **Blocking/Incubation buffer:** 1X PBS with 1% goat serum
- ✓ **Secondary Antibody:** example used is Goat-Anti-Mouse Alexa 488 from ThermoFisher
- ✓ **Mounting media:** ThermoFisher Histomount Cat #: 008030
- ✓ **Glass cover slip**

Before you begin

This protocol is intended for adherent cells grown in 6 well plates or in 35mm dishes using a glass coverslip placed on top of the cells. These work well with most immunofluorescence microscopes using lenses up to 100X magnification.

Protocol

1. Draw off culture medium with aspirator and add 1 ml of 3.7 % formalin in PBS solution to the dish. Incubate at room temperature for 1 minute.
   Tech Tip:
   a. To reduce background you can add 0.1% Tween 20 to PBS here and all subsequent steps. Use with caution as it may extract your antigen or wash cells off the dish.
2. Remove the neutral buffered formalin and add 1 ml of cold methanol. Incubate for 1 minute or less.
3. Remove methanol and add 1 ml of PBS, not letting the specimen dry out.
4. Remove PBS and add blocking buffer. Incubate for 30 minutes at room temperature.
5. Rinse cells with PBS 3 times, in 10 minute intervals.
6. Dilute Anti-Ubiquitin C-terminal Hydrolase 1 (Cat. # 2060-UCHL1) to 1:500 in incubation buffer. Incubate cells for 1 hour at room temperature or overnight at 4C.
7. Remove primary antibody and wash with 1 ml PBS 3 times, in 10 minute intervals.
8. Dilute secondary antibody in incubation buffer per manufacturer's recommendation. Incubate cells for 3 hours at room temperature or overnight at 4C.

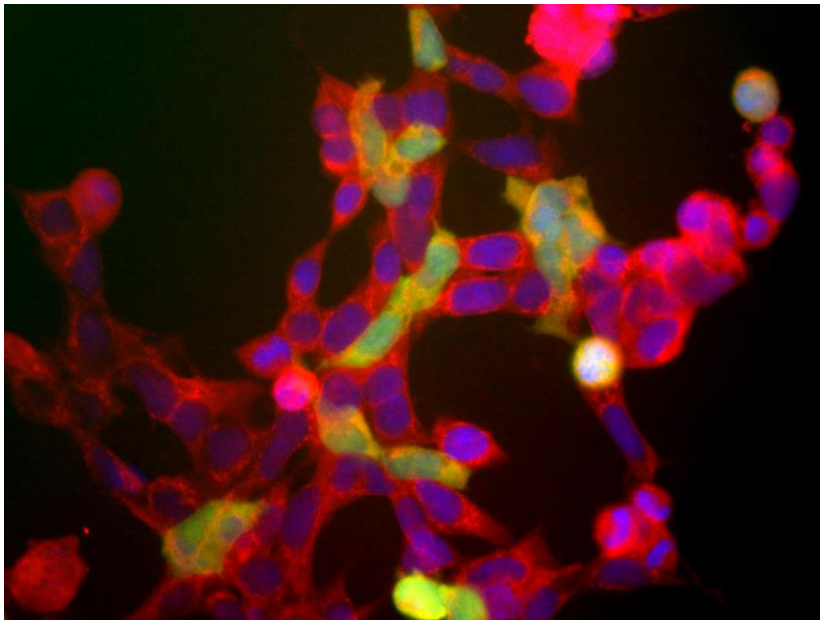
Tech Tip:

a. Alexa Fluor 488 dye diluted 1:2000 was used to produce image below.

9. Remove secondary antibody and wash with PBS 3 times, in 5-10 minute intervals.
10. Apply mounting medium onto dish and gently place glass cover slip before viewing under the microscope.

Tech Tip:

a. Any mounting media can be used, for this protocol ThermoFisher Histomount medium was used. Cat #: 008030.



Immunolabeling of HEK 293 cells labeled with anti-UCHL1 antibody (catalog #2060-UCHL1, green, 1:500) and anti-neuron specific enolase antibody (catalog #1520-NSE, red, 1:500). The blue in DAPI staining nuclear DNA.