Protocol: Inhibition of Autophagy and LC3B Antibody (NB100-2220) Immunocytochemistry (ICC)

Materials

- Chloroquine diphosphate (CQ) (10 mM) in dH_2O
- 1X PBS
- 4% paraformaldehyde in 1X PBS (make fresh)
- 1X PBS with 0.5% Triton X-100
- LC3 primary antibody (NB100-2220) (5 μg/mL) in 1% normal serum or BSA in 1X PBS
- fluorophore-conjugated anti-rabbit secondary antibody in 1% normal serum or BSA in 1X PBS
- 1-5% normal serum or BSA in 1X PBS
- DAPI or Hoechst (1-10 μg/mL)

Methods

Tip: For more information, see our Immunocytochemistry handbook at novusbio.com/icc-handbook.

1. Harvest cells from a semiconfluent culture and plate them on sterile glass coverslips. For optimal adhesion, some cell types may require coated coverslips (e.g. poly-L-lysine).

   Tip: Coverslips need to be sterilized with ethanol and flaming or by exposure to UV radiation. To facilitate handling and incubations, place several small circular coverslips in a single culture dish.

2. Grow cells to semi-confluency (70-75%).

   Tip: Gently move seeded coverslips to the incubator to ensure sufficient cell adhesion onto the coverslips. Do not let coverslips dry out and avoid adding solutions directly on the cells to reduce detachment.

3. Add CQ to culture dishes to a final concentration of 50 μM and incubate overnight (16 hours). Remember to include an untreated sample as a negative control.

4. Aspirate the culture medium from the dish, and gently wash with 1X PBS at room temperature.

5. Incubate the coverslips in freshly prepared 4% paraformaldehyde in 1X PBS at room temperature for 10 minutes.

6. Rinse coverslips with 1X PBS for 2 minutes.

7. Incubate the coverslips in 1X PBS, 0.5% Triton X-100 at room temperature for 5 minutes.

8. Wash away the permeabilization buffer by incubating in 1X PBS for 5 minutes.

9. Block the coverslips in 1-5% normal serum or BSA (in 1X PBS) for 1 hour at room temperature.

10. Dilute primary antibody in 1% normal serum or BSA (in 1X PBS). Incubate coverslips with ~5 μg/mL rabbit anti-LC3 primary antibody (NB100-2220) for 1 hour at room temperature (37°C is optional), or for 16 hours at 4°C.

    Tip: Antibody concentrations may range from 5-20 μg/mL.

11. Wash the coverslips in 1X PBS, 3 times for 5 minutes each.

12. Prepare an appropriate dilution of fluorophore-conjugated anti-rabbit secondary antibody in 1% normal serum or BSA (in 1X PBS). Antibody concentrations need to be optimized for every system.

13. Incubate the coverslips in the secondary antibody dilution for 1 hour at room temperature in the dark.

14. Wash the coverslips in 1X PBS, 3 times for 5 minutes each.

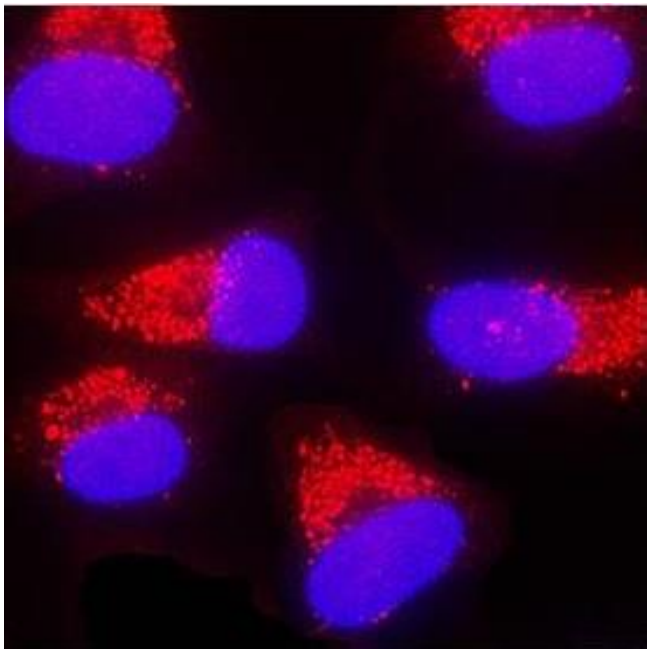
15. Once all washing steps have been completed, the coverslips can be counter stained with DAPI or Hoechst (1-10 μg/mL) to stain the nuclei.

16. Invert the coverslip onto a glass slip with mounting media. Use an antifade containing mounting media to reduce photobleaching.

17. Carefully remove any excess mounting media and seal as required with nail polish.

18. Use a fluorescence microscope to examine and image the cells.

    Note: Quantitation of LC3-II positive puncta may be performed manually or automated by the use of appropriate software for image analysis. However, a standardized approach should be applied across samples to prevent the introduction of bias and to ensure reproducibility.



Immunocytochemistry/Immunofluorescence: LC3B Antibody [NB100-2220] - LC3B detected in immersion fixed HeLa human cervical epithelial carcinoma cell line treated with Chloroquine using 1μg/mL rabbit anti-LC3B polyclonal (NB100-2220, Novus Biologicals). Cells were stained using donkey anti-rabbit IgG-NL557 and counterstained with DAPI (blue).