

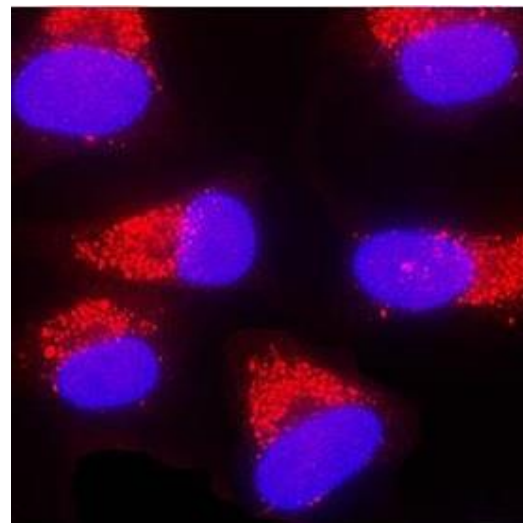
NB100-2220 Protocol

Immunocytochemistry/Immunofluorescent (ICC/IF)

Inhibition of Autophagy using Chloroquine diphosphate with LC3B Antibody (NB100-2220)

Materials

- Chloroquine diphosphate (CQ) (10 mM) in dH₂O
- 1X PBS
- **Blocking Buffer**: 1% normal goat serum in 1X PBS
- **Fixation Buffer (fresh)**: 4% paraformaldehyde in 1X PBS
- **Permeabilization Buffer**: 1X PBS with 0.05% Triton X-100
- LC3B primary antibody (NB100-2220) (2 µg/mL) in Blocking Buffer
- Fluorophore-conjugated anti-rabbit secondary antibody in Blocking Buffer
- **Nuclear Stain**: DAPI or Hoechst or DRAQ5™ (NBP2-81125)



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

Methods

Tip: Review our Immunocytochemistry handbook for technical tips and an easy to follow troubleshooting guide 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 Fixation Buffer at room temperature for 10 minutes.

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6. Rinse coverslips with 1X PBS for 2 minutes.
7. Incubate the coverslips in Permeabilization Buffer at room temperature for 5 minutes.
8. Wash in 1X PBS for 5 minutes.
9. Block the coverslips in Blocking Buffer for 1 hour at room temperature.
10. Dilute rabbit anti-LC3B primary antibody ([NB100-2220](#)) to ~2 µg/mL in Blocking Buffer and incubate on coverslips for 1 hour at room temperature. *Note: Coverslips can alternatively be incubated at 37°C for 1 hour or 4°C for 16 hours.*

Tip: Antibody concentrations may range from 1-10 µg/mL. The optimal concentration needs to be determined for every system.

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 Blocking Buffer. *Note: Antibody concentrations need to be optimized for every system.*
13. Incubate the coverslips in the secondary antibody solution 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 counterstained with a nuclear dye.

Option 1 (UV dye): Counterstain with 1-10 µg/mL DAPI or Hoechst in 1X PBS

Option 2 (Far-red dye): Counterstain with 5 µM DRAQ5™

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.

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