ImageStream® LC3 Puncta Staining Protocol

Samples: (1 x 10^6 cells per test)

Single fluorescent color control samples – unstained, LC3-AF647 (or LC3-AF488), DAPI

Experimental samples – untreated, positive control treatment, experimental treatment

Materials

1. 1x Phosphate buffered saline without Ca^{2+}/Mg^{2+} (PBS)
2. 0.005% saponin (Sigma-Aldrich Cat# S4521); Make the 0.005% saponin in 1x PBS
3. Wash buffer (PBS/0.1% azide/2% FBS)
4. 10% formaldehyde (Polysciences # 04018); Make working solution of 1% and 2% formaldehyde in 1x PBS
5. Mouse anti-LC3 (Medical & Biological Laboratories, Code No. M152-3); Make working solution at 1:100 in the 0.005% Saponin
6. Alexa Fluor 647 Donkey anti-mouse (Invitrogen Cat# A31571) or Alexa Fluor 488 donkey anti-mouse (Invitrogen Cat# A21202); Make working solution at 1:100 in wash buffer
7. 10x DAPI: 10µg/mL DAPI (dissolved in dH2O, Molecular Probes Cat# D3571) and 1% Triton X-100 (from 10% Calbiochem Cat # 648463) in 1x PBS
8. 1.5mL Siliconized polypropylene microcentrifuge tubes: Sigma (Cat. T4816)

Cell preparation

Treat cells to induce autophagy. After treatment wash cells with 1x PBS and resuspend them so the cell density is 1x10^6 cells per 100µL.

Staining Protocol

All washes done at 300 x g 10min 4°C in a swinging bucket rotor. Staining should be done in the dark at RT. Cell concentration should be 1 x10^6 cells per 100µL.

1. Treat cells to induce autophagy.
2. Wash cells with 1x PBS.
3. Fix cells in 2% formaldehyde (PFA) for 20 minutes.
4. Wash with 0.005% saponin, spin and resuspend in 100µL of the mouse anti-LC3 in 0.005% saponin solution for 30 minutes.
5. Wash with wash buffer, spin and resuspend in 100µL of the secondary donkey anti-mouse AF 647 (or AF488) in wash buffer solution for 30 minutes.
6. Wash with wash buffer, spin and resuspend in 100µL 1% formaldehyde.
7. Add 10x DAPI solution so the concentration is 1µg/mL.
8. Run directly on ImageStream or FlowSight in 1.5 mL microcentrifuge tubes.