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Immunofluorescence of ATP13A2 and CD63

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We use this protocol and it's working

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Abstract

This protocol describes the seeding, fixation and staining of samples for immunofluorescent visualization of ATP13A2 and CD63.

Materials

- 4% paraformaldehyde (Thermo Fisher Scientific): J61899
- BSA (Roth): 8076.4
- Saponin (Sigma): S-2149
- anti-ATP13A2 (Sigma): A3361
- anti-CD63 (ExBio): 11-343-C100
- alexa-488 goat anti-rabbit IgG (Invitrogen): R37116
- alexa-594 goat anti-mouse IgG (Invitrogen): A11005
- DAPI (Sigma): D9542
- FluorSave (Merck): 345789-20ml

1 Seed cells: 75 000 cells/well (SH-SY5Y) in a 12-well plate with coverslips. Allow the cells to attach and grow for 48:00:00 . 2d

2 Wash the cells with ice-cold PBS.

3 Fix the cells with 4% paraformaldehyde 00:30:00 at 37 °C. 30m

4 Wash the cells twice with ice-cold PBS.

5 Permeabilization and blocking with a mixture of 5% BSA (Roth) and 0.5% saponin (Sigma) (diluted in PBS) (referred to as blocking buffer) for 01:00:00 on a shaker. 1h

6 Incubate with primary antibody (anti-ATP13A2, A3361, Sigma; anti-CD63, 11-343-C100, ExBio) (diluted 1/200 in blocking buffer) for 02:00:00 (40 µl antibody mixture is sufficient per coverslip in a wet chamber). 2h

7 Wash with DPBS for 00:05:00 on a shaker, repeat this step 3x. 5m

8 Incubate the samples in secondary antibody (alexa-488 goat anti-rabbit IgG R37116, Invitrogen; alexa-594 goat anti-mouse IgG, A11005, Invitrogen) (diluted 1/1000 in blocking buffer) for 00:30:00 on a shaker. 30m

9 Wash with DPBS for 00:05:00 on a shaker, repeat this step 3x. 5m

10 Incubate the samples in 200 ng/ml DAPI (D9542, Sigma) for 00:15:00 . 15m

11 Wash with DPBS for 00:05:00 on a shaker, repeat this step 3x. 5m

12 Mount the samples with FluorSave (345789-20ml, Merck).

13 Acquire images using a LSM880 microscope (Zeiss) with a 63x objective and Airyscan detector.