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Liposome tubulation

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We use this protocol and it's working

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Keywords: Liposome tubulation, LRRK2, Electron microscopy, ASAPCRN, liposome tubulation experiment, induced liposome tubulation experiment, liposome tubulation, liposome tubulation this protocol details method, liposome, negative stained electron microscopy, electron microscopy, confocal fluorescence microscopy, analysis by confocal fluorescence microscopy, microscopy, lrrk2

Abstract

This protocol details methods for the LRRK2-induced liposome tubulation experiment and its analysis by confocal fluorescence microscopy and negative stained electron microscopy.

Attachments

DOCX

iuuebvk9p.docx

20KB

Confocal fluorescence microscopy analysis

30m

1 Prepare the samples in a PCR tube with [M] 300 nanomolar (nM) LRKK2 proteins (WT or mutant full length LRRK2 or RCKW), [M] 20 micromolar (µM) liposomes with or without [M] 1 millimolar (mM) GMPPNP (or other guanylnucleotides).

Note

Note: Liposome tubulation is sensitive to LRRK2 concentration. Too much protein results in more liposome aggregates.

2 Immediately deposit 6 µL - 10 µL samples of step 1 on a 35 mm glass bottom dish and incubate at 37 °C for 00:30:00 .

30m

Note

Note: Drop some buffer in the dish to prevent samples from drying out due to evaporation during incubation.

3 After incubation, capture images with a Spinning disk confocal (SDC) microscopy at Room temperature on a Nikon Ti-E inverted microscope using the Improvision UltraVIEW VoX system (Perkin-Elmer).

Note

Note: Movies were collected from time 00:00:00 .

Negative stained electron microscopy (EM) analysis

31m 25s

4 Glow-discharge carbon-coated grids (25 mA, 00:00:45).

45s

5 Place the discharged grids into a 35 mm glass bottom dish.

6 Prepare samples in a PCR tube with [M] 300 nanomolar (nM) LRKK2, [M] 80 micromolar (µM) liposomes and [M] 1 millimolar (mM) GMPPNP.

7 Immediately apply 6 µL of the mixture to the grid and incubate the mixture at 37 °C for 00:30:00 . 30m

Note

Note: Drop some buffer in the dish to prevent samples from drying out due to evaporation during incubation.

8 Blot the grid with filter paper after incubation and stain samples with 2% uranyl acetate for 00:00:40 . 40s

9 Dry the grid with filter paper.

10 Take images using a Talos L 120C TEM microscope at 80 kV with Velox software and a 4k × 4K Ceta CMOS Camera (Thermo Fisher Scientific).