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Protocol status: Working
We use this protocol and it's working

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Liposome tubulation

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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

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PROTOCOL integer ID:
68886

Keywords: Liposome tubulation, LRRK2, Electron microscopy

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 30m 00:30:00.

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 40s 00:00:40.

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).