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Transmission Electron Microscopy of Native Nanodiscs

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We use this protocol and it's working

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Abstract

This is a protocol for conducting transmission electron microscopy of native nanodiscs to determine population size distribution and morphology.

Troubleshooting



Staining grids

32m 35s

- 1 Dilute nanodisc samples into single molecule range, typically around between 1:10 and 1:100 if the samples have been through size exclusion chromatography using dilution buffer (1M 50 millimolar (mM) Tris HCl pH 7.4) .
- 2 Glow-discharge carbon-coated copper grids (200 mesh) for ⌚ 00:00:30 seconds. 30s
- 3 Apply ⌚ 5 µL sample to the grid and after ⌚ 00:01:00 minute blot off with Whiteman ashless filter paper. 1m
- 4 Wash grid once with ⌚ 5 µL uranyl formate for ⌚ 00:00:05 seconds. 5s
- 5 Stain grid with ⌚ 5 µL uranyl formate for ⌚ 00:01:00 minute and blot dry with Whiteman ashless filter paper. 1m
- 6 Allow to dry for ⌚ 00:30:00 minutes to ⌚ Overnight 30m

Image Acquisition

- 7 Take micrographs using a JEOL JEM 1400PLUS electron microscope at an operating voltage of 80 kV.