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Transmission Electron Microscopy of Native Nanodiscs

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Protocol status: Working
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.

Staining grids

32m 35s

1 Dilute nanodisc samples into single molecule range, typically around between 1:10 and 1:100 is the samples have been through size exclusion chromatography using dilution buffer ([M] 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.