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Preparation of Negative Stain Transmission Electron Microscopy Grids

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We use this protocol and it's working

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Abstract

This protocol details how to prepare negative stain protein samples for transmission electron microscopy (TEM) imaging.

Preparation of Negative Stain

3m 30s

1 Using an EM-grade forceps, place carbon film supported copper grids (Quantifoil) with the matte carbon side (other side, polished, shiny copper) facing up onto a filter paper inside a glass petri dish.

2 Glow-discharge the carbon grids using a plasma cleaner like PDC-3XG (Harrick) for 00:00:30 . 30s

3 Hold the grid with EM-grade forceps with the carbon side facing up above a filter paper.

4 **Blotting:** Pipette 4 µL of your sample onto the carbon grid and incubate for 00:01:00 . 1m

5 Wick away liquid with filter paper wedge.

6 **Stain:** Add 7 µL of 2% uranyl acetate solution (Electron Microscopy Sciences) on top of the carbon grid and incubate for 00:01:00 . 1m

Note

- Uranyl acetate is toxic and radioactive! Wear appropriate safety gear, prevent incorporation. Waste containing uranyl acetate must be discarded as radioactive waste.
- Before use, centrifuge the 2% uranyl acetate solution at 20000 x g, 00:10:00 in order to pellet possible precipitates.

7 Carefully dry the carbon grid using a filter paper.

8 Add 7 µL of 2% uranyl acetate solution (Electron Microscopy Sciences) on top of the carbon grid and incubate for 00:01:00 . 1m

9 Carefully dry completely the carbon grid using a filter paper wedge.

10 Air dry the carbon grid for a few minutes.

11 Store the carbon grid in a cassette until imaging by TEM.