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THE UNIVERSITY OF ARIZONA
RESEARCH, INNOVATION & IMPACT
Core Facilities

Imaging Cores – Electron

Negative Staining TEM SOP

Preparation performed by investigator prior to sample submission to the Core Facility

- [1] Fixation with 2.5 % glutaraldehyde in 0.1M PBS for 1 hr.
- Transfer to 0.1 M PBS buffer pH 7.2 - 7.4
- Submit the suspension to the core facility in the buffer, solution, or double distilled water.

Procedure

Load 5 µL of [2,3,4] sample suspension in a [5] carbon-coated grid	[6] 1min ☐ 2 min ☐ 5 min ☐
Remove excess with filter paper.	☐
Staining with 5 µL 2 % UA.	1 min ☐ 2 min ☐
Remove excess with filter paper.	☐
Rinse in 0.1 M PBS ☐ or ddH2O ☐	3 x 10 secs ☐☐☐
Rinse in ddH2O.	1 x 10 secs ☐
Let the grid dry.	5 min ☐

Notes:

1. **Fixation is required for bacteria, virus, protozoa microorganisms.**
2. **Exosomes and liposomes preparation in PBS, nanoliposomes, nanocellulose or proteins can be used for this procedure without prior fixation.**
3. **Powder (lyophilized) samples require to be resuspended in double distilled water, or specific solution.**
4. **Prepare several dilutions of the sample in the buffer or molecular grade water accordingly. Include an undiluted, and an unstained sample as well.**
5. **To increase the sample adhesion to the carbon-coated grids, glow discharge the grids for 30 sec at 15 mA.**
6. **Time of incubation will depend on the sample and the concentration of the suspension.**