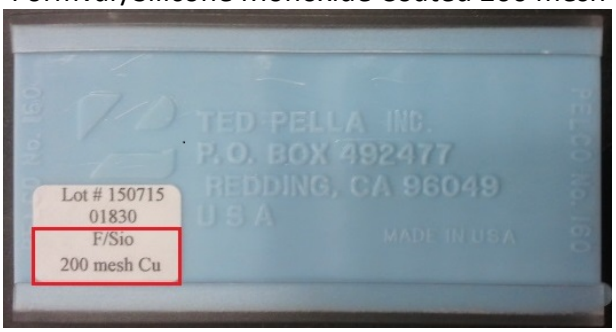
Negative Staining Protocol

This protocol is suitable for the following particles in suspension:

Viruses, Bacteria, Nanoparticles, Nanofibers, Vesicles, Lipid Micelles, Cell Fractions, etc.

Required Materials:

Formvar/Silicone Monoxide Coated 200 mesh Copper grids



#7 Self-Locking Forceps

Nanovan Negative Stain

Whatman #50 Filter Paper- 90mm Diameter
P10 Pipette
P200 Pipette tips
Parafilm
Petri Dish
Double Sided Tape

Procedure:

1. Begin by placing a 4x4 square of Parafilm down on the bench.

2. Slide open the grid box and remove a grid with the self-locking forceps. If the "A1" coordinate is in the top left corner, then the coated side of the grid will be facing the left-hand side of the container. The coated side should appear dark and shiny.

   Note: Ensure to grab the grid by the outer rim only. The grid can be picked up under the stereomicroscope to facilitate this.

3. Lay the forceps on the bench so that the grid is over the parafilm. Apply ~10µL of sample onto the grid and allow to adsorb for 2-5 min. Cover sample by making a tent with a folded piece of filter paper.

4. Blot off excess sample by inverting the forceps and touching only the edge of the grid to a clean piece of filter paper, leaving behind a thin film.

5. Allow to air dry for ~2min under filter paper tent.

6. On a clean piece of Parafilm, add 1 drop of Nanovan negative stain. The drop can be pipetted onto the grid at this point, or the grid can be floated directly on the drop for the staining step.

7. Stain for ~30s-1min under filter paper tent.

8. Blot off excess stain as before and allow to dry for 2min before viewing under TEM.

9. While sample is drying, take a 10cm Petri Dish and place 2 pieces of double-sided tape onto the lid. Stick a clean piece of filter paper onto the tape and use the bottom of the dish as the lid.

10. When sample has finished drying, use a wedge of filter paper to slide in between the arms of the forceps and gently push the grid out and onto your filter paper dish. This is to prevent the grid from being drawn between the arms by any liquid that may remain.

11. The sample is now ready to be viewed under the TEM

If there are questions or concerns at any point during this procedure, contact your friendly EMCF staff for assistance.