

## **Negative Staining**

1. Place the desired number of carbon coated grids onto metal grid prep holder, with the carbon-side facing upwards.
2. Glow discharge the grids using the maintained presets (15mA, 45 seconds).
3. While waiting, prepare parafilm with wash droplets of water on top, 1-3 for each grid.
4. Once complete, remove grids from the glow discharge unit and pick up your first grid a pair of tweezers.
5. Load between 4-10  $\mu\text{L}$  of sample onto grid and allow the sample to remain on the grid for 1-3 minutes, to ensure sample sticks to the carbon film (this will vary and needs to be optimized with each sample type).
6. Once the incubation period is complete, blot, using the edge of a piece of filter paper, to remove the sample, leaving only a thin layer of liquid.
  - a. Avoid over-blotting and do not let the grid dry out completely.
  - b. This will keep the sample hydrated.
  - c. This will be true for all subsequent blotting steps.
7. If performing washes, immediately place the grid, sample side down, onto the first wash droplet, for  $\sim 10$  seconds. This will dilute away any unwanted buffer components that may lead to a thicker/heavier background stain layer.
8. Blot away the water.
9. Repeat as needed for additional water washes.
10. Load the  $\sim 5\mu\text{l}$  of 2% uranyl acetate (aqueous) onto the grid for staining.
  - a. Pre-load the pipette with another  $5\mu\text{l}$  of stain for the next application (or next grid).
11. Blot the stain away; reminder, do not let the grid dry out.
12. Repeat 1x-3x; this may also need to be optimized by sample.
13. Perform a final blot and set grid aside to dry before placing it into the grid box.