Glow discharging protocol:

Materials:

1 grid (300 M F/C Cu)

1 self-closing tweezers

PELCO easiGlow glow discharge system

In addition to the above, it is best to have all other materials for sample prep, such as those used for negative staining and sample application, handy such that samples can be prepared immediately after glow discharging

Required PPE: gloves

1. Transfer clean grid to one of the parafilm-wrapped microscope slides next to the glow discharge system
2. The side of the grid facing up will be the side that is glow discharged. Place grid carbon side up (this is the shiny side)
3. Confirm that the plasma current and duration settings on the easiGlow are set to 15 mA and 30 seconds respectively
4. Place slide in the slot on the easiGlow and place the lid over the chamber.
5. Select "AUTO RUN"; after the chamber is evacuated, confirm that the glow discharge runs (purple plasma will be visible in the chamber).
6. Wait until the "AUTO RUN" option returns on the touch screen; remove the lid and recover samples.
7. Proceed to subsequent sample prep steps as soon as possible

Sample preparation protocol: negative stain, immediately following glow discharge of EM grids.

Materials:

1 glow discharged grid (300 M F/C Cu)

1 aliquot ferritin protein standard**

1 self-closing tweezers

1 aliquot of 2% uranyl acetate stain

1 aliquot of distilled H2O

5 pieces of small rectangular filter paper

1 large piece of filter paper OR piece of parafilm

Wear gloves & all other appropriate PPE

1. Prepare (or acquire) protein sample in compatible buffer.
2. Set up your workstation- use parafilm or a large piece of filter paper as a base to place under your tweezers (this will catch the grid if you drop it).
3. Grab grid from microscope slide using self-closing tweezers. Aim for the edge/border of grid.
4. Set tweezers down with grid.
5. Pipette 3uL of diluted ferritin sample.
6. Gently pipette onto grid (avoid touching grid- this will bend it).
7. Allow sample to sit for 2 minutes.
8. Use filter paper (small piece) to gently wick away sample from side of grid. *Avoid touching grid with filter paper!*
9. Pipette 3 uL of H2O onto grid.
10. Wick away water. Repeat 9 and 10.
11. Pipette 3 uL of 2% uranyl acetate stain onto sample. Let sit for 1.5 minutes.
12. Wick away stain. ***This is depleted Uranium (toxic heavy metal) waste.***
13. Let grid air dry for 2-3 minutes or until visibly dry.
14. Store samples.