



NEGATIVE STAINING

Materials:

Formvar carbon coated grids
Fine tip tweezers
Parafilm
Scissors
uA
ddH₂O-or rinsing buffer
specimen
filter paper
Pipettes
pipette tips

1. (Optional) Use the bottom of a petri dish and put a large piece of filter paper inside—dump your grids onto the paper—then place them onto a slide shiny side up...**OR**
1. Take grids out of grid holder and place them on slide shiny side up
2. Put slide in glow discharge machine—auto run

Tweezer method:

1. Dump grids off of slide back onto filter paper, pick up one grid shiny side up with self closing tweezers and rest on top of filter paper
2. Place one drop of specimen (5 microliters) onto grid—leave for 1 minute; use filter paper to suck off liquid
3. Place one drop of uA (5 microliters) onto grid—leave for 1 minute; use filter paper to suck off liquid

View in microscope and determine what if any variable needs to change

Variables that can change: dilution of specimen, rinses, times....

Parafilm method:

1. Place drop of specimen on a piece of parafilm; place drops of rinse to the right of the specimen drop, then uA drps to the right of the rinse drops---follow the above procedure and float grid on top of each drop for desired length of time.