



Manual Blotting Protocol

This protocol is for individuals that have been certified to operate the Vitrobot. Please refer to the following paper Serbynovskyi et al 2025 (<https://pubmed.ncbi.nlm.nih.gov/38328036/>) for details on assembling the filter tips.

- i. Alert Cryo-EM staff if any assistance or supervision is needed.
- ii. Reserve Vitrobot in iLab.
- iii. Bring any supplies and/or tools you wish to use instead of what is already provided by the Core.
- iv. Bring a facemask to protect samples from your breath (recommended).
- v. Refer to the “Vitrobot Handout” or “Vitrobot Protocol” for basic Vitrobot operation.
- vi. This protocol is for individuals that have been certified to operate the Vitrobot.

- **Gather Tools and Supplies for Making Filter Tips**

- o 20 μ L pipet tips at least as many as the number of grids you plan to manual blot
- o Filter paper at least one per 12 filter tips you intend to make
- o 20 μ L pipet tip box(es) as many as needed to house the completed filter tips
- o Scissors
- o Box cutter or razor
- o Ruler
- o Cutting surface
- o Clean disposable gloves



- **Make the Filter Tips**

1. Wear clean disposable gloves.
2. Cut a sheet of filter paper into quarters, and then those quarters into thirds, for a total of 12 pieces.
3. Lay pipet tips on the cutting surface.
4. Use the box cutter or razor to cut a pipet to the desired internal diameter (recommended: 3 mm).
5. Insert an uncut pipet tip into a cut one.
6. Cut the uncut pipet tip above the raw edge of the already cut pipet tip (recommended: 1 mm).
7. Remove the long-cut pipet tip and cover it in a filter paper piece.
8. Gently twist/shimmy a short-cut pipet tip over the filter-covered long-cut pipet tip.
9. Hold the filter paper tip at 45° angle against a tabletop filter paper.
10. Roll the filter tip to round out the edges.
11. Flatten the filter tip against the tabletop filter paper.



12. Gently twist/shimmy the long-cut pipet tip out of the filter tip.
13. Store the filter tip in a designated pipet box.

- Set the blot total to zero on the Vitrobot.
- Dry Run Vitrobot to Test that It's Working Properly (see steps directly below).
- Manual Blot Vitrobot Operation
 - o Operate Vitrobot as normal all the way through pipetting the sample onto the grid.
 - o Remove a filter tip from its box and place it on a pipetman.
 - o Use the pipetman to guide the filter tip into the Vitrobot chamber.
 - o Gently blot the grid, manually counting the seconds.
 - o Discard the filter tip.
 - o Tap "Next" or footpedal for Vitrobot to plunge, skipping WT, BT_i, BT_o, and DT.
 - o Resume normal Vitrobot operation.