

Preparing coated grids

in fume cupboard

Use the following conditions for Formvar or Pioloform (polyvinyl butyral).

1. Prepare the Pioloform solution in fume cupboard: 0.3% Pioloform diluted in chloroform:
 - 0.15 g of Pioloform in 50 ml chloroform mix well until the Pioloform has dissolved completely (sonicating water bath can be used).
 - the solution can be stored under light free conditions (brown glass bottle) and used for about 2 weeks, then prepare fresh.
2. Prepare the grids:
wash the grids with chloroform and ethanol / acetone
or:
put grids in beaker with acetone and leave in sonicator for a few min (less than 8 min, the beaker must not float).
or:
use the grids as they are, provided they are not too dirty (excessive washing can make it hard for the film to stick to the grids). Usually grids that haven't been used for anything else before (factory fresh) can be used as they are.
3. Before use, wash dip-miser with chloroform.
4. Fill the dip-miser with the Pioloform solution to a depth of about 3/4 of the height of a glass slide.
5. Place a clean slide (washed with acetone and lint-free tissue) into dip-miser.
6. Lift slide out of the dip-miser, making sure the Pioloform solution runs off without leaving horizontal lines or patches (in high humidity conditions, this will almost invariably happen). The faster the lift, the thicker the film will be.
7. Hold slide for 20-30 sec in the air space of the dip-miser, to allow film to thin down a bit and become more even.
8. Cut around film edge with new razor blade.
9. Breathe on both sides of the slide (the water vapour in the breath facilitates separation of the film from the slide when added to the water bath).
10. Let the film float onto the water surface by slowly lowering it vertically into a container with dH₂O, making sure there is no dust on the surface
11. Check the thickness of the Pioloform film: uniform grey is good, silver-gold coloured means that the film is too thick.
12. Put grids (shiny side up) on the Pioloform film
13. Clean a slide and cover the surface with a white paper label.
14. Collect the Pioloform film with the grids by catching the film edge with the slide and dropping the slide straight down completely submerging the film. Take the slide out of the water on an angle to avoid peeling the film off again.
15. Remove excessive moisture only on from the corner of the slide with filter paper. Dry for 1-5 days in a closed container .