

Cp3 Plunge Freezer Protocol

Setup:

- Turn on power switch on right side.
- Open nitrogen tank.
- Test plunger without sample: close safety shield, press “reset,” then “start.”
- Wet sponge with hot water and place into chamber. If necessary, re-wet sponge. Humidity should be ~80 – 90 %.
- Add fresh uncontaminated liquid nitrogen to workstation.
- Replace filter paper:
 - Gently pull out blotters, use tweezers to remove pin. Do not lose the plastic disk!
 - Place inverted pin, filter paper, and plastic disk in the filter paper loading station. Push down gently with blotter to attach.
- Slowly condense ethane into cup.
- Set blotting time.
- When set temperature (-174°C) and relative humidity is reached, insert cryo plunge freezer tweezers with a plasma cleaned grid into plunge rod.
- Add 2-3µl of sample to grid. Rotate plunge rod 90 degrees.
- Close safety shield, press “reset,” then “start” to blot and plunge.
- Open safety shield and raise plunge enough to remove tweezers by pressing the blue button. Quickly transfer grid from ethane to liquid nitrogen and into a cryo grid box.

Shutdown:

- Raise plunge rod into chamber before shutting off Cp3.
- Close the nitrogen tank.
- Close the ethane tank.
- Make sure the tanks for the Solarus glow discharger are closed.
- Remove sponge from chamber.
- Empty and invert LD4 to dry.
- Place LN2 and ethane cup in fume hood.
- Return all tools to drawer and clean up.

If you suspect any damage to the machinery, or encounter any problems during use, please tell Ashleigh or another EMG staff member.