

Homemade LN₂ AutoFill System

Setup (check list)

1. It must be confirmed that the loaded cryo-specimen is good for data collection and SerialEM has been set up for data collection before setting up LN₂ autofill system. You may want to run data collection at several points to make sure SerialEM is running ok.
2. Pause data collection in SerialEM and wait for Pause fully in effect (SerialEM will pause after finishing all operations with current item in Navigator).
3. Use both hands to move pump straight up out of the plastic storage tube and slowly dip it into the storage tank, the warm tube below the pump may cause vigorously bubbling of LN₂ and you must wear protection gloves during this step. You must finish this step in at least 20-30 sec before letting the O-ring sit on the storage tank opening.
4. Position the liquid nitrogen pipe at the right orientation so the pipe does not build up tension, fix the pump to the storage tank by the chain lock provided.
5. Remove original cap from cryo-specimen holder dewar.
6. Slowly push LN₂ storage tank with cart to the right side of microscope, gently move cart to position yellow LN₂ phase separator near the opening of specimen holder dewar, insert the engineered cap with funnel and sensor to the opening of holder dewar, make sure the funnel is right below phase separator to collect LN₂ (you may need to gently move the cart to do the alignment).
7. Secure the sensor cable on the metal arm of cart.
8. (Optional) Lock the wheel of cart. This step is optional.
9. Plug the cable to the serial port on the pump and secure it with the two screws.
10. Plug power supply cable to the power extension cord. Now the pump will beep 7 times and the first LED (amber) is flashing with long time intervals (3-4 sec).
11. (Optional) Turn on pump monitoring program on support PC. This step is optional. Once the pump is turned on it works with internal timer regardless of on/off status of the monitor program.
12. Press the green button on the pump and there is a long beep. The first LED (amber) is flashing with short time intervals (~1 sec) and second LED is now on with red. In 20-30 sec LN₂ starts to fill the holder dewar. Watch LN₂ flow carefully to make sure there is no spill of LN₂.
13. Pump stops when the sensor temperature reaches and stays below -180°C for 10 sec. Now the pump internal timer starts to count down for 3.5 hours for next refilling and the second LED on pump turns green (It turns red again when the sensor temperature rise above -180°C, which takes less than 10 min).
14. Wait for 3-5 mins and resume data collection in SerialEM.
15. Setup LN₂-spill-collection apparatus (Aluminum foil, metal funnel with styrofoam box and empty LN₂ tank) beneath the cryo-specimen holder.

How to stop (check list):

Check the pump monitor program on the support PC, find out when the last refill happened, it is highly recommended to disassemble the filling system at least 20-30 mins from the last refill in order to warm up the engineered cap, sensor and pipe.

1. Stop the data collection **completely** in SerialEM on the K3 computer and back up data if not started:

2. Close column valves, retract K3 camera, put down fluoro screen, center stage, close shutter on cryo-specimen holder, retract Obj aperture if used in TUI, and retract CryoBox.
3. Make sure the pump is not pumping LN₂.
4. Move the LN₂-spill-collection apparatus (Aluminum foil, metal funnel with styrofoam box and empty LN₂ tank) away from the microscope.
5. Close pump monitoring software on Support PC. This step is optional.
6. Press green button on the pump and make sure the second LED from top is off and the first LED (amber) is flashing with long time intervals (3-4 sec).
7. Unplug the pump power cable.
8. Unfasten the two screws and unplug the serial cable from the pump.
9. Unsecure sensor cable from metal arm of cart, **slowly** remove the holder cap with the funnel from the holder, **and secure the cap on top of the metal arm with tape**. This step must be **gentle** and do not use force to remove the cap, watch carefully when facility staff is doing the demo.
Note: if the cap cannot be removed from the holder, it is probably frozen to the cryo-specimen holder dewar, wait 5-10 mins and try again.
10. Now move the pump cart away from the microscope. Watch overhead while moving the cart to make sure that the metal arm, LN₂ pipe and phase separator donot hit anything else.
11. Remove the chain lock that secures the pump on the LN₂ tank, **slowly** pull the pump straight up until all the tube are out of the tank, inter the pump into the plastic tube on the side of the tank **slowly**.
12. Remove the cryoholder from TEM as usual. Return stage tilt to 0°.