

**SP004.2**  
**Modified 8-24-22 by KB**

## **Lyophilization**

### **Materials and Reagents:**

1. Lyophilizer (note 1)
2. Container suitable for lyophilization
3. Sample for lyophilization
4. -80°C freezer
5. Freeze dry flask (optional, note 1)
6. Freeze dry flask lid with metal stem (optional, note 1)

### **Protocol:**

#### **Lyophilizer Start-Up**

1. \_\_\_\_\_ Turn on power button on the front of the lyophilizer to turn on/wake up, or touch the touch screen (note 2).
2. \_\_\_\_\_ Check the drain pan inside the lower cabinet to ensure it is empty, and check for any large buildups of ice. The drain pan slides out and can be poured out into the sink.
3. \_\_\_\_\_ Be sure that all sample ports on the left side of the machine are closed (horizontal), and the vacuum release valve on the front of the machine is closed (note 1).
4. \_\_\_\_\_ Press the Manual button on the touch screen (note 3).
5. \_\_\_\_\_ Choose your desired temperature for the inside of the cabinet, OR if using a side mounted flask, choose “in-flask” (note 4).
6. \_\_\_\_\_ Press OK
7. \_\_\_\_\_ Wait until both the refrigeration and the collector are at appropriate temperatures before placing samples on the lyophilizer (note 5).

#### **Putting Sample on Lyophilizer**

8. \_\_\_\_\_ Make sure that your sample is compatible with the machine (note 6).
9. \_\_\_\_\_ Transfer sample into a container suitable for lyophilization (note 7).
10. \_\_\_\_\_ Place container in -80°C freezer for a minimum of 2 hours (note 8).
11. \_\_\_\_\_ Remove sample container from freezer and loosen the lid of the sample container so that gas can escape.
12. \_\_\_\_\_ Place the container in the cabinet or a freeze-dry flask of appropriate size. If using a flask, ensure that the lid forms a tight seal around the glass lip of the flask.
13. \_\_\_\_\_ Close the cabinet door and rotate the handle of the door until the seal is air tight. If using a flask, open the sample valve at the point of connection to the machine (note 1).
14. \_\_\_\_\_ On the touch screen, press the vacuum button. On the next screen, press the “start” button. On the shelf directly below the lyophilizer, the vacuum light will switch on. Press the green start button on the left facing side of the vacuum to start (note 9).
15. \_\_\_\_\_ Fill out the lyophilizer sign-up sheet.
16. \_\_\_\_\_ Inspect the lyophilizer daily to see when sample is completely dried (note 10).

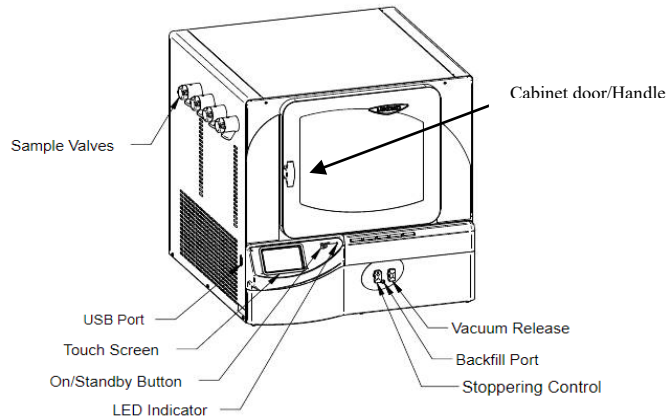
17. \_\_\_\_ When ready to remove sample, turn off the vacuum by pressing the “vacuum” button on the touch screen, and then pressing “stop”. Open the vacuum release valve on the front of the machine. The door to the cabinet will not open until pressure is equalized.
18. \_\_\_\_ Take out sample, making sure to reseal the lid of your container.
19. \_\_\_\_ Fill out the lyophilizer sign up sheet.

**Lyophilizer Shut-Down**

20. \_\_\_\_ Close the vacuum release valve and sample valves.
21. \_\_\_\_ If a large amount of material/solvent was lyophilized, run the vacuum with the gas ballast open for 1-2 hours, or overnight. (note 11)
22. \_\_\_\_ If the cabinet was set to below freezing, there may be ice buildup on the cooling coils within the cabinet. In the case of ice buildup after lyophilizing a sample, press the “Defrost” button next to the “Vacuum” button.
23. \_\_\_\_ Clean any debris on the shelf in the cabinet.
24. \_\_\_\_ Clean and drain any liquid pooling in the water trap in the cabinet.

**Notes:**

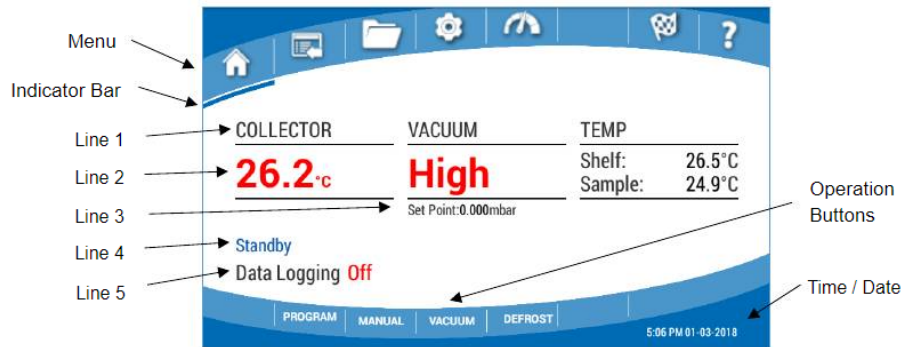
1. There is one lyophilizer available in the lab. Samples can be placed either within the cabinet (front facing door) of the lyophilizer or in a flask with a rubber and metal lid and hooked to the left side of the machine. If the sample is placed in the cabinet, a glass flask and metal stemmed top are not necessary. All components of the lyophilizer setup are compatible with aqueous solvents. Sample valves are located on the left had side of the machine. The vacuum release valve is located on the front of the machine below the cabinet door.



2. Layout of the front screen and power button on the front of the lyophilizer: 1-the touch screen, 2-the power button, 3-light will be on and green if the lyophilizer is powered.

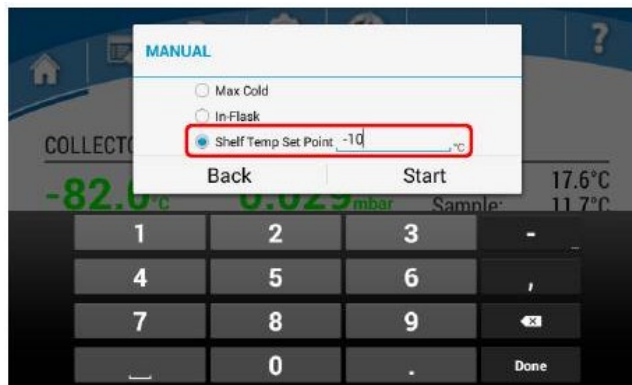


- The “Manual”, “Vacuum”, and “Defrost” buttons are all along the bottom row of the touch display.



Line 1, 2 and 3 display the collector temperature in Celsius, the vacuum level in milliBar and its set point, and the temperature of the inside of the cabinet. The display color of the collector temperature and vacuum pressure will turn green when -60C and 1.50 mBar are reached, respectively. Line 4 displays the optional data logging and its status.

- Pressing the “Manual” button will bring up three options. The “Shelf Temp Set Point” will keep the cabinet at the desired temperature. The temperature can be set to up to -50C and up to +50C. If using a flask only, use the “in-flask” option. “Max Cold” will lower the temperature of the cabinet to approx. -70C. **Do not lyophilize samples while the shelf temperature is set to max cold. It draws refrigeration from the collector, which must remain at -80C or below. Use this setting to pre-freeze samples without vacuum only.**



- The collector must reach -80C before lyophilizing. This can take around an hour or more.
- No organic solvents, phenol, glycerol or radioactivity may be placed on the lyophilizer, nor any solvents which could interfere with freezing.
- Examples of suitable containers are: 15 ml and 50 ml Falcon centrifuge tubes, Oakridge centrifuge tubes, eppendorf microcentrifuge tubes, cryovials and 250 ml or smaller filter unit receiver bottles. 13 x 100 mm and 16 x 100 mm glass tubes, and small round-bottom flasks can also be used, but use caution since they can easily crack during freezing. It is critical not to overfill the container for lyophilization because of expansion during the freezing process. It is advisable to fill plastic containers no more than 50% full, and glass containers no more than 30% full. Alternatively, samples may be shell-frozen using acetone containing dry ice; this will prevent expansion of the sample during the freezing process.

8. Samples can also be frozen overnight at -80°C. If tube expansion is a concern, the sample can be “step frozen” to reduce sudden expansion. To step freeze, place sample at -20°C until completely frozen (usually 1-3 hours) before freezing at -80°C.
9. The vacuum will be loud at first, this is normal. The sound will slowly taper off as pressure within the lyophilizer decreases. If the vacuum does not reach 0.120 mBar, your sample, along with any other samples on the lyophilizer will thaw and can boil out of the tube. Therefore, it is critical that the vacuum gauge is monitored until an appropriate vacuum level has been reached, about 5 minutes. If the vacuum never reaches an acceptable level, follow troubleshooting steps below.
10. It may not be possible to visually determine if a sample is dry. If not, it will be necessary to remove the flask and inspect the sample tube for the presence of ice.
11. It is possible for condensation to accumulate within the vacuum. If this happens, the vacuum may not be able to hold 0.120 mBar of pressure and the vacuum must be dried out. Running the vacuum with the gas ballast open will pull more air into the vacuum and push condensation and liquid out. The gas ballast is a black knob on the front of the vacuum.

### Troubleshooting Guide:

Problem	Possible Cause	Explanation/Remedy
Sample thawing	Sample buffer not compatible with freezing	Some buffer components, such as residual glycerol in cells, can prevent proper freezing. Try refreezing the sample and monitor closely during lyophilization.
		Samples containing acetonitrile may exhibit thawing when initially placed on the lyophilizer, but can refreeze under vacuum. Monitor closely. If sample is at risk of leaking out of the tube and/or does not refreeze after ~30 minutes, remove and place back at -80°C for several hours before trying again.
	Vacuum did not reach 0.120 mBar	Always monitor vacuum until it comes down completely. If it does not, see vacuum troubleshooting below.
	Sample lid is too tight	Loosen the lid and refreeze sample. Some tube types do not allow the lid to be loose enough to allow proper vacuum.
	Overcrowding in flask	If too many tubes are placed in the same flask, they may press on each other and cause lids to become wedged onto the top of the tube. Similarly, a flask that is too short can force the lid back down onto the tube. Try a larger flask or placing tubes into multiple flasks.
	Sample tube overfull	Tall/narrow tubes can result in thawing at the bottom of the tube, especially when overfilled. Try freezing the tube at an angle to maximize the surface area being exposed to the vacuum. Alternatively, switch to a large sample tube.
Vacuum does not reach 0.120 mBar (or takes too long to reach 0.120 mBar)	Poor seal	Ensure that the door is closed tightly and/or the lid is properly seated on the flask and in the sample port.
		Check the position of the vacuum release valve on the front of the machine
	Excessive ice build-up	Excessive ice build-up can prevent vacuum from reaching samples. Shut down and defrost lyophilizer, then go through start-up method.
	Condensation buildup in the vacuum	See note 11
	Maintenance needed	If the troubleshooting steps outlined above do not resolve the issue, follow the protocol to remove all flasks and return all samples to -80°C. Contact the lab personnel responsible for lyophilizer maintenance and notify the owners of any samples that were removed.

