Labconco FreeZone Triad Benchtop Freeze Dryer

Standard Operating Procedure

Rev. No. 2 by Ana Bohorquez and Gary Scheiffele

1. Purpose

This standard operating procedure is intended as a summary of basic instrument operation. This includes safety, setup, sample loading, instrument settings, cleaning, and shutdown.

This SOP does not supersede the Labconco operating manual. The full instrument manual includes theory, all cautions, and detailed explanations of parameters and options.

2. Overview

The Labconco Triad Freeze Dryer is intended to dry a frozen sample through sublimation. The advantage over oven or vacuum drying is that, if done correctly, there are no capillary forces or solvent migration involved that would distort the sample and reduce surface area. The Triad can freeze dry on either an adjustable temperature shelf or in standard freeze drying flasks.

Warning: If the liquid being removed is not entirely aqueous, consult staff.

How the sample is frozen will affect the process due to the size/shape of water crystals. Always remember that the process is a heat balance between the heat of sublimation (dropping the sample temperature) and conductive and radiative heating (raising the sample temperature). If sublimation is proceeding too slowly and the sample thaws, then the process becomes simple vacuum drying. If the heat of sublimation is not replaced, the sample temperature drops such that sublimation slows, and the process takes much longer than it should. Normally one aims for sample prep with more than the minimum exposed surface area.

Note: the Triad manual seems to assume that the chamber is under vacuum while initial cooling is occurring. **Vacuum should be pulled only after the condenser reaches its -80°C operating temperature.**

3. Prerequisites

1. Use of the Labconco Triad requires:
2. Becoming an RSC user at https://rsc.aux.eng.ufl.edu.
3. Requesting training through https://rsc.aux.eng.ufl.edu.
4. Reading the full instrument manual and this SOP.
5. Successfully completing hands on training under the supervision of RSC staff.

4. Safety

4.1 Cryogen/Dewar:

Samples may be frozen using liquid nitrogen. When using liquid nitrogen for this purpose, wear at minimum safety glasses and cryo gloves and a full face shield is recommended. Slowly pouring liquid nitrogen over the samples will cause less splashing than plunging the samples into liquid nitrogen.

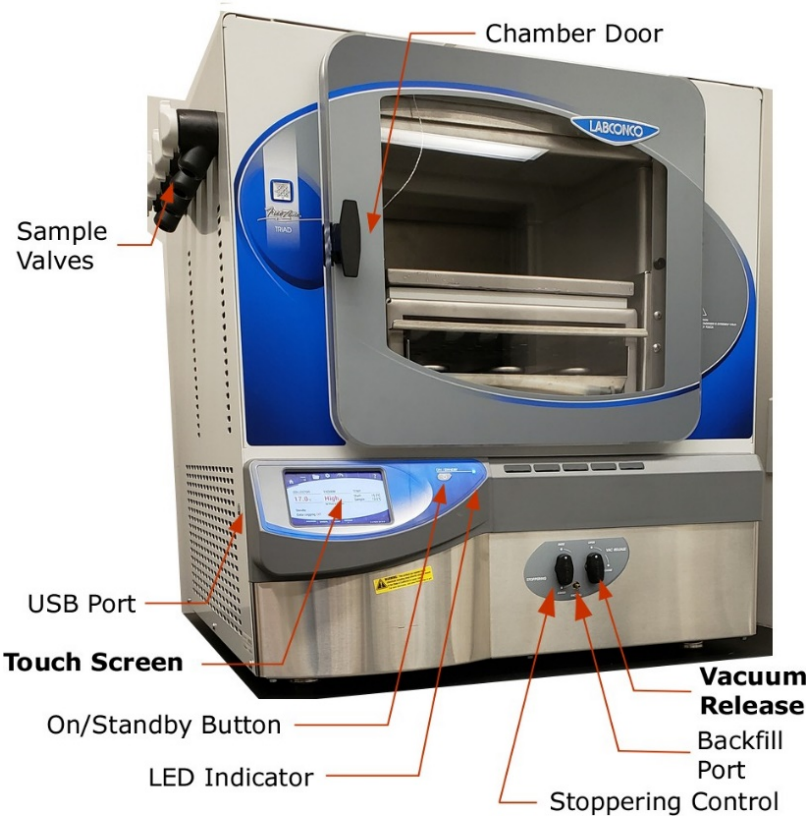
4.2 Vacuum:

The instrument will pull a full vacuum (roughly 15 pounds per square inch) on your frozen sample. The dimensions of the open end of the chamber are roughly 14.5”x15.5” HxW, so the door is supporting approximately 3,300 pound of force when under sublimation pressure. If the door fails, there will be a major implosion and subsequent rebounding of shards. Wear your safety glasses, and pay attention to any changes to the door’s appearance.

5. Operation

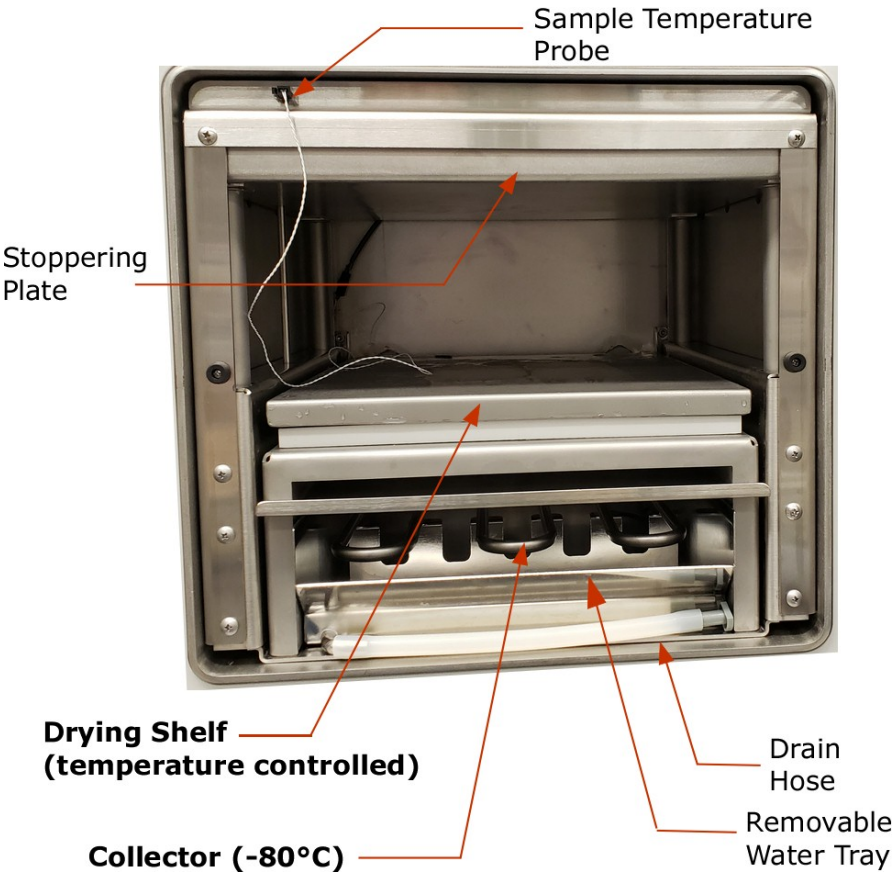
NOTE: One must log into the TUMI in order for the vacuum pump to turn on the vacuum pump.

5.1 Freeze Dryer Exterior



Use the diagram above to familiarize yourself with the basic layout of the freeze dryer exterior. You will likely use the touch screen the most. Vacuum release should be closed when vacuum is on, and the backfill port may be used to fill the chamber with inert gas. The USB port is for data logging or program backup.

5.2 Freeze Dryer Interior



Use the diagram above to familiarize yourself with the basic layout of the interior of the freeze dryer. Freeze drying will take place on the shelf (unless one uses the exterior side ports) and up to 2 L of liquid can be collected by the collector. After defrosting, the collector tray can be drained into a bucket using the drain hose, or the water tray can be carefully removed and emptied into either hazardous waste (if using other than purely aqueous .liquids) or the sink. Note that it will take ~6 h to completely freeze 2 L of water on the shelf under the "Max Cool" condition.

5.3 Touch Screen

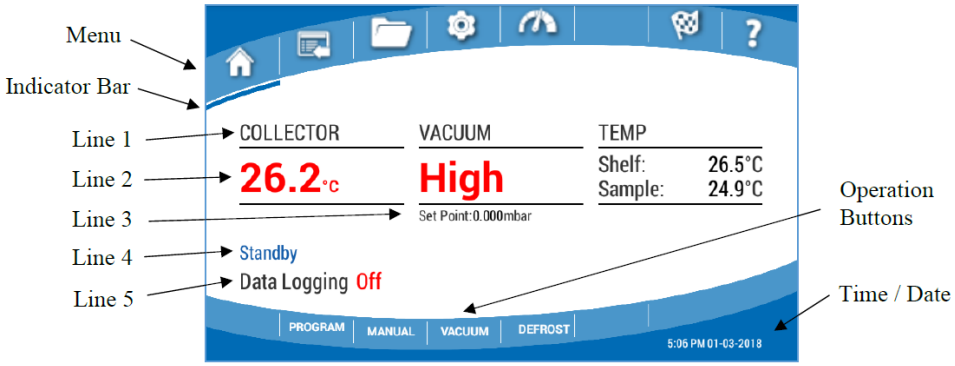
5.3.1. Touch Screen Display – Capacitive touch screen displays system operating parameters, set-up parameters and alarm messages. All user interface will take place via the touch screen.

5.3.2. On / Standby Button – If the display is illuminated pressing this button will put the display into low power mode (display backlight will be turned off). If the display is in low power mode, pressing this button will illuminate the display (Note: the display can also be brought out of low power mode by touching any location on the screen).

5.3.3. On / Standby LED Indicator – Blue LED indicator light to designate the current status of the system.

- 1 flash per second = Initial power up (loading operating system)
- Slow pulse = Touch screen in low power mode (screen timeout)
- ON = Unit is plugged in and touch screen is in active mode
- Off = Unit is unplugged

5.4 Touch Screen Operation



Menu icons are displayed across the top of the screen. Their descriptions for our freeze dryer are (from left to right):

Home **Programs** **Data Logging** **Settings** then **Sensors** (Empty) (Empty) **Help**

Operation modes are displayed across the bottom of the screen. Their descriptions are:

Program	Full program mode
Manual	Manual mode for starting cooling
Vacuum	Manual mode for starting vacuum
Defrost	Manual mode to start collector defrost

You can move between menu screens by either touching the menu icons or swiping across the screen from left to right or right to left

5.5 Manual Mode with Pre-Frozen Samples

1. Go to the Home Screen and press **MANUAL**
2. **Enter** the desired **shelf set point temperature** and press **Start**.
3. The system will begin ramping the shelf temperature until the set point temperature is achieved. After reaching the set point temperature, the system will hold at this temperature indefinitely.
4. **When the collector temperature reaches –80°C and the shelf temperature reaches the desired set point**, open door, add sample(s), close and latch door. **Make sure that vacuum release valve is closed.**
5. If using sample temperature probe, wait until sample temperature stabilizes.
6. Press **Vacuum** and set the desired vacuum level, then press **"Start".**
7. Shelf set point temperature and vacuum set point can be changed at any time during the manual freeze dry process.
8. To stop, press MANUAL, then "Stop". This will turn off the refrigeration system and heater.
9. Finally, press VACUUM, then "Stop". Open vacuum release.

5.6 Manual Mode with Unfrozen Samples

1. Place unfrozen samples on the shelf and close the door. The sample probe can be placed in the sample (or one of the samples) if desired.
2. Make sure that the **vacuum is OFF**.
3. Go to the Home Screen and press **MANUAL**

4. **Enter** the desired **shelf set point temperature (or Max Cool)** and press **Start**.
5. The system will begin ramping the shelf temperature until the set point temperature is achieved. After reaching the set point temperature, the system will hold at this temperature indefinitely.
6. When the collector temperature reaches –80°C and the shelf temperature reaches the desired set point **make sure that the vacuum release valve is closed**.
7. If necessary, change the shelf set point to the freeze drying process temperature.
8. Press **Vacuum** and set the desired vacuum level, then press **"Start".**
9. Shelf set point temperature and vacuum set point can be changed at any time during the manual freeze dry process.
10. To stop, press MANUAL, then Stop. This will turn off the refrigeration system and heater.
11. Finally, press VACUUM, then "Stop". Open vacuum release.

5.7 Manual Mode with Pre-Frozen Samples in the side ports

1. Go to the Home Screen and press **MANUAL**
2. Click on the "**In-Flask**" button and press **Start**. Cooling will commence.
3. **When the collector temperature reaches –80°C** vacuum can be started. **Make sure that vacuum release valve is closed.**
4. Press **Vacuum** and set the desired vacuum level, then press **"Start".**
5. Once the vacuum level is reached the sample flask can be attached to the side port valve and the valve opened.
6. Wait for freeze drying to complete.
7. To stop, press MANUAL, then Stop. This will turn off the refrigeration system and heater.
8. Finally, press VACUUM, then "Stop". Open vacuum release.

5.8 Program Mode

Supplied Example Program:

Program Name: 9421 012919

Step	Ramp Rate (°C/min)	Shelf Temp. (°C)	Time (hh:mm)	Vacuum (mbar)	Delete
1	--	MC	1:00	--	remove
2	1.50	-55	1:00	0.000	remove
3	3.00	50	1:00	0.000	remove
4	0.25	-34	1:00	0.000	remove
5	3.00	0	1:00	0.500	remove
6	0.70	24	1:00	0.000	remove

Save Back

Any of the above manual modes can be done in sequence and with more reproducibility using the program mode. The above example program is starting out with the shelf in "max cool" and no vacuum, then changing to -55°C with vacuum at 0.0 mbar. There are then examples of programed ramps with different shelf temperatures and vacuum levels.

Up to 30 programs with up to 16 segments per program can be stored. Note in the below example of a simple 3 step program that vacuum levels are not mentioned.

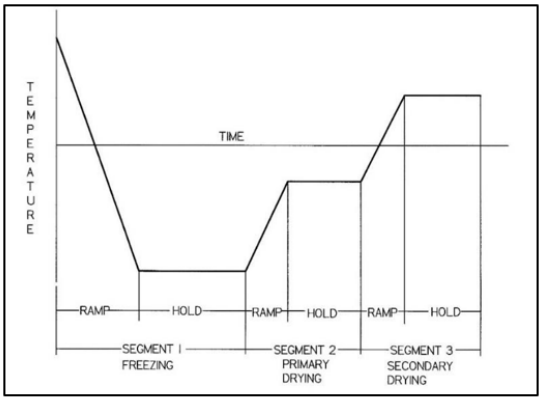


Illustration of a simple 3 Step Program

5.9 Data Logging

1. Process conditions (sample temperature, shelf temperature, vacuum level) can be logged and later copied to a USB flash drive.
2. New/Stop will start a new log file or stop one in progress
3. Data View will list the data in table format.
4. Chart will present the data as a chart, but with vacuum and only one temperature sensor at a time.
5. Copy will allow the copy of a selected log file to a flash drive.
6. Delete is used to remove a log file from the freeze dryer memory

5.10 Shutdown

1. Prior to leaving your session, the freeze dryer chamber should be clean and dry.
2. A "Defrost" cycle can be done if enough ice was collected. This will finish if the collector temperature reaches 60°C or after 2 h.
3. After any work with acidic or basic solutions all surfaces in the chamber (and especially the collector) should be wiped down and if determined by staff to be necessary – neutralized.
4. Again, melted ice can go down the sink only if it is only water. Any biohazard should go into 20% bleach first. Any additional organic liquids can make it hazardous waste.

6. Considerations for vacuum setpoints

6.1 Vapor Pressure above Ice

The vacuum level may be set by the user to optimize the freeze dry process. Normally, the sublimation rate will increase as the pressure increases in the Freeze Dryer. A good starting place is to set the vacuum so its level is equivalent to about 10°C colder than the eutectic or collapse temperature of the sample. The reference table below shows the relationship between ice temperature and vapor pressure.

Vapor pressure above ice

Temp. (°C)	Pressure (mbar)	Temp. (°C)	Pressure (mbar)	Temp. (°C)	Pressure (mbar)	Temp. (°C)	Pressure (mbar)	Temp. (°C)	Pressure (mbar)
0	6.11	-11	2.37	-21	0.94	-31	0.34	-50	0.039
-1	5.63	-12	2.17	-22	0.85	-32	0.31	-60	0.011
-2	5.18	-13	1.98	-23	0.77	-33	0.28	-70	0.003
-3	4.76	-14	1.81	-24	0.70	-34	0.25	-80	0.001
-4	4.37	-15	1.65	-25	0.63	-35	0.22		
-5	4.02	-16	1.50	-26	0.57	-36	0.20		
-6	3.69	-17	1.37	-27	0.52	-37	0.18		
-7	3.38	-18	1.25	-28	0.47	-38	0.16		
-8	3.10	-19	1.13	-29	0.42	-39	0.14		
-9	2.84	-20	1.03	-30	0.38	-40	0.13		

6.2 Examples for Eutectic/Pre-Freeze/Vacuum

Adjustments to the vacuum level must be made for various freeze drying conditions. Factors that must be considered are whether the sample is freeze dried on heated shelves or in glassware attached to sample valves, the volatility of the sample itself, the size of the sample and the heat energy supplied to the sample. When the vacuum control is set to operate at less vacuum (higher pressure), the ice holding capacity of the collector may be decreased. Some guidelines for setting the vacuum level are shown below. Exact protocols must be determined by the user for the specific samples that are being freeze dried.

Material	Solidification/Eutectic Temperature	Pre-Freeze Temperatures (°C)	Vacuum Set Point (mbar)
Bacteria, Virus	≤ -40°C	≤ -50°C	≤ 0.04
Milk	-5 to -13	-15 to -23	1.65 to 0.77
Fungi	≤ -40	≤ -50	≤ 0.04
Vegetable Tissue	-25 to -50	-35 to -60	0.22 to 0.01
Human Tissue	-30 to -40	-40 to -50	0.12 to 0.04
Blood Plasma	-10 to -25	-20 to -35	1.03 to 0.22
Vaccine	-30 to -40	-40 to -50	0.12 to 0.04

7. Maintenance

7.1 User Maintenance

1. The user has the responsibility for carrying out appropriate decontamination if hazardous material is spilled on or inside the equipment. This may be done by wiping the contaminated surfaces with a soft cloth dampened with alcohol. *However, alcohol may craze the acrylic door.*
2. Clean up all spills; remove liquids from the chamber.
3. Clean door and gasket using soft cloth, sponge or chamois and a mild, nonabrasive soap or detergent.
4. The use of acids requires immediate cleaning and neutralization after a run or physical damage will result.
5. When freeze drying biological substances, it may be necessary to decontaminate the system. A surface decontaminant should be used to clean the accessible surfaces.

7.2 RSC Staff Maintenance

Clean refrigerant condenser – every 12 months
Replace vacuum pump oil – every 1000 hours
Check vacuum pump oil – monthly
Check rubber hoses and gaskets – monthly
Clean cabinet and door (mild non abrasive cleaners) - monthly

8. Appendix

https://www.labconco.com/articles/video-how-to-program-freezone-freeze-dryers

https://www.labconco.com/articles/common-mistakes-using-a-laboratory-freeze-dryer

https://www.labconco.com/articles/how-to-freeze-dry-faster

https://www.labconco.com/articles/why-are-my-freeze-dry-flasks-breaking-4-pre-free

https://www.labconco.com/articles/top-5-lyophilization-mistakes

https://www.labconco.com/articles/how-long-will-it-take-to-freeze-dry-my-samples

Note for the following application note: our collecter is -80 °C, our door is not glass, and we don't have teflon coated components.

https://www.labconco.com/articles/how-to-freeze-dry-the-6-most-challenging-samples

Request "Büchi Freeze Drying Adviser" from RSC staff or from Büchi. Some information from this document follows.

1. Freeze drying principles

Freeze drying is the first step in preparation for many analytical procedures in materials characterization. In contrast to oven- or air-dried samples, freeze drying causes less sample damage and avoids shrinkage or agglomeration of the samples, as can be seen in Figure 2. Thus, freeze drying is a very gentle process and particularly ideal for heat-sensitive and delicate samples. The cost to freeze dry depends on the number of samples that fit in the freeze dryer, the amount of water in the samples, and the open surface area of the samples. For faster freeze-drying times, samples should be in containers that provide a large surface area. Samples must first be frozen, then freeze dried (sometimes for multiple days) until dry.

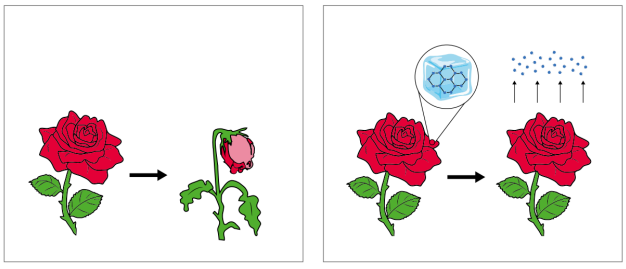


Figure 2. (Left) Common preservation methods apply heat to the product leading to structural changes, loss of color, taste and smell as well as nutritional constituents; (Right) In freeze drying, ice directly transitions into water vapor by sublimation allowing to maintain product structure and characteristic. Freeze drying is a very gentle process. Adapted from: *https://parsanalitik.com.tr/wp-content/uploads/2018/06/Freeze-Drying-Adviser-for-Freeze-Drying.pdf*

2. Freeze dryer setup

2.1 Components

The main components of a freeze dryer are the *drying chamber or specific drying attachments*, *the vacuum pump* and the *ice condenser*. The sample to be freeze dried is either placed in a system of shelves inside the drying chamber or it is filled into single flasks that are attached to the manifold. The vacuum pump is connected to the drying chamber via ice condenser and is responsible for evacuation of the drying chamber. Additionally, the vacuum pump acts as remover of all non-condensable gases that did not aggregate at the ice condenser. The main task of the ice condenser is the collection of the water vapor and all other condensable gases. Water molecules travel naturally towards the ice condenser encouraged by the difference in vapor pressure. The ice condenser temperature must be significantly lower than the frozen product temperature, i.e. minimum 15 °C colder.

3. Freeze dryer pressure and temperature working conditions

Sublimation can only occur at low pressure and a low temperature. The ideal freeze-drying conditions depend on the sample type as well on the solvent to be removed from the product. In most of the cases here, the main solvent will be water which is presented in three states; ice, water and water vapor. In the case of water, the freeze-drying process is based on the water's phase diagram (Figure 3).

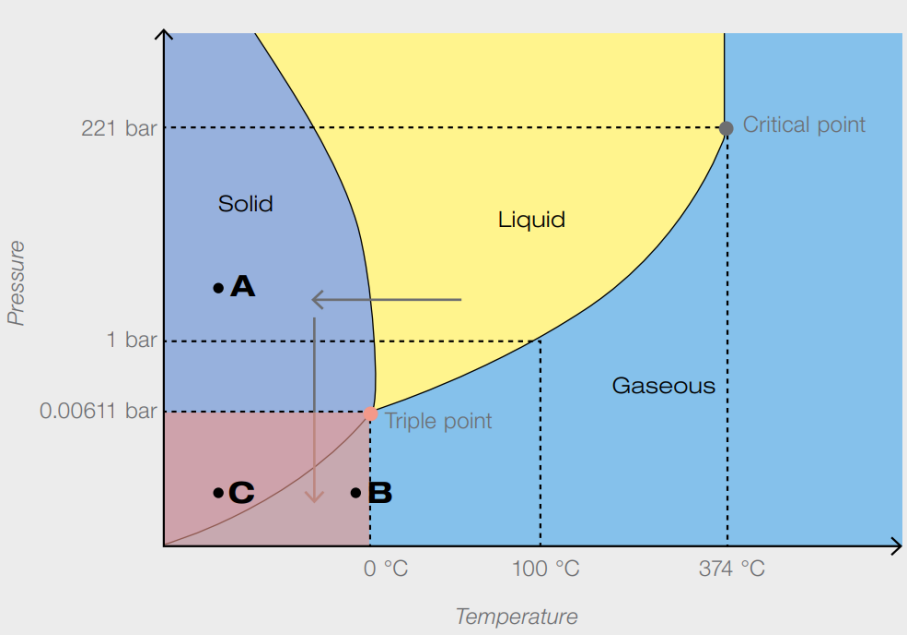


Figure 3. Phase diagram of water as a function of pressure and temperature. Adapted from: *https://parsanalitik.com.tr/wp-content/uploads/2018/06/Freeze-Drying-Adviser-for-Freeze-Drying.pdf*

At ~ 6 mbar, the three states coexists (triple point), and below 6 mbar water occurs only as ice or water vapor. The sample to be freeze dried is at the start a liquid. As can be seen in Figure 3, the water freeze drying process is the result of two-phase changes, first to the solid and further to the gaseous phase. Finally, for sublimation to take place once the sample is completely frozen, the pressure must be below the triple point (< 6 mbar).

4. Freeze dryer process stages

By now Labconco FreeZone Triad Benchtop Freeze Dryer's user should be aware that pressure and temperature are crucial variables in the freeze-drying process. The freeze-drying process involves three stages; freezing, primary drying and secondary drying and each process stage will require precise requirements of pressure and temperature (Figure 4).

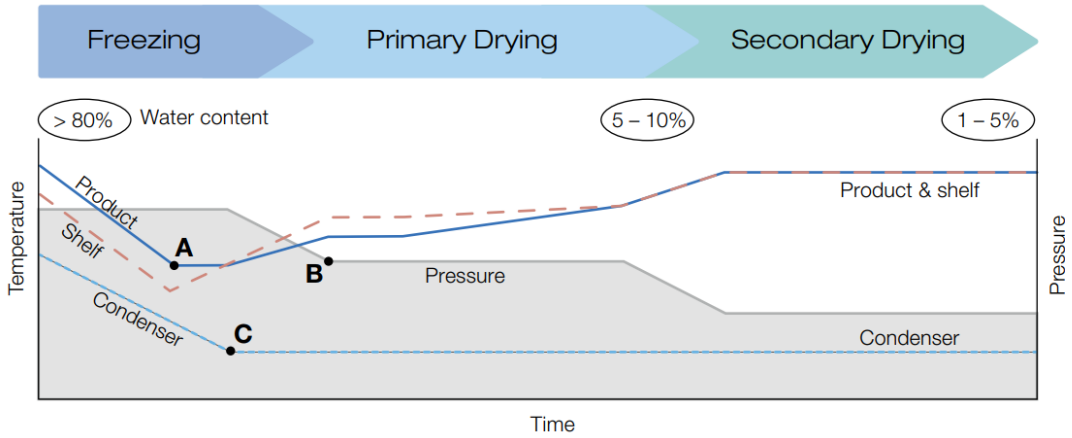


Figure 4. Stages of the freeze-drying process. The initial water content is usually reduced to more than 95%. Adapted from: *https://parsanalitik.com.tr/wp-content/uploads/2018/06/Freeze-Drying-Adviser-for-Freeze-Drying.pdf*

Stage 1: The sample is frozen at a temperature low enough to guarantee it is completely frozen (Figure 4, point A).

Stage 2: The drying chamber pressure is decreased to activate the drying process. The prevailing pressure and temperature readings are now both below the triple point (Figure 4, point B).

Stage 3: Sublimation creates water vapor in the drying chamber. If not removed from the system, the water vapor equilibrates and no further ice particles sublimate. The vapor particles are removed by means of the ice condenser, a cooling device running at temperatures far below the critical product temperature (Figure 3, point C).

5. Common mistakes when using a laboratory freeze dryer (https://www.labconco.com/articles/common-mistakes-using-a-laboratory-freeze-dryer)

5.1 Incompatible Samples

Often a sample is placed on a freeze dryer without any consideration as to the compatibility of the sample with the specifications of the freeze dryer. When lyophilizing, it is important to identify the components of the sample and their requirements for lyophilization. *Incompatibility can result in decreased quality of the freeze-dried sample and, more importantly, expensive damage to the freeze dryer or vacuum pump.*

5.2 Collector Temperature (by default set to -80° C)

Although it is not critical to know the precise freezing point of a sample, estimating the general freezing point or eutectic temperature of your sample is important. ***The collector temperature of the freeze dryer is recommended to be 15° C to 20° C below the freezing point of a sample.***

Vacuum Pump Damage

Maintaining deep vacuum levels is the most common problem in laboratory freeze drying. A damaged vacuum pump is the leading cause of inadequate vacuum levels in a freeze dry system. ***If the vapors are not completely collected on the coils in the collector, they will enter the vacuum pump.*** Oil vacuum pumps are the most susceptible to damage from these vapors. Often the vapors will condense in the pump and mix with the oil. Once mixed in the oil, water can cause extensive damage to a pump while solvents and acids can cause even more damage. Combination rotary vane/diaphragm and scroll pumps are more resistant to harmful vapors but still can be damaged from exposure.

Damage to any pump will be avoided by preventing vapors from entering the pump. Ensuring the compatibility of the samples to the freeze dryer, as discussed earlier, is the most important preventative step to vapors entering the pump. Here are more suggestions.

5.3 Sample Freezing

Before the freeze dry process can occur, the products to be dried must be in a frozen state. This can be accomplished in a freezer separate from the freeze dryer or on the shelf inside the Freeze Dryer. If the shelf inside the Freeze Dryer will be used to pre-freeze samples, make sure that the vacuum is turned off until the samples are fully frozen.

The sample container volume should be at least two to three times greater than the sample volume. The temperature required for pre-freezing is dependent on the characteristics of the sample. Pre-freezing temperature is typically at least 10° to 20°C below the eutectic or collapse temperature of the sample. Pre-freezing on the Triad shelf can be done in Manual or Program modes as long as the shelf set point temperature is adequate to fully freeze the sample.

In MANUAL mode, simply set the Shelf temperature to the desired value for freezing the sample, and allow an appropriate amount of time for the sample to be fully frozen before starting the vacuum and the freeze-drying process.

ACB/acb April 2019