



Labconco FreeZone[®] 4.5 Liter Freeze Dry System

Bergen County Technical Schools
Stem Cell Lab





Labconco FreeZone® 4.5 Liter Freeze Dry System Information Sheet

The Labconco FreeZone® 4.5 Liter Freeze Dry System is an instrument used in sample preparation, and for the preservation and storage of biological specimen, pharmaceuticals, and foods. Freeze drying, or lyophilization, is one of a number of dehydration methods, but is especially suited for substances that are heat sensitive. The process of freeze drying works by freezing the material, and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from its solid phase to a gas.

The Freeze Dry Process

Freeze drying has been used extensively in the development of pharmaceuticals, such as antibiotics, and preservation of biological samples, such as proteins, plasma, viruses, and microorganisms. This process has been shown to be nondestructive in nature, which has been demonstrated by the retention of viability in freeze dried viruses and microorganisms.

As already stated, freeze drying is a process of freezing a sample and reducing the pressure, followed by a heating that causes the solid water to sublime. The basis of the sublimation process involves the absorption of heat by the frozen sample in order to vaporize the ice, the use of a vacuum pump to enhance the removal of water vapor from the surface of the sample, the transfer of water vapor to a collector, and the removal of heat by the collector in order to condense the water vapor.

Freeze Dry Rates

The efficiency of the freeze drying process is dependent upon the surface area and thickness of a sample, the collector temperature and vacuum obtained, the eutectic point (melting point), and solute concentration of the sample. See the table below for examples of materials and dry times.

In most cases, the greater the surface area of a sample is, the faster the rate of freeze drying. In contrast, the thicker a sample is, the slower the rate of freeze drying. The heat of sublimation is often absorbed on one side of a frozen sample and must travel through the frozen layer to vaporize the water at the other surface. The water vapor must also travel through a layer of dried material. Therefore, the thicker a sample is, the greater the chance that the dried layer may collapse, causing a decrease in the rate of drying.

The process of sublimation is accomplished using the collector and vacuum pump. The collector should always be set as 15 to 20°C colder than the eutectic point of the sample. This traps the vapor as ice. The low pressure caused by the vacuum then draws the vapor to the collector. This vacuum is essential to maintain an efficient rate, since diffusion occurs very slowly under normal atmospheric conditions.

The rate of freeze drying is directly proportional to the vapor pressure, and the vapor pressure of a sample is dependent upon both the eutectic temperature and the solute concentration of a sample.

SAFE TEMPERATURE AND DRYING TIMES FOR SELECTED MATERIALS			
Material 10mm Thick	Safe Temperature °C	Collector Temperature °C	Hours (Approx.)
Milk	-5	-40	10
Urea	-7	-40	10
Blood Plasma	-10 to -25	-40	16
Serum	-25	-40	18
Vaccinia	-30 to -40	-50	22
Influenza Vaccine	-30	-50	24
Human Tissue	-30 to -40	-50	48
Vegetable Tissue	-50	-80	60



Labconco FreeZone[®] 4.5 Liter Freeze Dry System Quick Start Guide

Before starting the Labconco FreeZone[®] 4.5 Liter Freeze Dry System, the user must:

1. Wipe the interior of the collector chamber with a soft cloth or paper towel to remove any accumulated moisture.
2. Using a soft, lint free cloth or paper towel, wipe the collector chamber gasket to remove any dirt and contaminants that could cause a vacuum leak.
3. Check the collector chamber drain hose to ensure the hose is free of moisture and that the drain plug is securely installed.



Note: The Freeze Dryer will not start if any moisture is detected.



4. Check that each sample valve is closed, or in the "Vent" position.
 - a. The "Vent" position is shown to the right. The bevel on the valve knob is facing upward when in the "Vent" position.



5. Make sure that the oil level in the instrument's vacuum is not low, and that the oil is clear.



Once all of these items have been checked, the user can proceed to using the instrument.

Turn the instrument on by pressing the **Power Button**, located on the lower right side of the instrument.



Configuring the Freeze Dryer

The Freeze Dryer is already set-up with lab standards. If these conditions need to be altered for a freeze dry protocol, proceed with the following steps under instructor supervision. Otherwise, continue to the next section.

1. Press the **Menu Button** on the *Control Panel*.



- a. The LCD Screen will show **Vacuum Units**.



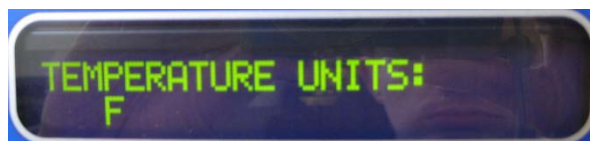
- i. Press **Select** until the desired unit (mBar, Pa, or Torr) is flashing. Press **Menu** to select and advance to the next screen.



- b. **Vacuum Set Point** will be the next item on the LCD Screen. This is used to tell the instrument how low the vacuum needs to be set for the sample being used.



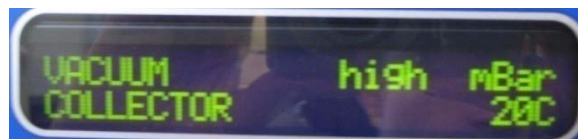
- i. Press **Select** until the desired set point is shown on the screen. Press **Menu** to select and advance to the next screen.
- c. Temperature Units is the next item on the LCD Screen.



- i. Press **Select** to select whether to display the temperature in Celsius or Fahrenheit. When the item is flashing, press **Menu** to select and advance to the next screen.
- d. The next two screens show the total hours that the **Refrigeration** and the **Vacuum** have been used. No changes need to be made on these screens. Press **Menu** to advance past each.



- e. The next screen is the **Transmission Rate Screen**. This is used to change the time between data transmissions.
 - i. Press **Select** to change the time interval to **10, 30, 60, 300, or 600** seconds. Press **Menu** to select and advance to the next screen.
2. After moving through these screens, the instrument will return to the **LCD Main Screen**, and show the conditions of the instrument. At this point, the user will be able to proceed to the **Freeze Drying Procedure**.



Starting Up a Freeze Dry Protocol

The Freeze Dryer can be operated in one of two modes: **Automatic** and **Manual**. Determine which mode to use, and follow that procedure.

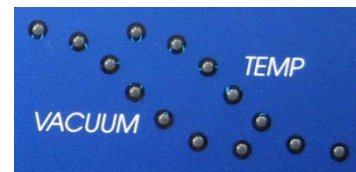
Automatic Start-Up

1. To run the **Auto Mode**, press the **Refrigeration Auto Button** on the *Control Panel*. This will start the instrument's refrigeration system.



- a. The **Green LED** above the button will illuminate.
2. When the collector temperature reaches **-40°C**, the vacuum pump will automatically start.

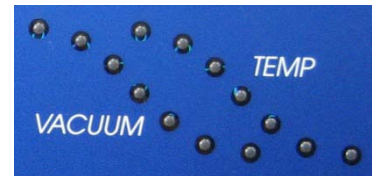
- a. The **Temperature Graph** will illuminate LED lights incrementally as the temperature of the unit drops to -40°C.



- b. The **LCD Display** will show the actual temperature of the instrument at all times.

3. Once the vacuum starts, the pressure in the instrument will begin dropping until it reaches **<0.133mBar**.

- a. The **Vacuum Pressure Graph** will illuminate LED lights incrementally as the pressure in the unit drops to < 0.133 mBar. The last light will blink when between 0.45 and 0.133 mBar, and then become steady once it reaches 0.133 mBar.



- b. The **LCD Display** will read "High" when the pressure in the instrument is above 5 mBar. It will then display the actual vacuum once the pressure drops below 5 mBar.

4. Once the last green LED light is illuminated for the vacuum, samples can be added.

Manual Start-Up

1. To run the **Manual Mode**, press the **Refrigeration Manual Button** on the *Control Panel*. This will start the instrument's refrigeration system.



- a. The **Green LED** above the button will illuminate.

2. When the collector temperature reaches **-40°C**, the vacuum pump can be started by pressing the **Vacuum Button** on the *Control Panel*.



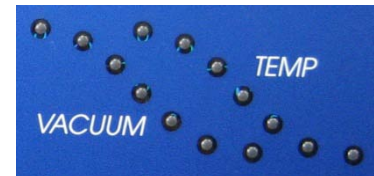
- a. The **Temperature Graph** will illuminate LED lights incrementally as the temperature of the unit drops to -40°C.



- b. The **LCD Display** will show the actual temperature of the instrument at all times.

3. Once the vacuum starts, the pressure in the instrument will begin dropping until it reaches **<0.133mBar**.

- a. The **Vacuum Pressure Graph** will illuminate LED lights incrementally as the pressure in the unit drops to < 0.133 mBar. The last light will blink when between 0.45 and 0.133 mBar, and then become steady once it reaches 0.133 mBar.



- b. The **LCD Display** will read "High" when the pressure in the instrument is above 5 mBar. It will then display the actual vacuum once the pressure drops below 5 mBar.

4. Once the last green LED light is illuminated for the vacuum, samples can be added.

Freeze Drying Samples

Note: The samples should always be pre-frozen. The sample container should always be two to three times the sample size. The freeze drying temperature should always be at least 10 - 20°C colder than the eutectic temperature of the sample.

1. Connect a pre-frozen sample to a **Sample Valve** on the **Drying Chamber** using an adapter.



2. Turn the plastic valve knob from the “**Vent**” position to the “**Vacuum**” position to open the valve.

- a. The bevel on the knob should be positioned **Downward** toward the sample to apply vacuum to the sample.



3. Before adding another sample, allow the system vacuum to return to **0.133 mBar** or lower. Repeat for each sample.

Note: Adding the samples one at a time and allowing the pressure to return to 0.133 mBar is necessary to ensure that the samples do not melt.

4. Allow the Freeze Dryer to run. Once all of the frost has disappeared from the outer surface of the sample container, and no cold spots can be detected upon handling, the sample is nearly dry (This may take several hours). Continue to dry the sample for several more hours to ensure low moisture content.

5. To remove a container from the instrument after drying is complete, turn the knob back to the “**Vent**” position. The sample container can then be removed.



Shut Down and Defrosting

To shut down and defrost the instrument after the freeze dry procedure is completed:

1. Release the vacuum by turning the plastic knob on a valve to the **Open**, or “**Vacuum**” Position.



2. Press the **Vacuum Button** on the *Control Panel* to turn the vacuum pump off.

3. Press the **Refrigeration Button** next to the illuminated LED to turn the refrigeration system off.



4. Turn off the instrument power by pressing the **Power Switch** on the lower right side of the instrument.



5. Pull the **Collector Chamber Drain Hose** out from the left side of the instrument and remove the **Drain Plug**. Place the hose in a container to collect any condensate from the instrument.



6. Remove the **Collector Chamber Lid** and allow room temperature air to melt the ice.
7. Once all ice is melted, flush the collector chamber with water and wipe the chamber clean.
8. Once all liquid is cleared from the instrument, close the drain plug and reinsert the hose into the instrument.
9. **Make sure the station is neat before leaving the instrument!**



Labconco FreeZone® 4.5 Liter Freeze Dry System Safety Sheet

1. Samples should be handled according to good laboratory procedures and methods in order to prevent accidents.
2. Dispose of all waste solutions according to waste disposal procedures.
3. Do not remove any panels or cords from the instrument to avoid electrical shock. Do not remove any vacuum hoses from the instrument.
4. Food and drinks should not be placed on or near the instrument.
5. Do not operate the instrument if the vacuum oil level is low.
6. Do not operate the instrument if the vacuum oil is cloudy, discolored, or contains particles.
7. Never operate the instrument or start the vacuum when there is liquid in the collector chamber. This could result in damage to the vacuum pump.
8. Some of the rubber and plastic components of the instrument can be attacked and degraded by certain chemicals and solvents. Thoroughly rinse the components with water after use of these chemicals or solvents in the instrument. **A list of these chemicals and solvents is included on the next page.**
9. The instrument's collection chamber should be wiped with a soft cloth or paper towel prior to use to remove any accumulated moisture. The collection chamber drain hose must be checked to ensure that it is free of accumulated moisture before use.
10. Dirt, dust, or contaminants on the collection chamber lid gasket can lead to a vacuum leak. Use a soft, linen free cloth or paper towel to remove any dirt or dust prior to use.
11. The instrument will achieve extremely low temperatures (as low as -40°C) and pressure (<0.15 mBar). Be aware of the temperature and pressure when operating the instrument to avoid injury.
12. After a sample had been dried completely, the collection chamber and cooling coil must be defrosted and dried of accumulated liquid. Never attempt to chip ice from the collector coil. Damage to the coil can result.

Chemical Degradation of Instrument Parts

Component	Material	Acids			Buffers		Solvents							
		Acetic Acid 20%	Formic Acid	Trifluoroacetic Acid (TFA)	Calcium Chloride	Sodium Phosphate	Acetone	Acetonitrile	Carbon Tetrachloride	Cyclohexane	Dioxane	Methyl t-Buryl Ether (BTBE)	Pyridine	
Valve Stem	Acetal (Delrin)	C	D	D	D		D							
Collector* Lid	Acrylic			D			D	D	D					
Hoses, Gaskets & Valve Bodies	Neoprene	C	D	D			C	C	D	D	D	C	D	
Flask Top	Silicon Rubber		C	D		D			D	D	D	C	D	
Chamber & Fittings	Stainless Steel				C									

C – Moderate degradation; Limited use.

D – Severe degradation; infrequent use recommended; immediate thorough cleaning required.



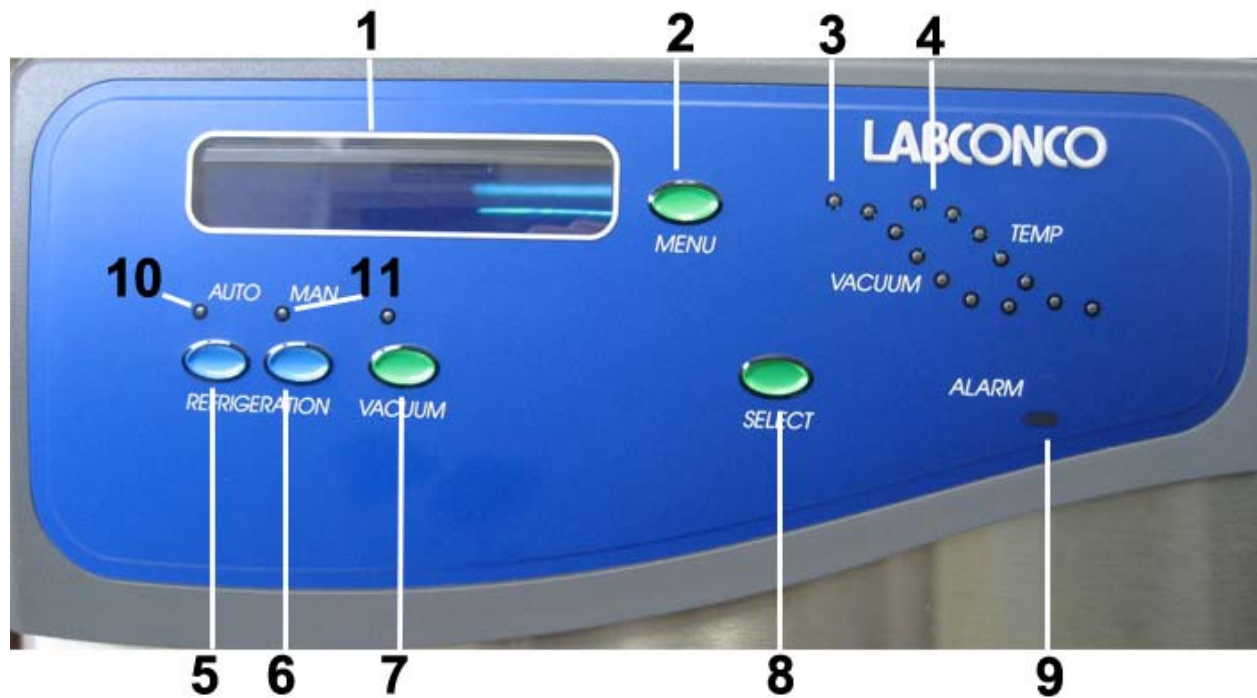
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Labconco FreeZone 4.5 Liter Freeze Dry System Overview:



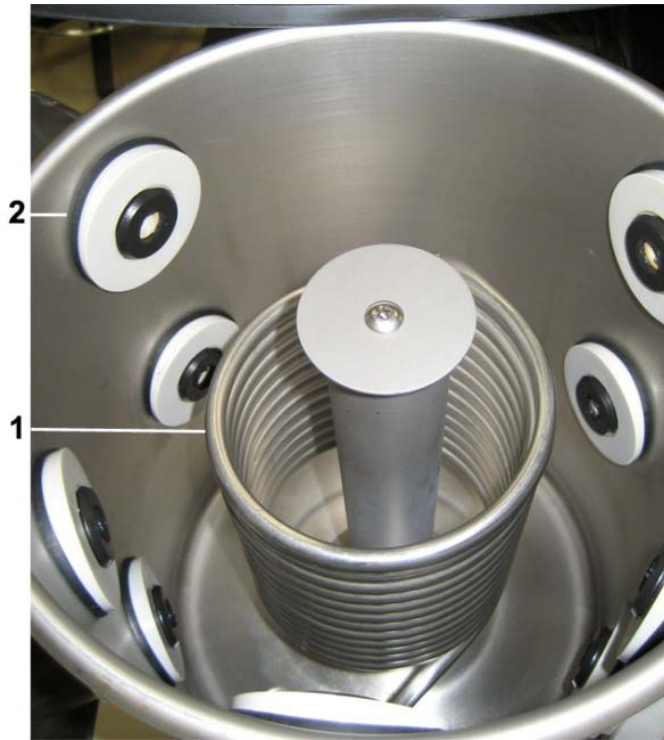
1. FreeZone Control Panel
2. Drying/Collector Chamber and Cooling Coil
3. Sample Valves and Ports
4. Power Button

FreeZone Control Panel Overview:



1. LCD Display
2. Menu Button
3. Vacuum Graph Display
4. Collector Temperature Graph Display
5. Automatic Refrigeration Mode Button
6. Manual Refrigeration Mode Button
7. Vacuum Button
8. Select Button
9. Alarm Indicator
10. Automatic Refrigeration Mode Indicator
11. Manual Refrigeration Mode Indicator

Drying/Collector Chamber Overview:



1. Cooling Coil inside of the Drying/Collector Chamber
2. Sample Port (internal)
3. Sample Port Connection Adaptor (external)
4. Port Valve
5. Sample Container
6. Sample Container Adaptor

