

Freeze Drier – Labconco Freezone

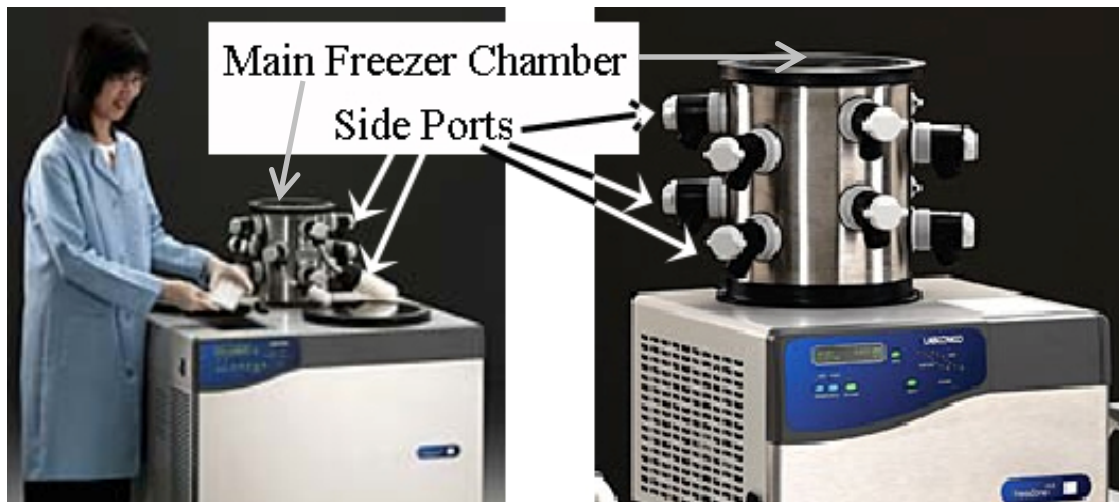
ALL USERS MUST ENTER THE FOLLOWING IN THE LOG BOOK:

- NAME
- DATE
- LENGTH OF USE

SAMPLES SHOULD BE FROZEN BEFORE FREEZE DRYING

Turning on the system

- If “Defrost” is still selected, press the button to deselect it.
- Cover the chamber containing the coils and the main freezer chamber
- Plug the black hose coming out of the bottom of the freeze drier with the metal stopper
- Press the “**REFRIGERATION AUTO**” button to start the refrigeration and compressor.
 - The system will be ready to use in about 10 min.
- When the temperature reaches -40°C the compressor will start automatically.
- Below 2000×10^{-3} mBar the display will show the actual vacuum reading. There is a vacuum graph indicator for quick inspection.
- Frozen samples can be dried once the vacuum is $\leq 133 \times 10^{-3}$ mBar by two methods:
 - in the **freezer chamber**
 - this is good for bulky specimens
 - in a **flask attached to a freezer port**
 - this is faster for solutions, which can be “shell frozen” in liquid nitrogen or dry ice-cooled acetone so that they coat the inside of the flask



Adding frozen samples

(Samples should be pre-frozen for more efficient freeze drying)

- **To the freezer chamber (for bulky specimens):**
 - turn vacuum pump **off**
 - press the “**vacuum auto**” button
 - Open a flask attachment port until the cover can be removed from the chamber. Make sure the **valve is opened slowly** so air bleeds in; close vacuum port
 - remove cover from main chamber; add samples; replace cover
 - turn vacuum on
 - Vacuum should be below 2000×10^{-3} mBar in ~60 seconds. If not, check the seals.

- **To a port (for small frozen samples or frozen solutions)**
 - o Leave the vacuum pump running
 - o Attach a vacuum flask with the frozen sample to a port using an adaptor, preferably metal,
 - o Open the port by turning the portion that has the bevel (see diagram next page). Vacuum pump will start to evacuate the flask, which should feel cool
 - o Remember to **support the flask with a rubber ring** to reduce stress on the neck
 - o Vacuum should be below 2000×10^{-3} mBar within about 30 seconds

How to tell when a sample is dry?

If the sample is within the main **freezer chamber**:

- The top and sides of the chamber will be relatively warm.
- Be patient as big samples can take **days to dry**.

You can remove the sample and check directly – If it is dry it will be room temperature

In a **flask attached to an external port** – The outside of the flask will be warm and dry.

Removing samples

- **From the main chamber**
 - o Turn off the vacuum
 - o Bleed the system to atmospheric pressure using one of the ports.
 - Make sure this is done slowly so that there is no sudden change in pressure that could damage the system, or break your specimens.
 - If the specimen feels cool, it has not dried completely – continue to lyophilize
 - o Remove dry samples and any debris
 - o Add another sample or shut down the system (see below)
- **From a port**
 - o Turn the beveled valve to the hole on the black port connection until air bleeds into the vacuum flask – see ‘vent/backfill port’ on the upper diagram
 - o Remove the flask
 - o Replace with another sample or shut down the system (see below)

Shutting down the system

- Vacuum pump and refrigeration should be turned **OFF**.
- The upper and lower chambers should be left **clean** and **dry**, at **atmospheric pressure**.

DEFROSTING THE CONDENSER COILS If there is ice on the condenser coils,

- Turn off the refrigeration and vacuum
- Turn on the defrost cycle and pull out the drain hose (bottom left on the front of the machine) and unplug it. Put the tube into a bucket.
 - o The condenser coil will warm to +10°C within a few minutes.
- Allow the ice to melt. Ice can sometimes be removed in bulk when partly melted.
 - o Never put stress on the coils or use a sharp object – this can damage the coils
- Dry the chamber thoroughly. If necessary, clean out debris. Replace all covers

NEVER LEAVE THE SYSTEM UNDER VACUUM WITHOUT THE PUMP RUNNING

NEVER LEAVE THE VACUUM PUMP RUNNING UNLESS YOU ARE FREEZE DRYING

ENTER YOUR NAME+DATE+LENGTH OF USE IN THE LOG BOOK.

Theory

Freeze drying – also called lyophilization – is a non-destructive method of preserving biological samples. It can even preserve viable samples of viruses and certain microorganisms, such as yeast.

Freeze drying (lyophilization) = removing water from frozen samples held in a vacuum chamber. The frozen water is converted directly into water vapor without an intermediate stage involving liquid water – this is the process of **sublimation**. (Although slower and less controlled than freeze drying, **sublimation** is what happens when ice cubes “disappear” after being stored in a domestic freezer for a long time, or frozen foods appear to be partially dehydrated). Lyophilization preserves the lifelike appearance of cells - otherwise the surface tension of the evaporating water causes shrinkage. A lyophilized grape resembles a grape; a dried grape is a raisin.

During lyophilization (controlled sublimation) samples are held at about -50°C in a vacuum of at least 133×10^{-3} mBar (one Bar is about one atmosphere of pressure). If one atmosphere is 760 mm Hg, then the vacuum in a lyophilizer is about 100×10^{-3} mm Hg or 100 μm of Hg.

In essence, the freeze drying process requires a balance between 1) the heat absorbed by the sample to sublimate the frozen water in the sample, 2) chilling by the refrigeration system to keep the sample from melting, and 3) maintaining a vacuum to enhance the sublimation rate. Water sublimates from the sample and the vapor condenses onto the refrigerator coils (which are somewhat colder). This is why the refrigerator coils become encrusted in ice.

The **rate of freeze-drying is directly proportional to the vapor pressure of water in the sample**, which is dependent on chemical type and solute concentration of the sample. The most important factor is the **eutectic** temperature of the sample. A **eutectic** or **eutectic mixture** is a mixture at such proportions that the melting point is as low as possible, and that furthermore all the constituents crystallize simultaneously at this temperature from molten liquid solution (http://en.wikipedia.org/wiki/Eutectic_point). For example, the eutectic temperature of pure water is 0°C . Adding table salt (NaCl) to pure water lowers the freezing point of the solution. The freezing point of saturated NaCl is -17.8°C (0°F), which is the eutectic temperature of an aqueous solution of NaCl. Even if you are lyophilizing what initially is a dilute aqueous solution of NaCl, eventually you will have removed enough water to reach a frozen, saturated NaCl solution (whose volume is dependent on the amount of NaCl). Since more concentrated samples have proportionally less water, the sublimation rate falls as dryness approaches.

Large concentrated samples should be lyophilized one at a time. This is particularly important for biological samples that contain sugars, whose eutectic temperature can be -30 to -50°C . Fortunately, the Freezone lyophilizer is ideal for biological samples.

In order for water to sublimate or melt it must absorb heat from its surroundings, which, under uncontrolled conditions, can lead to sample thawing. This is not a problem for most biological materials, unless the lyophilizer malfunctions or too long is spent in transferring frozen samples. However, melting during lyophilization can be a problem for certain highly concentrated biological samples (for example, containing high concentrations of glycerol or other “antifreeze” components). Melting can also be a problem for samples containing highly volatile solvents (e.g., acetic acid, formic acid, pyridine). Add these samples one at a time - or - dilute them with water so that the volatile solvent can escape from a frozen aqueous matrix - or - use a machine with a specialized refrigeration system.