

Best Practices for Ultra-Low Temperature Freezer Use

PHCbi VIP Series, VIP ECO Natural Refrigerant Series and TwinGuard® Series Ultra-Low Freezers

The Significance of Ultra-Low Temperature Storage

When you want to preserve biological samples and cell viability it is critical that cell metabolism be slowed or suspended entirely. Colder is usually better. Many facilities use protocols for storing specimens at temperatures of -70°C or -80°C. In some cases, specimens are preserved in liquid nitrogen (LN₂) in the liquid phase at -196°C, or in the vapor phase within a range of -196°C to -120°C or warmer, depending on the vapor phase gradient.

Because the recrystallization temperature of water is -129°C, metabolic activity ceases because enzymes cannot be exchanged to promote cell growth and, thus, cell aging is suspended. Newer mechanically refrigerated cryogenic freezers reach temperatures as low as -156°C to create what is known as an "air phase" storage medium. Regardless of the temperature and method, the need for uniform storage, or storage of all specimens at or near the same temperature, is important.

Frost Free, Always

Specimens covered with frost are unlikely to be as cold as desired. Frost can act as an insulating medium and reduce the efficiency of the refrigeration system. A freezer should always be free of frost around the walls, gaskets, doors or sub-lids (chest).

Frost Creates Problems

If frost is keeping your freezer door from closing correctly, both temperature uniformity and operating costs will suffer. Frost mitigates the transfer of energy through the walls which causes the refrigeration system to work harder than it should. Frost also inhibits the ability of a freezer to recover temperature to setpoint following a door or lid opening.

Know What You Want Before You Open the Freezer

When you know where your samples are before opening the freezer you minimize exposure to ambient temperature in the laboratory. Elective use of available inventory racks and boxes will help in navigating what is stored where. Minimize how long you keep your racks out of the freezer; grab what you need and return the rack and remaining boxes as soon as possible.

Pre-Freeze Samples Before Transferring to an Ultra-Low or Cryogenic Freezer

When possible, place samples in a standard lab freezer such as a -20°C or -40°C freezer to get a head start on the ultra-low process. This is called "pre-freezing". For large volume storage, many facilities, especially in pharmaceutical applications, have specially designed "blast" freezers developed to rapidly cool large loads using ultra-low temperature coils and high internal airflow. These freezers reduce specimen temperatures much more quickly. Care should be taken to understand the process of pre-freezing and the concept of "controlled rate freezing" as it applies to the particular cellular materials stored and how they are prepared.

Never Ignore an Alarm

Most freezers include internal alarm systems that warn of any temperature deviation from setpoint, a door left ajar, a power failure caused by an outage, a tripped circuit breaker or a disconnected electrical cord. It is never advisable to power any other laboratory appliance or instrument on the same receptacle or circuit breaker as an ultra-low freezer. It is always wise to use independent alarm systems with independent temperature sensor probes for both local and cloud-based monitoring. When possible, the use of liquid CO₂ or LN₂ backup systems are always recommended.



Choose Your Storage Temperature

Some users prefer to use temperature setpoint as an energy-saving strategy. On older ultra-low freezers, a 10°C difference between -70°C and -80°C or lower can save energy. Newer ultra-low freezers with more efficient refrigeration systems also operate more inexpensively at warmer temperatures, but the idea of having a 10°C margin from storage temperature and ambient allows more time to remediate a problem in case of a power failure. Peer-reviewed data from others with experience in managing cell viability from a -70°C repository can be helpful in determining the best overall temperature for your application.

Don't Skip Preventive Maintenance

Regular preventive maintenance is important to keep your freezer functioning properly. Your facility team should perform regular cleaning and removal of frost from around the door or lid, taking care to avoid damage to the door gaskets. This applies to sub-lids and inner doors as well.

If your freezer has a conventional condenser, airflow filters across the refrigeration system fan must be cleaned regularly to maintain proper cooling and to keep the compressors running efficiently.

It is always wise to check your freezer for proper temperature calibration so that you are sure the internal control and temperature indicating probe(s) are true and accurate.

Preventive Maintenance At-A-Glance

Condenser Filter and Coil

Use a vacuum to remove dust from the condenser filter every two to three months, more often if required. At the very least, schedule this for whenever you change the batteries in your smoke alarm or set your clocks ahead or back for daylight saving time. More often if the laboratory area is dusty.

Clean and Protect the Door Gasket

Your door gasket is specially designed for both durometer (softness) and contact (multi-point). The gasket creates a series of air pockets that help protect against the extreme temperature differential inside and outside the cabinet. You can spot air leaks by looking at frost streaks along the periphery of the door or lid; these indicate a gasket failure. Use a cloth to remove frost build-up. Use of a plastic scraper is common, but extreme care is required to prevent damage to the gasket. When your door or lid is closed, make sure it is, in fact, closed. If there is any resistance due to frost, remove it immediately.

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Fresh Batteries are a Must

If your freezer control system includes a battery back-up, make sure the batteries are charging properly. A certified service technician should change your rechargeable batteries every two years or so. This battery check-up also applies to any CO₂ or LN₂ back-up systems, or remote alarm systems that may been added to your installation.

Know and Understand Function Codes

Newer freezers include "predictive performance" metrics integrated into the system. These platforms feed data to the microprocessor controller where it is interpreted by algorithms to spot trends or minute deviations over time that signal a change in performance. More comprehensive control systems include menu displays and fault warnings similar to automobiles that provide a real-time view of how the system is working and what might need attention.

Keep a shortcut copy of function codes from your operator's manual near your freezer for quick reference. These codes also help technicians diagnose problems over the phone when you have questions.

Managing Ice and Frost

Frost is unavoidable, so regular cleaning is an easy and costeffective way to avoid problems. The inner chamber will build up frost and then ice over time. The more you remove frost, the less you have to remove ice. This is caused by the quick condensation of humidity from your lab ambient on the ultracold surfaces that attract moisture. Make sure you check outer and inner door gasket surfaces and handles.

Tips to Minimize Frost:

- **1.** Make sure your freezer is installed away from direct airflow of heating and cooling vents.
- 2. Minimize the number of door openings.
- Know what you want and where it is before you open the door or lid.
- Make sure your inner and outer door latches are secured after opening and closing.
- Make sure your outer door latch assembly has not been bent or twisted from over torque due to previous frost or ice build-up events.



Vacuum Relief on Upright Freezers

The exterior door gasket provides an excellent seal that protects specimens and provides an energy efficient thermal barrier. Because this door gasket seals very well, a vacuum is created whenever the warmer air enters the interior and contracts after a door opening, pulling the door against the gasket and making it hard to open. Most freezers now include vacuum relief ports that can equalize the pressure and permit fast re-entry to the freezer if necessary. It is important to inspect this relief port for frost and ice build-up as well, especially in high humidity installations.

Calibration is Critical

Calibration service should be performed yearly by a qualified service technician. This is necessary to follow good storage practices, GMPs or other criteria, and to comply with your local facility protocols. Calibration requires the use of a probe certified by the NIST and placed into the interior center of the freezer. PHC Corporation of North America offers calibration and validation services by arrangement.

Defrost if Necessary

If frost or ice overrun your freezer you must defrost. While time-consuming, it is absolutely necessary to keep surfaces free of frost, "snow" or ice. Because it involves transferring the stored protect, this is a process you will want to plan and arrange for in advance.

- Remove all product and place it in another ultra-low freezer.
- Turn the unit off along with the battery switch.
- Open all doors and allow unit to defrost.
- Wipe up all water on the interior ceiling, walls, base and inner and outer doors.
- Follow with a non-chlorine detergent wipe-down if desired.
- Turn the freezer ON and allow to operate overnight before reloading the removed product.

Note - Recommended ambient conditions for PHCbi brand ultra-low temperature freezers are from 5°C to 35°C (40°F to 90°F) and 80% humidity. Installations outside of these parameters may affect the performance of your freezer.



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