

# Lyophilizer Standard Operating Procedure

## Bioengineering Department

### Scope

This Standard Operating Procedure (SOP) applies to the use of the Labconco FreeZone 6 plus Lyophilizer located in Bourns Hall Room B156.

### Hazards

The consequences of improper use of this lyophilizer are significant and can impact both personnel and the environment.

Certain Organic Solvents, if not fully trapped, can polymerize vacuum pump oil and turn it into sludge resulting in binding of the moving parts inside the pump.

Improper handling of cryogenics can result in injury, explosion and death



### Controls

#### **Administrative**

All protocols to be used in the preparation of samples for lyophilization must be reviewed by the supervising faculty member and then submitted to the Bioengineering Department Chair for final review and approval. Each user must notify the chair if there is any change from the approved protocol and obtain approval for that modification.

Prior to any independent use of the lyophilizer training by the by Bioengineering Laboratory Safety Officer or designee is required. This training includes the observation of the entire sample preparation and lyophilization process. All approved users must sign in the log book and only one user can use the lyophilizer at the scheduled time.

#### **Organic Solvent Use Restrictions**

Samples with only traces of organic solvent can be lyophilized in our FreeZone 6 lyophilizer because the freezer trap of this lyophilizer can only go down to -86°C. Dichloromethane is the toughest of solvent to handle because it does NOT freeze at -80°C or even -88°C. Fortunately, Acetonitrile and most acids, which can also cause oil polymerization, are fully trapped at -80°C.

#### **Acid Usage Restrictions**

Some of the major components of the FreeZone Dry system are susceptible to degradation. For example, 20% acetic acid can create moderate degradation and trifluoroacetic acid (TFA) can create severe degradation. Thus the lyophilizer can not be run for more than two days for samples with traces of acid.



## **Shell Freezing Requirement**

All samples need to be shell frozen either in dry ice or liquid nitrogen. Shell freezing as a method of prefreezing the product can increase the surface area to volume ratio by spreading out the frozen product inside the vessel.

### **Freezing the sample in liquid nitrogen (cryogen)**

Liquid Nitrogen Dewar is required for storing and dispensing small quantities of liquid nitrogen. Do not use Styrofoam containers to hold liquid nitrogen because they are likely to become brittle and can present an extreme fire/explosion hazard. If it is necessary to hold the sample when placing it in the nitrogen, it is best to use forceps (big tweezers).

### **Precautions**

- Liquid nitrogen is a hazardous material. Liquid nitrogen boils at 77 Kelvin (-194 Celsius). Wear safety goggles, lab coat and CRYO gloves at all times while handling liquid nitrogen.
- Liquid nitrogen will condense oxygen which can cause severe explosion. Never place liquid nitrogen or dry ice in a sealed container or any object that could cause entrapment of the gas. Never place liquid nitrogen in a container that is easily oxidized such as Styrofoam.
- Never mix dry ice or liquid nitrogen with water or water ice or pour it down the sink. Ice can solidify around it, trapping the gas at a high pressure.
- Never use liquid nitrogen in an area that is not well ventilated. Therefore, in closed spaces liquid nitrogen is an asphyxiation hazard. It is advised to open the door in B360 while taking the liquid nitrogen from the tank.

## ***Engineering Control***

A charcoal filter has been installed in this lyophilizer to capture organic vapors but it works best with only one solvent at a time. If multiple organic volatiles are present, the one with the greatest affinity to charcoal carbon will displace any other organics adhering to the carbon particles. Thus it is critical to remove the organic solvents in the sample preparation procedure. To deal with this issue, the vacuum pump oil will be changed frequently, which will be established empirically.

## **Authorized Contact People:**

- 1) Bioengineering Department Chair Jerome Schultz (2-2111)
- 2) Bioengineering Laboratory Safety Officer Hong Xu (2-7235)

## **Emergency Contact:**

- 1) Bioengineering Laboratory Safety Officer Hong Xu (2-7235)
- 2) Bioengineering Department Chair Jerome Schultz (2-2111)
- 3) Any medical emergencies, fires or explosions, please call 911 from the lab phone immediately.