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Standard Operation Procedures (SOPs) for the Palla VM-KT Vibrating Cryomill: Production of Fresh Frozen Standard Reference and Control Materials (SRMs/CMs) at the NIST Reference Material Production Facility



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U.S. Department of Commerce
Penny Pritzker, Secretary

National Institute of Standards and Technology
Willie May, Under Secretary of Commerce for Standards and Technology and Director

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Standard Reference and Control Material Production at the NIST Marine Environmental Specimen Bank

The National Institute of Standards and Technology (NIST) maintains the Marine Environmental Specimen Bank (Marine ESB) at the Hollings Marine Laboratory (HML) in Charleston, South Carolina. The Marine ESB is an environmental specimen bank that cryogenically archives specimens (e.g., marine sediments, fish tissues, mussels, oysters, marine mammal tissues, and bird eggs and feathers) collected as part of several monitoring and research programs supported primarily by other US government agencies, including, the National Oceanic and Atmospheric Administration (NOAA), US Geological Survey (USGS), and US Fish and Wildlife Service (USFWS). The Marine ESB is specifically designed to store environmental specimens over long periods of time (50 to100 years). A systematic well-designed specimen bank program, is not only a valuable component of real-time monitoring and basic research, but it also enables future investigators to extend their research into the past (hind casting) and provides for future verification of analytical results (quality assurance).

The Marine ESB facility consists of a 73.2 m^2 (788 sq. ft.) ISO certified Class 5 Cleanroom, a 79.4 m^2 (855 sq. ft.) ISO certified Class 7 Reference Material (RM) Production Facility, a 146.3 m^2 (1,575 sq. ft.) ISO certified Class 7 Cryogenic Storage Facility, and 46.8 m^2 (504 sq. ft.) of support facilities consisting of ante rooms and office space. Specimen bank and cleanroom protocols for projects of the Marine ESB are described in NISTIR 7389 (Pugh et al, 2007).

In addition to maintaining the Marine ESB in Charleston, NIST also develops and certifies Standard Reference Materials (SRMs) and Control Materials (CMs) to support accurate and compatible measurements for industry. SRMs and CMs are valuable in industry settings to establish instrument calibrations to ensure quality assurance parameters, to validate the accuracy of specific measurements, and to support the discovery and development of new measurement procedures. The Reference Material Production Facility, housed within the Marine ESB, provides the equipment and capabilities to produce SRMs/CMs for certification. These capabilities include developing lyophilized (freeze-dried) materials using a Millrock Quanta Series Freeze Dryer PC/PLC, and cryogenic materials (such as a fresh, frozen powder) using a Palla VM-KT Vibrating Cryomill (cryomill). The Standard Operating Procedures (SOPs) for the cryomill are described below.

Safety

The Marine ESB has been equipped with several safety features to ensure safe laboratory operating procedures. The HML where the Marine ESB is located is a NOAA facility; therefore, all personnel must abide by NOAA safety rules and policies. Because NIST and NOAA are both federal agencies under the Department of Commerce, many safety policies and procedures are similar. The HML Safety Committee has written a Laboratory Chemical Hygiene Plan and

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Emergency Preparedness Plan that are strictly enforced throughout the entire laboratory. A Job Hazard Analysis has also been completed for cryogenic homogenization using the cryomill and can be found in Appendix A. The Job Hazard Analysis includes information on work activities completed in the Marine ESB and what Personal Protective Equipment (PPE) are required to be worn during specific laboratory activities. All personnel who work in the laboratory must read, understand, and sign the HML Job Hazard Analysis before conducting work in the laboratory. Additionally, the cryogenic processing using the cryomill has been approved through the NIST Hazard Review System and can be found at https://mmlweb.nist.gov/safety/.

Oxygen Sensors

The Marine ESB facility (Class 5 Cleanroom, Class 7 RM Production Facility, and Class 7 Cryogenic Storage Facility) are equipped with oxygen (O_2) sensors that are monitored by the REES Centron Monitoring System. In the event that O_2 is depleted in any of the areas of the Marine ESB, an audible alarm will sound, as well as flashing alarm lights, warning individuals of O_2 depletion.

Personal Protective Equipment

There are two ISO Class 6 ante-rooms (Rooms C102 and D103) that provide access to the Class 5 Cleanroom and the Class 7 Reference Material (RM) Production Facility through the Freezer Room. These anterooms are used by Marine ESB personnel to change into proper 'clean' garments before entering the clean-air laboratories. Disposable laboratory coveralls, hoods, and shoe covers are provided in this room and must be worn at all times while in the clean-air spaces. The material used to make these garments is non-woven Tyvek, a highly effective contamination control fabric that is resistant to penetration by airborne particles. These garments are processed and packaged in a clean environment by the manufacturer and are disposable, but can be worn several times before soiling and tears occur. Additionally, hearing and eye protection must be worn at all times when operating the cryomill.

Safety Data Sheets

Several hazardous chemicals are referred to and used during cryohomogenization using the cryomill. Refer to Appendix B for applicable SDS documents.

Palla VM-KT Vibrating Cryomill

The cryomill is designed to cryogenically homogenize large quantities of frozen material under liquid nitrogen (LN_2) vapor-phase conditions (-120 °C to -180 °C), Figure 1. The flow of LN_2 and motor vibration are controlled by an electrical control panel, Figure 2. Titanium rods are rotated

within the material grinding chamber (300) when the cryomill motor is on and in motion, Figure 3. Teflon bellows connect the material grinding chamber (300) and motor to the stationary cryomill parts, and allow material to freely flow in and out of the material grinding chamber (300), Figure 4.



Figure 1. Palla VM-KT Vibrating Cryomill



Figure 2. Palla VM-KT Vibrating Cryomill Electrical Control Panel

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Figure 3. Titanium Rods contained inside Material Grinding Chamber



Figure 4. Teflon Bellow

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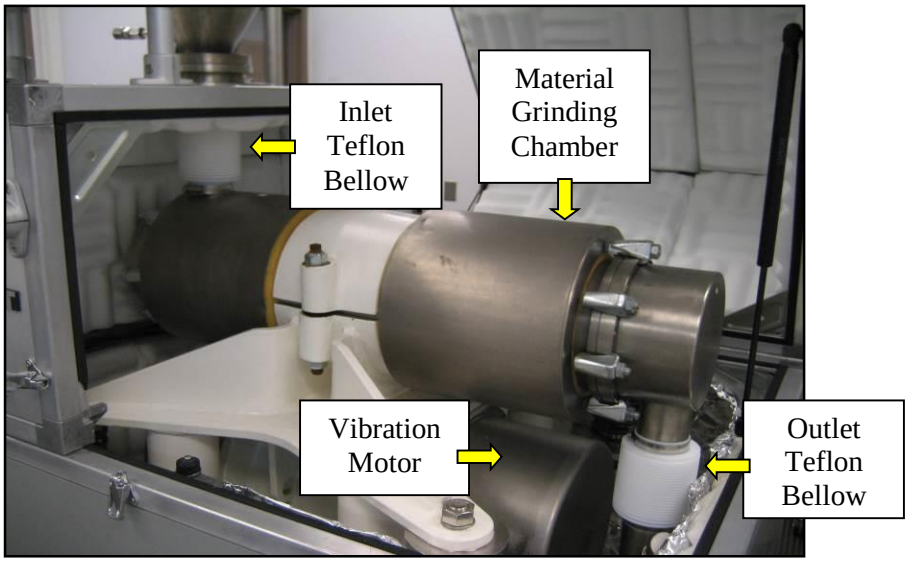


Figure 5. Palla VM-KT Cryomill Material Grinding Chamber and Motor

Compressed Air Smashing Device

To ensure a consistent and uniform flow of material through the cryomill, the frozen material must be crushed into <2.5 cm diameter pieces in order for the material to pass uniformly into the material grinding chamber (300) of the cryomill. Large pieces of material cause an inconsistent flow of material and clog the cryomill inlet socket (100), Figure 6, preventing subsequent material from being distributed into the material grinding chamber (300). To eliminate this problem, large pieces of frozen material are crushed using the Marine ESB compressed air defragmenting device, CAD, Figure 7. Details about this procedure have previously been described in Pugh et al., 2007.



Figure 6. View Inside the Inlet Socket

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Figure 7. Compressed Air Defragmenting Device

LN_2 Piping System

The cryomill is connected to a LN_2 in-line piping system. This system provides a vacuum-jacketed, insulated pipe along with a cryovent device, the Gordinier Electronics Keep Cold (Model 279) thermocouple sensor controller, located in the specimen bank to ensure that LN_2 is maintained in the piping system and gas is vented out. Liquid nitrogen is brought into the specimen bank through an intricate piping system that distributes LN_2 to multiple freezer stations. Each freezer station consists of a single supply line with either one or two connection valves, totaling forty-one connection valves. These valves provide LN_2 to 30 LN_2 vapor-phase (-150 ºC) freezers, 10 ultra-cold (-80 ºC) electric freezers, and cryomill. The LN_2 bulk storage tank for the Freezer Room, Reference Material Production Facility, and Cleanroom operations is stored behind the HML building in a 12 m3 (3,170 gallons) vertical storage tank with a telemetry system to the LN_2 commercial supplier (Figure 8). The main distribution lines are equipped with multiple flow valves strategically placed to ensure safety. Furthermore, a permanent LN_2 vapor vent system was installed on the cryomill to safely vent LN_2 out of the Reference Material Production Facility. The REES Centron Monitoring System: Monitoring, Alarming, and Data Management System is used to ensure correct temperatures and LN_2 levels are maintained for each freezer. In addition, this system monitors room conditions (i.e. temperature and humidity) and percent oxygen levels within the Freezer Room, Reference Material Production Facility, and Cleanroom.

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Figure 8. LN_2 Bulk Tank

Cryomill Assembly

Most parts of the cryomill are numbered and locations shown in an illustration developed by the cryomill manufacturer, Figure 9.

Table 1 indicates the name and item number assigned to parts of the cryomill. These part names and numbers are used in assembly steps. Steps for cryomill assembly are divided into Outlet Assembly and Inlet Assembly and are listed below.

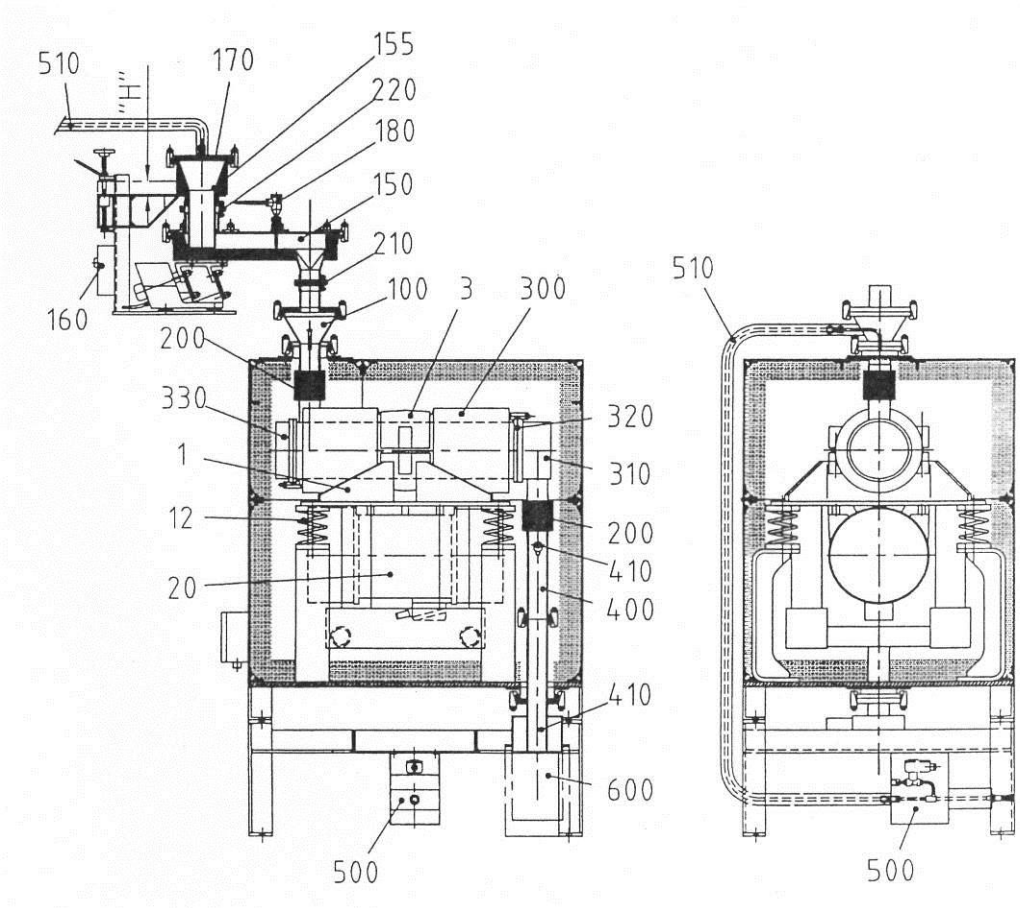


Figure 9. Palla Cryomill Schematic

Photo credit: Technical Machinery Documentation, Vibrating Mill Palla VM-KT

Item Number	Item
1	Vibrating Body Mount
3	Clamping Yoke
12	Pressure Springs
20	Vibration Motor
100	Inlet Socket
150	Vibrating Trough Conveyor
155	Filling Socket
160	Thyristor Control
170	Mount Cover
180	Temperature Pick-up
200	Inlet & Outlet Bellow
210	Mount Bellow
220	Vibrating Trough Conveyor Inlet Bellow
300	Material Grinding Chamber
310	Material Chamber Outlet Head
320	Material Chamber & Material Chamber Outlet Junction
330	Material Chamber End Cap
400	Outlet Tube
410	Temperature Pick-up
500	Liquid Nitrogen Valve Box
510	Flexible Liquid Nitrogen Supply Line
600	Collection Basin

Table 1. Palla MT-KM Cryomill Schematic Part Numbers

Outlet Set-Up

1. Open both the large and small protective covers of the cryomill, Figure 10.

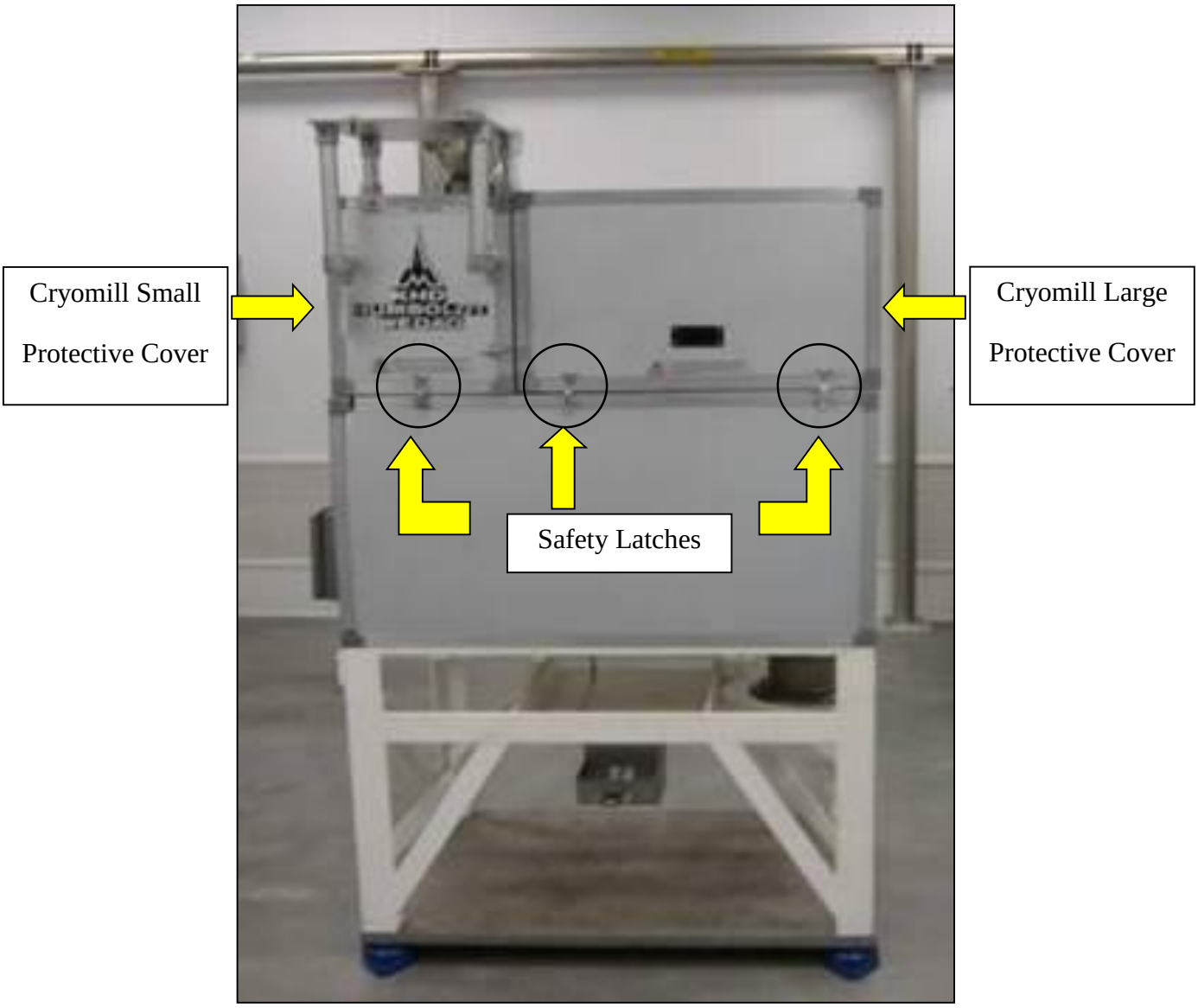


Figure 10. Palla Cryomill Large and Small Protective Covers

2. Place the clean titanium rods inside the clean material grinding chamber (300). Attach the material chamber outlet head (310) to the material grinding chamber (300) using the provided securing clamps. Tighten the nuts on the securing clamps, ensure that the 6 securing clamps are in a “star pattern” and secure the material chamber outlet head (310) to the material chamber end cap (330), Figure 11.

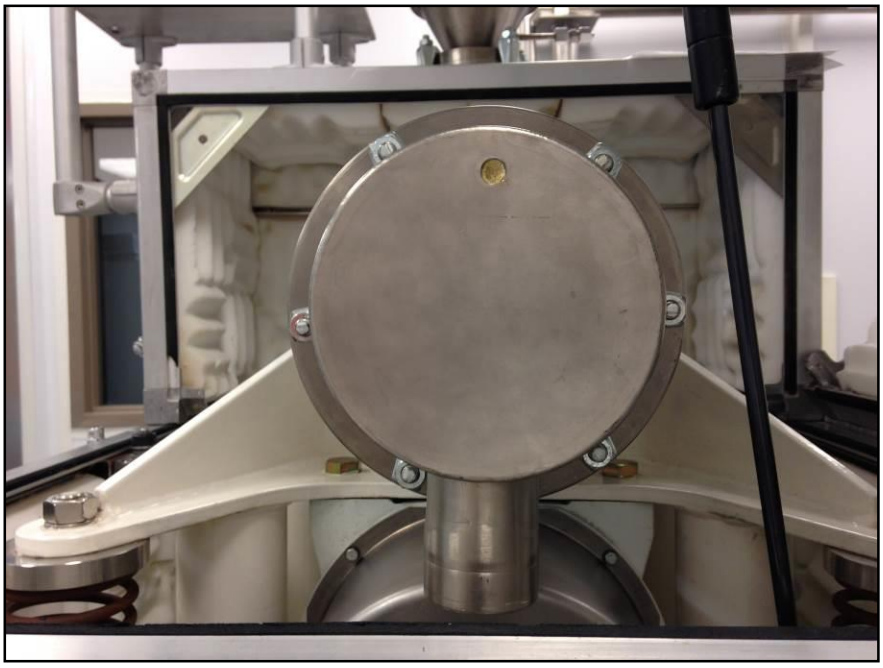


Figure 11. Outlet Head

3. Secure the outlet tube (400) to the cryomill body using the 2 securing clamps. Also, tighten the temperature pick-up (410) to the outlet tube, Figure 12.



Figure 12. Outlet Tube and Temperature Pick-up

4. Place a lower metal band onto the lower portion of the outlet bellow (200) and an upper metal band onto the upper portion of the outlet bellow. Ensure that the extra metal(s) piece of the lower and upper metal bands are facing away from the body of the bellow. Position the outlet bellow between the material chamber end-cap (330) and the outlet tube (400). You will need to compress the bellow to position it properly. Expand the bellow until it overlaps the grooves on both the material chamber outlet head (310) and the outlet tube (400). Tighten the screws on the metal bands of each bellow to secure it in place. See Figure 13and Figure 14.



Figure 13. Outlet Bellow Upper Metal Band



Figure 14. Outlet Bellow Lower Metal Band

Attach the outlet socket to the outlet tube (400) using the 4 clamps provided. Ensure that the LN_2 exhaust hose is secured to the outlet socket using the two metal bands provided,

5. Figure 15. The LN_2 exhaust hose should always be secured to the exhaust pipe installed on the cleanroom floor.

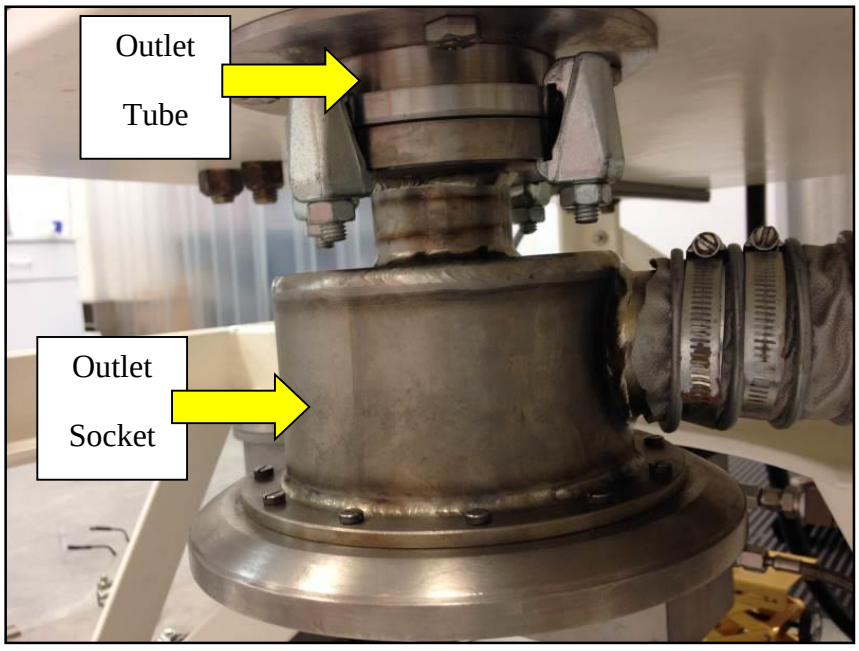


Figure 15. Outlet Socket with LN_2 Exhaust Hose

Inlet Setup

6. Attach the material chamber end cap (330) to the material grinding chamber (300) using the provided securing clamps. Tighten the nuts on the securing clamps ensuring that the 6 securing clamps are in a "star pattern" on the material chamber end cap (330) and material grinding chamber (300), Figure 16.

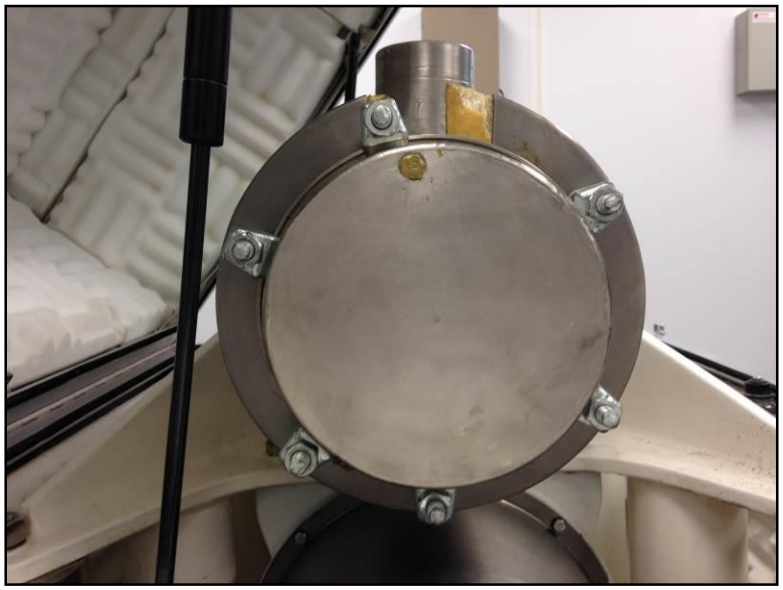


Figure 16. Material Chamber End Cap

7. Close the small protective cover of the cryomill.

8. Place a lower metal band onto the lower portion of the inlet bellow (200) and an upper metal band onto the upper portion of the inlet bellow (200). Position the inlet bellow (200) with the upper and lower metal bands loosely attached between material chamber end-cap (330) and the inlet tube. Again, ensure that the extra metal(s) piece of the lower and upper metal bands are facing away from the body of the bellow. You may need to compress the bellow to position it properly between the between material chamber end-cap (330) and the inlet tube. Expand the bellow between the material chamber end cap (330) and the inlet socket tube. Tighten the screws on the metals bands to ensure that the bellow will remain securely attached, Figure 17.



Figure 17. Inlet Bellow

9. Place the inlet socket (100) on top of the inlet tube of the cryomill. Position the LN_2 line on the inlet socket (100) so that it is pointed away from the mount platform. Secure the inlet socket to the Inlet tube using the 4 securing clamps provided, Figure 18.

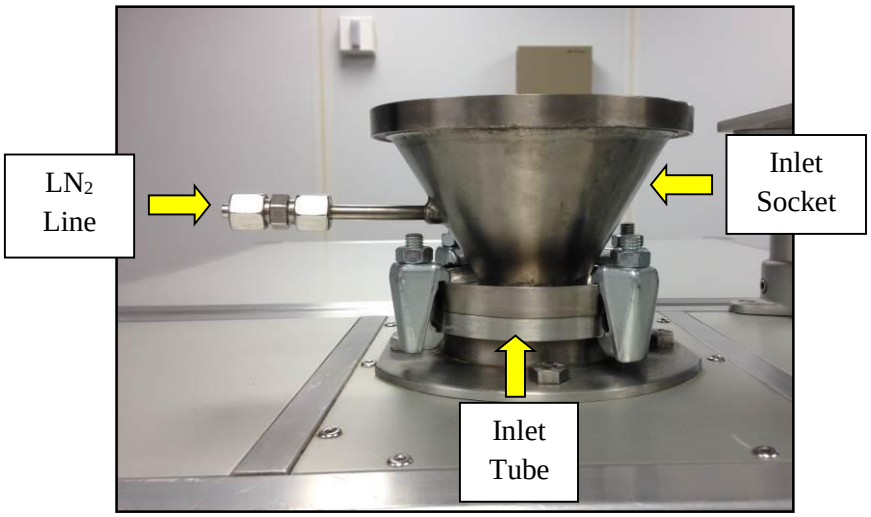


Figure 18. Inlet Socket Attached to Inlet Tube

If the vibrating trough conveyor is not being used, the initial cooling of the cryomill using LN_2 can commence at this point. In this case, proceed to the section titled, ***Cryomilling Setup without Vibrating Trough Conveyor****. If the vibrating trough conveyor is being used, proceed to step 10.*

Cryomilling Assembly with Vibrating Trough Conveyor

10. Place the inlet socket head cover on top of the inlet socket (100) and secure it in place using the 4 securing clamps, Figure 19.

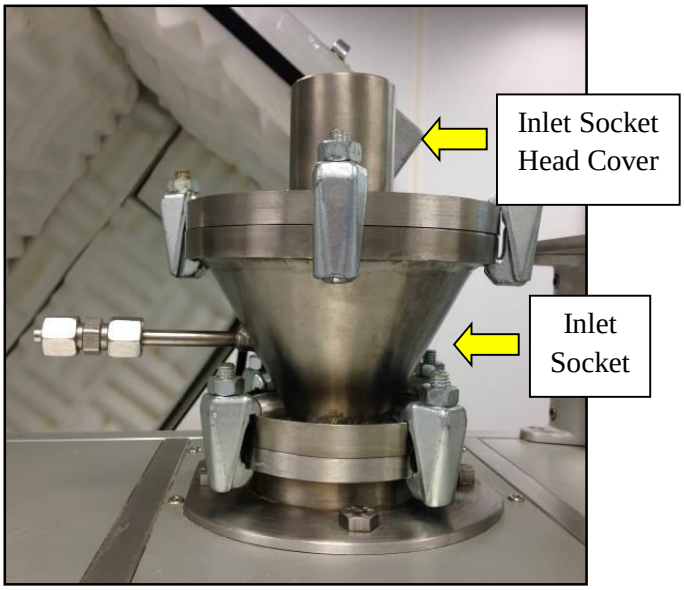


Figure 19. Inlet Socket with Inlet Socket Head Cover

11. Locate the vibrating trough conveyor (150) and secure the vibrating trough conveyor lid to the vibrating trough conveyor base using the 10 securing clamps provided. The securing clamps attach in specific places as indicated in Figure 20.

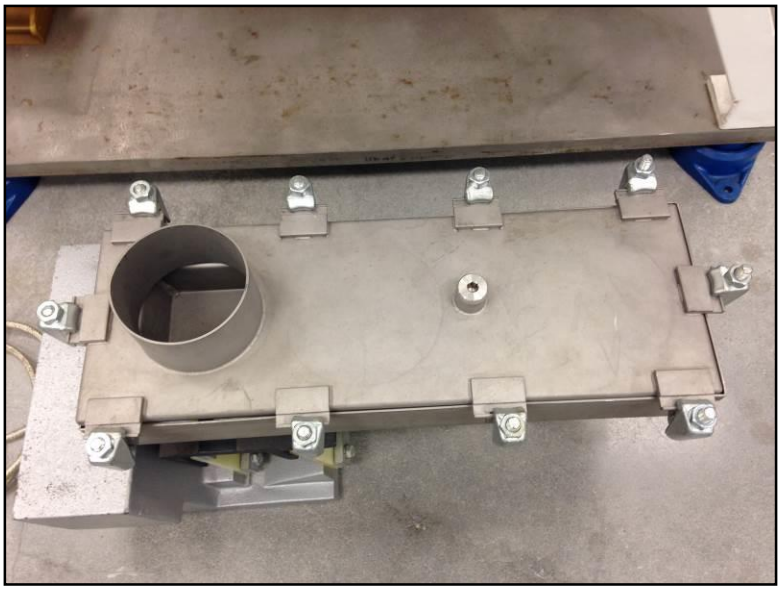


Figure 20. Vibrating Trough Conveyor

12. Attach the conveyor support to the mount platform using the 4 nuts provided, Figure 21.

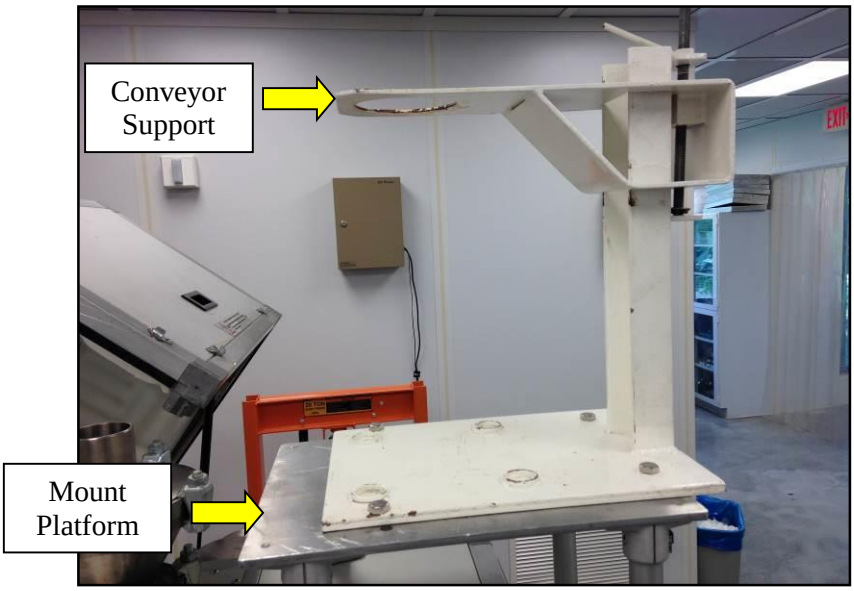


Figure 21. Conveyor Support and Mount Platform

13. Next, place the vibrating trough conveyor onto the conveyor support. **Be cautious when lifting the vibrating trough conveyor as it is very heavy!** Position the four rubber feet on the vibrating trough conveyor into the appropriate spots on the conveyor support.

14. Locate the mount bellow. Place a lower metal band on the base of the mount bellow (210) and an upper metal band on the top portion of the mount bellow (210). Ensure that the extra metal piece(s) of the metal band is not facing the bellow body. Position the mount bellow (210), with the metal bands attached, between vibrating trough conveyor (150) and the inlet socket head. Tighten the screws on the upper and lower metal bands of the mount bellow (210) to secure it in place, Figure 22.

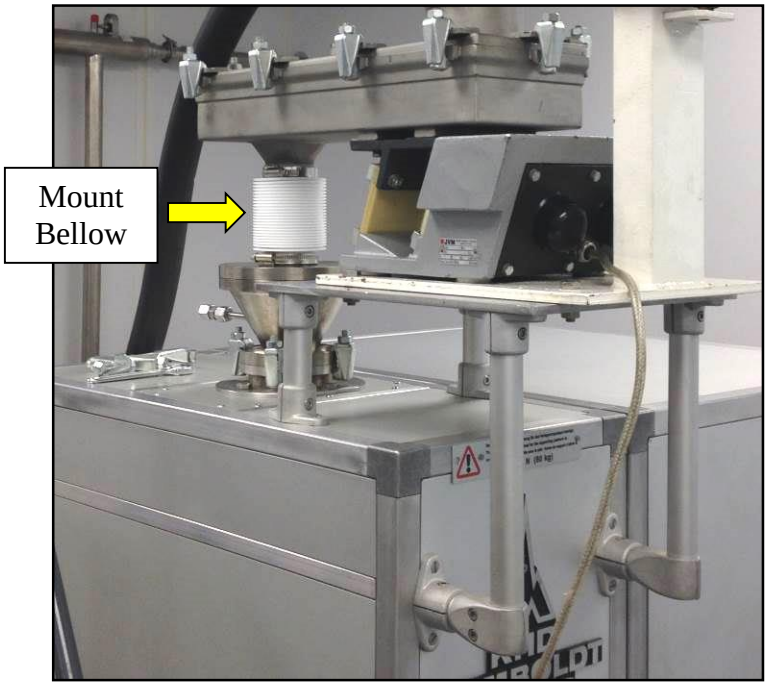


Figure 22. Mount Bellow Attachment

15. Locate the vibrating trough conveyor inlet bellow (220), Figure 23. Place an upper and lower metal band onto the vibrating trough conveyor inlet bellow (220). Ensure that the extra metal piece on the metal bands is not facing the bellow body. Place this vibrating trough conveyor inlet bellow (220), onto the large opening at the top of the vibrating trough conveyor. Do not tighten the metal bands yet; they will be tightened in a later step.

16. Position the filling socket (155) into the large bellow opening. The filling socket will rest on the conveyor support. There should be a gap between the base of the filling socket (155) and bottom of the vibrating trough conveyor (150). This gap allows material to flow from the filling socket (155) through the vibrating trough conveyor (150) and into the material grinding chamber (300). If a gap is not present, turn the knob on the conveyor support to increase the gap. Expand the vibrating trough conveyor inlet bellow (220) sufficiently between the filling socket and the vibrating trough conveyor. Tighten the metal bands on the vibrating trough conveyor inlet bellow (220) to ensure that the bellow will remain securely attached.

17. Place the mount cover with the flexible LN_2 supply line (170 and 510) onto the filling socket (155). Loosely secure this assembly onto the filling socket using 2-3 securing clamps, Figure 23.

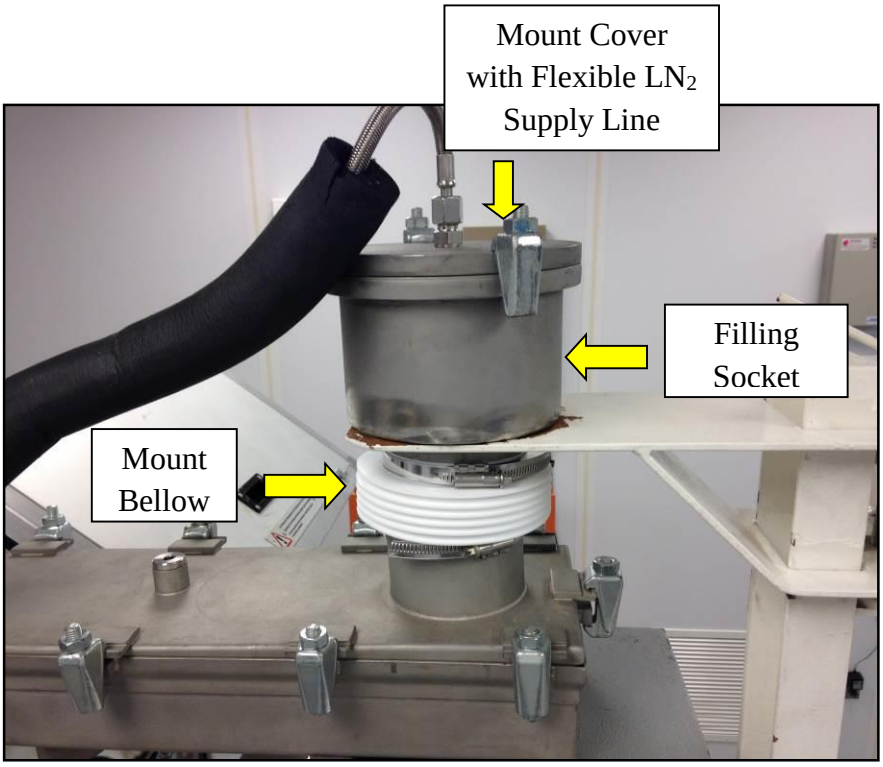


Figure 23. Flexible LN_2 Supply Line Attached to Filling Socket

18. Check that all securing clamps on the cryomill are tightened.

19. Close the large protective cover and secure the safety latches on both the large and small protective covers, Figure 10 . When the large protective cover is closed, the cryomill safety button is depressed allowing the cryomill to be operational, Figure 24. The cryomill will not operate with the large protective cover open.



Figure 24. Cryomill Safety Button

20. Plug the electrical line from the vibrating trough conveyor (150) into the thyristor control (160) attached to the outside of the cryomill. The thyristor control (160) controls the vibration speed of the trough conveyor which controls the material flowing into the material grinding chamber (300), Figure 25.



Figure 25. Thyristor Control

21. The cryomill is now assembled *with* the vibrating trough conveyor (150) and ready to be cooled using LN_2.

Cryomilling Assembly without Vibrating Trough Conveyor

Depending on the material size to be cryohomogenized, the vibrating trough conveyor (150) may not be required for use. In the event that the vibrating trough conveyor (150) is not being used to distribute material into the cryomill and the material will be manually added to the material grinding chamber (300), the following steps should be performed.

1. Secure the inlet socket (100) to the inlet tube using the 4 securing clamps provided.
2. Place the flexible LN_2 supply line attached to the mount cover (510 and 170) onto the inlet socket (100). The cryomill is now assembled without the vibrating trough conveyor (150) and ready to be cooled using LN_2, Figure 26.



Figure 26. Flexible LN_2 Supply Line Attached to Inlet Socket

Preparing Material and Supplies

Material chosen for cryogenic homogenization utilizing the cryomill must be frozen before cryogenic homogenization. The frozen material must then be crushed into <2.5 cm diameter pieces in order to flow uniformly into the material grinding chamber (300) of the cryomill.

Additionally, collection basins are needed and should be pre-cooled prior to being used to collect homogenized material as it flows from the outlet head of the cryomill. Collection basins may be stainless steel buckets, stainless steel baskets lined with Teflon bags, or plastic basins. Depending on the material being cryohomogenized and the type of analysis being conducted, it is advantageous to have several collection basins available for use to eliminate certain contamination issues.

Palla Cryomill Operation

After the cryomill is successfully assembled, it is cooled using the LN_2 supply from the NIST LN_2 bulk tank. Below are steps to begin cooling and operating the cryomill.

1. Ensure that all nuts and securing clamps are tightened. Close both cryomill protective covers and secure the safety latches. There are three safety latch points that must be secured before proceeding with the cryomill process, Figure 10.

2. The LN_2 control valve box (500) controls the flow rate of LN_2 into the cryomill, Figure 27. Ensure that the black knob is turned to the right (closed) prior to operation and during set-up.



Figure 27. LN_2 Control Valve Box

3. Place the flexible LN_2 supply line attached to the mount cover (170 and 510) onto the desired resting place, either the filling socket (155) or the inlet socket (100). In the event that the vibrating trough conveyor is being used, secure the LN_2 hose to the filling socket (155), Figure 23. In the event that the vibrating trough conveyor is *not* being used, secure the LN_2 hose to the inlet socket (100), Figure 26.

4. Turn the LN_2 supply on by turning the knob that is closest to the floor on the house piping system, Figure 28. Opening this valve allows LN_2 to flow to the LN_2 control box.

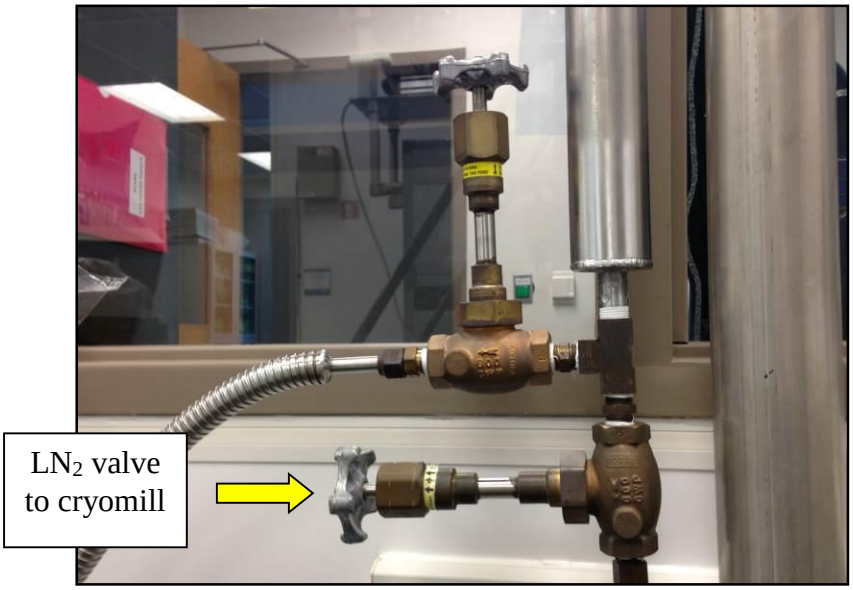


Figure 28. LN_2 House Supply Valve

5. Next, turn the black knob on the LN_2 valve box (500) at least 5 rotations counterclockwise, Figure 27. This allows LN_2 to flow from the LN_2 control box, through the LN_2 supply line, and into the cryomill.

6. Next, on the electrical control panel, turn the power to the cryomill ON. In Figure 29, turn the large red knob clockwise so the "I" is visible in the knob view window. It will be necessary to wait several seconds for the control panel to run machine diagnostics.



Figure 29. Cryomill Electrical Control Panel

7. Place a collection receptacle at the base of the cryomill underneath the outlet socket to catch any residual LN_2 that may flow out once LN_2 is flowing into the cryomill. Any residual LN_2 vapor will be exhausted through the exhaust hose.

8. Turn the nitrogen release key to "I". This key starts, and stops, the flow of LN_2 into the cryomill. Liquid nitrogen should begin to flow from house supply line, to the LN_2 valve box (500), through the flexible LN_2 supply line (510), then through the material grinding chamber (300). If LN_2 does not begin to flow, make sure that the house LN_2 valve, Figure 28, is open and the flow rate valve on the LN_2 control valve box (500), Figure 27, is opened.

9. A preprogrammed temperature setting for LN_2 (-190 °C) has been set using the electrical control panel temperature settings. To program a different set temperature, see the Palla cryomill technical documentation for instruction.

10. Allow several hours for the cryomill to reach the desired pre-set temperature. Upon reaching the desired temperature, the LN_2 control valve box will begin "clicking." This clicking is an LN_2 valve in the LN_2 control box opening and closing controlling the flow of LN_2 into the cryomill, thereby maintaining the set temperature of -190 °C.

11. After the desired temperature has been reached and maintained for at least 30 minutes, the LN_2 key can be turned off to the "O" position.

12. With the LN_2 key in the off position, depress the white button labeled "unbalance motor on." The vibration motor (20) will start and the titanium rods will begin rotating. Allow the motor to run for 20 to 30 seconds. The temperature inside of the material grinding chamber (300) will dramatically increase, or warm. The increase in temperature is due to the titanium rods initial rotation. **CAUTION**- This step causes the titanium rods inside the material grinding chamber (300) to rotate and without material flowing through, is very loud. Participants in the immediate cryomilling vicinity should wear ear/hearing protection.

    NOTE - If the vibration motor does not start, make sure that the large and small protective covers are closed.

13. Depress the red button labeled "unbalance motor off." The vibration motor will stop, therefore stopping the titanium rods from moving.

14. Turn the nitrogen release key to "I" to begin the flow of LN_2 until the desired temperature is reached again. After reaching desired temperature a second time, the cryomill is ready to be used.

Cryohomogenizing Material

After the cryomill has reached and maintained the desired temperature, the pre-frozen material can be distributed into the material grinding chamber (300). The following steps describe how to distribute material into the cryomill *with* and *without* the vibrating trough conveyor (150).

Cryomilling with the Vibrating Trough Conveyor

1. Ensure the LN_2 key is in the off position and remove the mount cover (170) with the flexible LN2 supply line (510) from the filling socket (155) and place it below the cryomill base out of the way. Place a pre-cooled collection basin (600) below the outlet socket to collect cryohomogenized material as it flows out of the cryomill.

2. Depress the white button labeled "unbalance motor on." The vibration motor (20) will start and the titanium rods will begin rotating.

3. Turn the black knob on the thyristor control (160) to the desired vibration speed.

4. While standing at the top of the 4-step safety ladder, begin evenly and continuously distributing material into the filling socket (155), Figure 30, using a stainless steel scoop. The material will flow through the vibrating trough conveyor, through the material grinding chamber (300), and out through the outlet head, Figure 31.

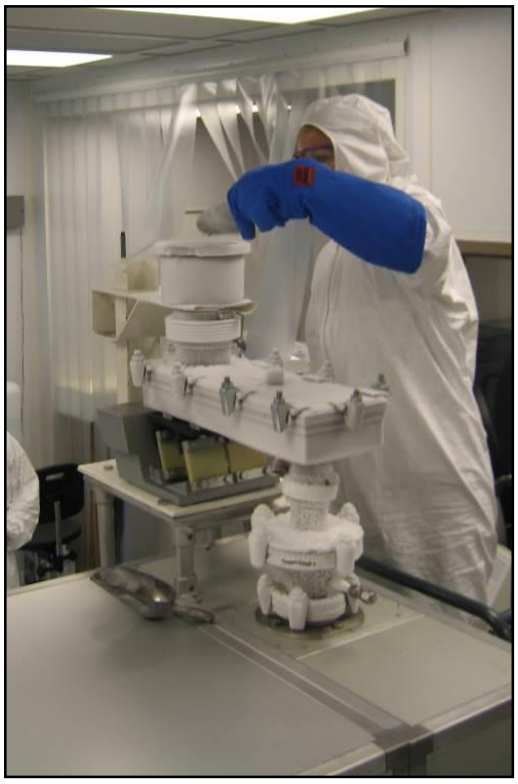


Figure 30. Cryomilling with the Vibrating Trough Conveyor



Figure 31. Cryohomogenized Material Flowing from the Outlet Head

5. Once a collection basin is full of frozen cryohomogenized material, it should be placed quickly into either an LN_2 freezer, or the LN_2 cryocart, and a new collection basin placed under the outlet head in its place to continue catching material.

6. Repeat step 4 until all material has passed through the cryomill, or the cryomill temperature reaches -80°C. See Figure 32.

7. When the cryomill reaches -80°C, it will be necessary to depress the "unbalance motor off" and cool the cryomill again.

8. Place the mount cover (170) onto the filling socket and secure it using a securing clamp.

9. Turn the LN_2 key to the on position to allow LN_2 to begin flowing through the cryomill again. Allow the LN_2 to flow until the desired temperature is reached again. Repeat any necessary steps to complete homogenization. It is not recommended to have LN_2 flowing into the cryomill while the vibration motor is on.

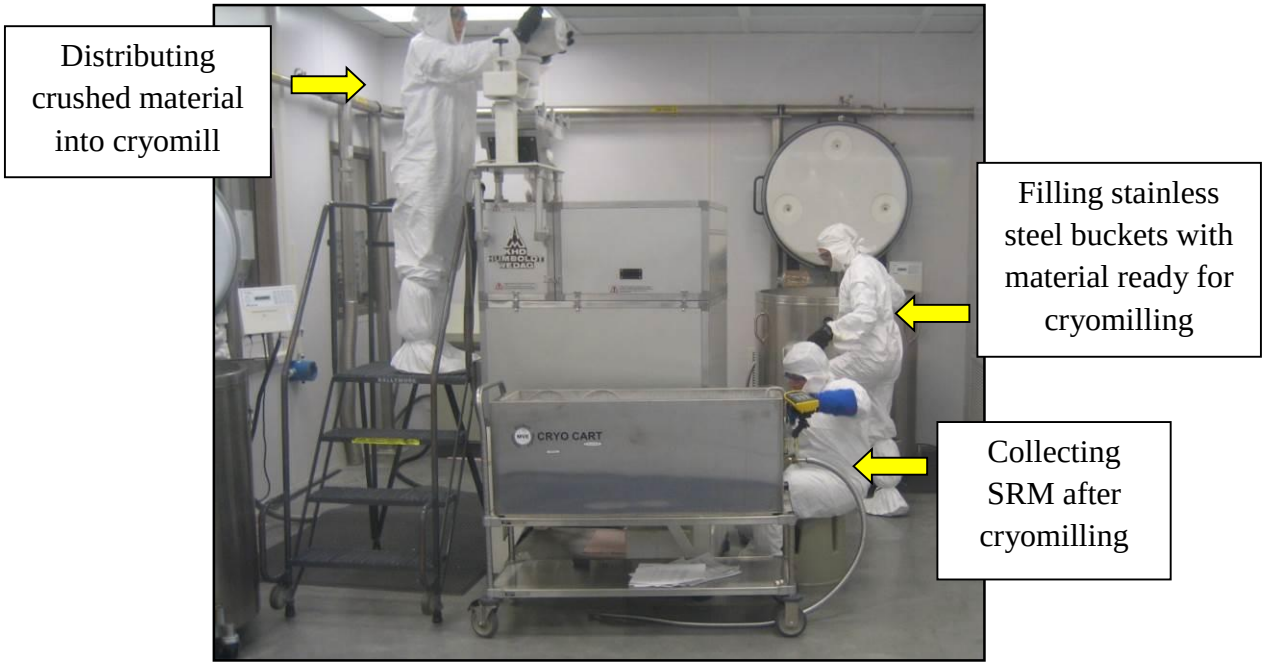


Figure 32. Cryomilling material with the Vibrating Trough Conveyor

Cryomilling without the Vibrating Trough Conveyor

1. With the LN_2 key in the off position, remove the mount cover (170) with the flexible LN_2 supply line (510) from the inlet socket (100) and place it below the cryomill base out of the way. Place a pre-cooled collection basin (600) below the outlet socket to collect cryohomogenized material as it flows out of the cryomill.

2. Depress the white button labeled "unbalance motor on." The vibration motor will start and the titanium rods will begin rotating.

3. While standing at the top of the 4-step safety ladder, begin to continuously distribute material into the Inlet Socket (100), Figure 33, using a stainless steel scoop. The material will flow directly into the material grinding chamber (300) and out through the outlet head, Figure 31.

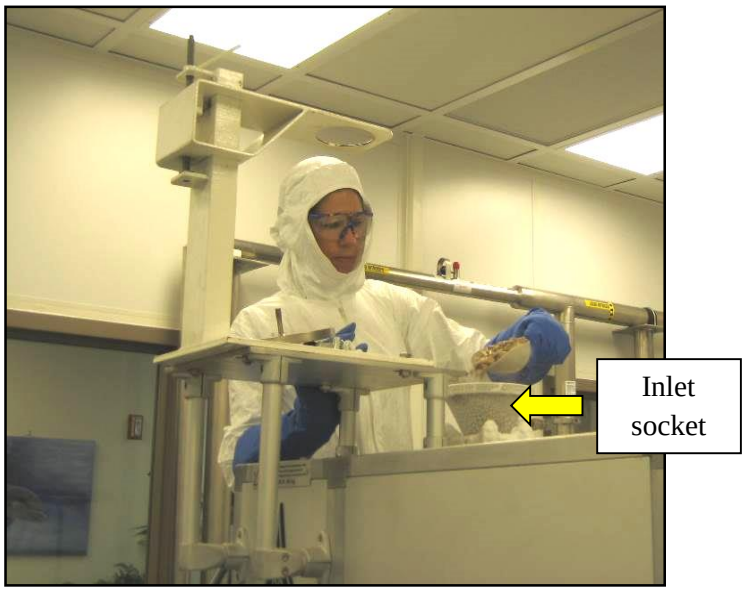


Figure 33. Cryomilling without the Vibrating Trough Conveyor

4. Once a collection basin is full of frozen powder material, it should be placed quickly into either an LN_2 freezer or the LN_2 cryocart and a new collection basin placed under the outlet head in its place to continue catching material.

5. Repeat step 3 until all material has passed through the cryomill, or the cryomill temperature reaches -80 °C.

6. When the cryomill reaches -80 °C, it will be necessary to depress the "unbalance motor off" and cool the cryomill again.

7. Place the mount cover (170) onto the inlet socket and secure it using a securing clamp, Figure 26.

8. Turn the LN_2 key to the on position to allow LN_2 to begin flowing through the cryomill again. Allow the LN_2 to flow until the desired temperature is reached again. Repeat any necessary steps to complete homogenization. It is not recommended to have LN_2 flowing into the cryomill while the vibration motor is on.

Particle Size Measurement

Once cryomilling has been completed, the particle size distribution of the material is measured using the Malvern Mastersizer 3000. The instrument uses laser diffraction to determine the sizes of particles within the range of 0.01 to 3500 µm in diameter. As particles pass through the instrument, scatter produced by two lasers gives the measure of particle size. See ROA 646.06-14-309 for additional instructions.

Disassembling, Thawing, and Clean-up Procedures

Upon completion of cryomilling, all parts of the cryomill and RM Production facility must be thoroughly cleaned following the steps below, as well as the cleanroom maintenance steps described in NISTIR 7389.

1. Turn the cryomill off by depressing the red button labeled "unbalance motor OFF." The motor and titanium rod movement will stop.

2. Turn the LN_2 house supply valve off, Figure 28. Turn the LN_2 control valve box knob , Figure 27, clockwise to close the valve and stop any additional flow of LN_2 into the cryomill.

3. Open the large protective cover of the cryomill to allow thawing to begin. Using a socket wrench, loosen the nuts on the securing clamps attached to the outlet head. The nuts on the securing clamps may still be frozen and may take several minutes to thaw before they can be loosened.

4. Meanwhile, the inlet set-up may be broken down. All pieces secured to the top of the small protective cover must be removed before the cover can be opened.

5. Remove the mount cover with flexible LN_2 supply line (170 and 510) from either the filling socket (155) or the inlet socket (100), and place it on the floor. Disassemble the remainder of the cryomill inlet set-up, place all items in the sink, and thoroughly rinse items with warm water. After rinsing, wipe the items with Deconahol and a Texwipe, and place the cleaned cryomill parts aside to dry on absorbent mats.

6. After all parts, including the conveyor support, have been detached from the small protective cover, open the protective cover to thaw and clean the material grinding chamber (300). Do not remove the mount platform from the small protective cover; it is permanently attached, Figure 21.

7. Loosen and remove thawed securing clamps located on the material chamber end cap (330) and outlet head and rinse if necessary. Remove titanium rods from the material grinding chamber (300) and rinse with warm water. Thaw and clean the material grinding chamber (300) with warm water. After drying, wipe the material grinding chamber (300) with a Texwipe and Deconahol.

8. Follow the Labconco Steam Scrubber (steam scrubber) steps below to clean items using the steam scrubber.

Labconco Steam Scrubber

Some cryomill pieces are best cleaned using the steam scrubber, Figure 34. These pieces include: titanium rods, Teflon bellows, stainless steel collection buckets, and stainless steel scoops. These items must be thoroughly rinsed with warm water before cleaning in the steam scrubber.

Do <u>not</u> clean any insulated parts from the cryomill using the steam scrubber: filling socket, inlet socket, end cap, outlet head, temperature pick-up, vibrating trough conveyor, securing clamps, outlet tube, and/or outlet socket. Instead, clean the above listed insulated items using warm water and Deconahol as previously described.



Figure 34. Labconco Steam Scrubber

1. Roll the steam scrubber to the sink located in the RM Production facility.
2. Attach the distilled (DI) water line from the steam scrubber to the HML sink DI water faucet, and attach the tap water line from the steam scrubber to the HML sink tap water line, see Figure 35 and Figure 36.

3. Place all drain lines inside the sink then plug the steam scrubber electrical cord into an electrical outlet

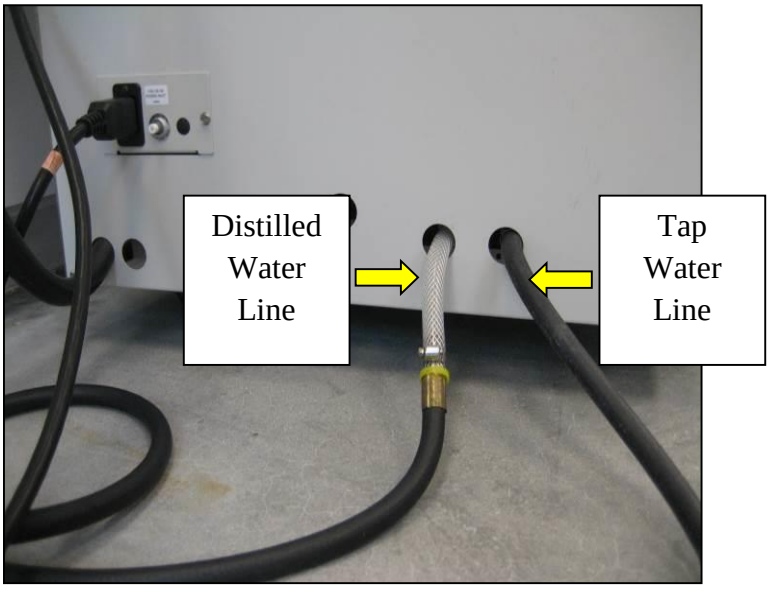


Figure 35. Labconco Steam Scrubber Distilled and Tap Water Lines

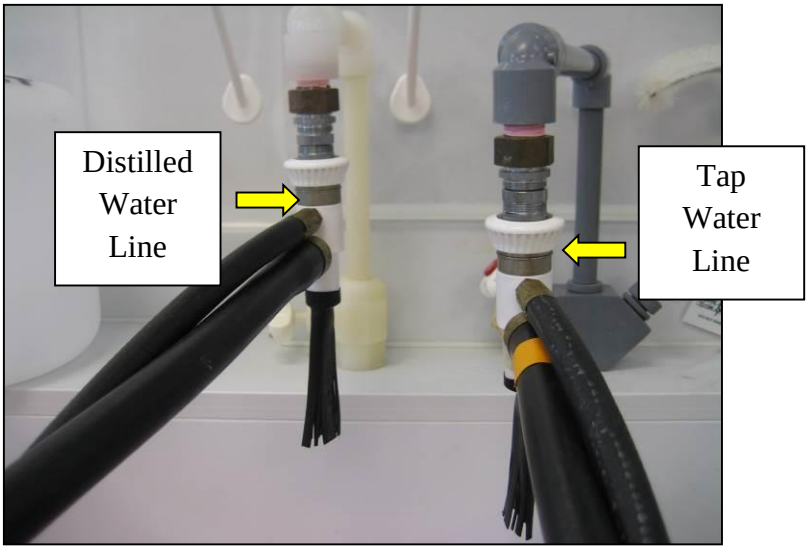


Figure 36. HML Distilled and Tap Water Lines

4. Load the cryomill parts inside the steam scrubber, add LabSolutions detergent to the detergent cup, and LabSolutions neutralizing acid rinse to the neutralizing solutions port, Figure 37. Close both the detergent cup and neutralizing acid rinse port. Safety Data Sheets (SDS) for the LabSolutions detergent and neutralizing acid rinse are available in Appendix A.

5. Gently close and latch the steam scrubber door, but **do not force** the door closed. Items may need to be removed or rearranged in order for the steam scrubber door to close. Once the door is securely closed, the Science Plus program will appear on the steam scrubber screen (Figure 38). Turn on the DI and hot tap water from the sink knobs. Press "Run/Cancel" button to start the wash cycle.

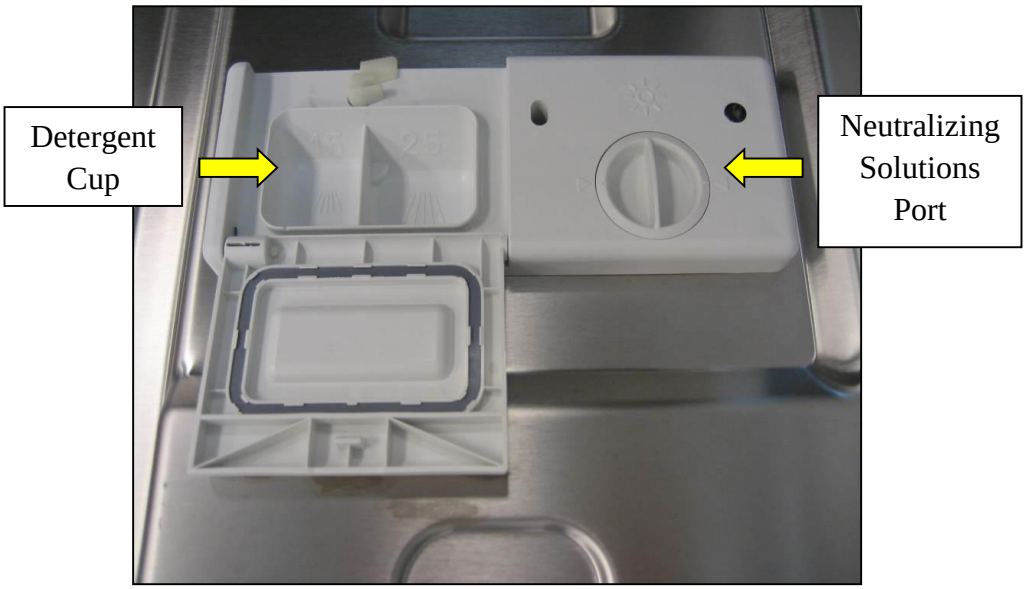


Figure 37. Labconco Steam Scrubber Detergent Cup and Neutralizing Solutions Port



Figure 38. Labconco Steam Scrubber Display Screen

Cryomilling Overview

1. Frozen material must be crushed into <2.5 cm in diameter pieces in order to fit into the cryomill.
2. The vibrating trough conveyor (150) can be attached to the cryomill to ensure that the material uniformly enters the cryomill. Or, material can be distributed directly into the material grinding chamber (300).
3. A vacuum jacketed, insulated LN_2 hose (510), which is connected on one end to the LN_2 piping system in the Marine ESB, is tightly attached to the cryomill. After reaching the desired cryomilling temperature, the LN_2 is turned off.
4. The cryomill vibration motor (20) is turned on and the mill begins vibrating and rotating the titanium rods located within the material grinding chamber (300).
5. Material is distributed into the material grinding chamber (300) through the vibrating trough conveyor (150), or the inlet socket (100) for cryohomogenization.
6. The material travels through the material grinding chamber (300) where the vibrating titanium rods pulverize the material into powder.
7. The cryohomogenized material falls from the outlet tube (400) at the bottom of the machine and is collected in pre-frozen containers which in turn are quickly placed in an LN_2 freezer when full.
8. The cryohomogenized material is passed through the cryomill as many times as necessary to ensure homogeneity. A particle size analysis should be conducted to verify homogeneity.
9. The cryomill, cryomill parts, and RM production facility are cleaned.
10. Upon completion, the cryohomogenized material can be bottled, or if necessary, packaged for shipment to the NIST Reference Material Facility in Gaithersburg, MD, for further processing.

References

Pugh, R.S., Ellisor, M.B., Moors, A.J., Porter, B.J., and Becker, P.R. Marine Environmental Specimen Bank: Clean Room and Specimen Bank Protocols. NISTIR 7389, National Institute of Standards and Technology. 2006, 46 pp.

Palla VM-KT Cryomill Technical Documentation

Ness, Jennifer M., Standard Operating Procedure for Particle Size Characterization of Seabird Egg Homogenate NIST ROA 646.06-14-309

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Appendix A

Hollings Marine Laboratory Marine ESB Job Hazard Analysis

Hollings Marine Laboratory Marine ESB Job Hazard Analysis

JOB HAZARD ANALYSIS
Hollings Marine Laboratory

Program: **National Institute of Standards and Technology**

Area Supervisor: **Paul Becker**

Room number: **C102-C105**

I. Work Activities: *(list below)*

1) Samples will be cryogenically homogenized inside of liquid nitrogen (LN_2) vapor-phase freezers
2) Cleanup of Teflon and titanium materials using chemicals (acids and solvents)
3) Processing and sub-sampling of marine animal tissues
4) Marine animal tissues will be freeze dried
5) Samples will be cryogenically homogenized using a large capacity cryomill

II. Listing of protective equipment that may be required for these tasks:

Equipment	Applicable Work Activity Number
a. Closed Toe Shoes	1, 2, 3, 4, 5
b. Lab Coat	
c. Safety Goggles	1, 2, 3, 4, and 5
d. Gloves	1, 2, 3, 4, and 5 (1 and 5 require thermal gloves)
e. Fume Hood	2
f. Apron	
g. Dust Mask	
h. Radiation Badge	
i. Face Shield	
j. Hearing Protection	
k. Other: Coverall w/ hood, booties	1, 2, 3, 4, and 5
l. Other: Oxygen Monitor*	1, 2, 3, 4, and 5
m. Other: N-95 Mask	1, 3, 4, and 5 (when handling marine mammal tissues)

* May be required

III. Location of the nearest emergency equipment for this area:

1. Fire: Fire pull alarm is near the exterior door in hallway, near room **inside Room C103** .
   Fire extinguisher mounted on the wall near door to room **C108.**
2. Eyewash and safety shower: Eyewash is in the sink in room **C103** .
   Shower is in room **outside Room C106**.
3. SDS sheets are available online at: http://hq.msdsonline.com/noaa/Search/Default.aspx (NO ID or PASSWORD required) and on the wall in the ante-room , Room **C102**.
4. Spill Kit and First Aid Kit is in Room **C102**.

This analysis has been identified for the activities in the area by:

Supervisor: _____ Date 5/13/2014

Area Safety Representative: _____ Date 5/13/2014
R. Mueller 6-30-14

I have been informed of the possible hazards in this work area and of the Personal Protective Equipment that is required. **Before undertaking any new task which does not appear to be specifically addressed by this hazard analysis, I will seek guidance from my immediate supervisor.**

Signed: _____ Date 5/13/2014
(Person Working in the Laboratory)

Signed: _____ Date 5/13/2014
(Person Working in the Laboratory)

Signed: _____ Date 5/13/2014
(Person Working in the Laboratory)

Signed: _____ Date 5/21/14
(Person Working in the Laboratory)

Signed: _____ Date 5-21-14
(Person Working in the Laboratory)

Signed: _____ Date 5/21/14
(Person Working in the Laboratory)

Signed: _____ Date 6/17/14
(Person Working in the Laboratory)

Signed: _____ Date 6/17/14
(Person Working in the Laboratory)

8/29/14

For questions concerning completion of this form, call Martin Burnett @ 762-8808.

Appendix B

Liquid Nitrogen (LN_2) Safety Data Sheet

LabSolutions Detergent Safety Data Sheet

LabSolutions Neutralizing Acid Rinse Safety Data Sheet

BOC GASES

MATERIAL SAFETY DATA SHEET

PRODUCT NAME: NITROGEN, REFRIGERATED LIQUID

1. Chemical Product and Company Identification

BOC Gases,
Division of
The BOC Group, Inc.
575 Mountain Avenue
Murray Hill, NJ 07974

TELEPHONE NUMBER: (908) 464-8100
24-HOUR EMERGENCY TELEPHONE NUMBER:
CHEMTREC (800) 424-9300

BOC Gases
Division of
BOC Canada Limited
5975 Falbourne Street, Unit 2
Mississauga, Ontario L5R 3W6

TELEPHONE NUMBER: (905) 501-1700
24-HOUR EMERGENCY TELEPHONE NUMBER:
(905) 501-0802
EMERGENCY RESPONSE PLAN NO: 20101

PRODUCT NAME: NITROGEN, REFRIGERATED LIQUID
CHEMICAL NAME: Nitrogen
COMMON NAMES/SYNONYMS: Nitrogen, liquid
TDG (Canada) CLASSIFICATION: 2.2
WHMIS CLASSIFICATION: A

PREPARED BY: Loss Control (908)464-8100/(905)501-1700
PREPARATION DATE: 6/1/95
REVIEW DATE: 6/7/96

2. Composition, Information on Ingredients

INGREDIENT	% VOLUME	PEL-OSHA[1]	TLV-ACGIH[2]	LD_{50} or LC_{50} Route/Species
Nitrogen FORMULA: N_2 CAS: 7727-37-9 RTECS #: QW9700000	99.995 to 99.999	Simple Asphyxiant	Simple Asphyxiant	Not Available

[1] As stated in 29 CFR 1910, Subpart Z (revised July 1, 1993)

[2] As stated in the ACGIH 1994-95 Threshold Limit Values for Chemical Substances and Physical Agents

3. Hazards Identification

EMERGENCY OVERVIEW

Simple Asphyxiant - This product does not contain oxygen and may cause asphyxia if released in a confined area. Maintain oxygen levels above 19.5%. Contact with product may cause frostbite or freeze burns in exposed tissues. Nonflammable.

ROUTE OF ENTRY:

Skin Contact	Skin Absorption	Eye Contact	Inhalation	Ingestion
Yes	No	Yes	Yes	No

HEALTH EFFECTS:

Exposure Limits	Irritant	Sensitization
No	No	No
Teratogen	Reproductive Hazard	Mutagen
No	No	No
Synergistic Effects None reported		

Carcinogenicity: -- NTP: No IARC: No OSHA: No

EYE EFFECTS:
Contact with evaporating liquid may cause tissue freezing.

SKIN EFFECTS:
Contact with rapidly evaporating liquid can cause cryogenic "burns" or frostbite. Frostbite effects are a change in color of the skin to gray or white, possibly followed by blistering.

INGESTION EFFECTS:
Ingestion is unlikely. Contact with product may cause tissue freezing.

INHALATION EFFECTS:
Product is a non-toxic simple asphyxiant. Effects of oxygen deficiency resulting from simple asphyxiants may include: rapid breathing, diminished mental alertness, impaired muscular coordination, faulty judgement, depression of all sensations, emotional instability, and fatigue. As asphyxiation progresses, nausea, vomiting, prostration, and loss of consciousness may result, eventually leading to convulsions, coma, and death.

Oxygen deficiency during pregnancy has produced developmental abnormalities in humans and experimental animals.

NFPA HAZARD CODES	HMIS HAZARD CODES	RATINGS SYSTEM
Health: 3	Health: 3	0 = No Hazard
Flammability: 0	Flammability: 0	1 = Slight Hazard
Reactivity: 0	Reactivity: 0	2 = Moderate Hazard
		3 = Serious Hazard
		4 = Severe Hazard

4. First Aid Measures

EYES:
Never introduce ointment or oil into the eyes without medical advice! In case of freezing or cryogenic "burns" caused by rapidly evaporating liquid, DO NOT WASH THE EYES WITH HOT OR EVEN TEPID WATER! Remove victim from the source of contamination. Open eyelids wide to allow liquid to evaporate. If pain is present, refer the victim to an ophthalmologist for treatment and follow up. If the victim cannot tolerate light, protect the eyes with a light bandage.

SKIN:
For dermal contact or frostbite: Remove contaminated clothing and flush affected areas with lukewarm water. DO NOT USE HOT WATER. A physician should see the patient promptly if the cryogenic "burn" has resulted in blistering of the dermal surface or deep tissue freezing.

INGESTION:
A physician should see the patient promptly if the cryogenic "burn" has resulted in blistering of the dermal surface or deep tissue freezing.

INHALATION:
PROMPT MEDICAL ATTENTION IS MANDATORY IN ALL CASES OF OVEREXPOSURE. RESCUE PERSONNEL SHOULD BE EQUIPPED WITH SELF-CONTAINED BREATHING APPARATUS. Victims should be assisted to an uncontaminated area and inhale fresh air. Quick removal from the contaminated area is most important. Unconscious persons should be moved to an uncontaminated area, and if breathing has stopped, administer artificial resuscitation and supplemental oxygen. Further treatment should be symptomatic and supportive.

5. Fire Fighting Measures

Conditions of Flammability: Nonflammable		
Flash point: None	Method: Not Applicable	Autoignition Temperature: None
LEL(%): None	UEL(%): None	
Hazardous combustion products: None		
Sensitivity to mechanical shock: None		
Sensitivity to static discharge: None		

FIRE AND EXPLOSION HAZARDS:
None. Nonflammable

EXTINGUISHING MEDIA:
None required. Use as appropriate for surrounding materials.

6. Accidental Release Measures

Evacuate all personnel from affected area. Use appropriate protective equipment. If leak is in user's equipment, be certain to purge piping with inert gas prior to attempting repairs. If leak is in container or container valve, contact the appropriate emergency telephone number listed in Section 1 or call your closest BOC location.

7. Handling and Storage

Use only in well-ventilated areas in accordance with manufacturer's and BOC instructions. These cylinders must ALWAYS be kept upright. Specialized trucks are needed for their movement. Do not drag, slide or roll cylinders. Stationary customer site vessels should be operated in accordance with the manufacturer's and BOC's instructions. Do not attempt to repair, adjust or in any other way modify the operation of these vessels. If there is a malfunction or other type of operations problem with the vessel, contact the closest BOC location immediately for assistance.
Liquid nitrogen is delivered into stationary vacuum jacketed vessels at the customer's location or in portable vacuum-jacketed "liquid" cylinders requiring special handling methods. Consult manufacturer's instructions.

Due to the extremely cold liquid, uninsulated transfer lines may condense air. The liquefied air may flash of nitrogen, leaving an oxygen enriched liquid. Do not allow the liquefied air to contact oils, greases, or other combustible materials such as asphalt and motor oil.

Vessels for liquid nitrogen are designed specifically for nitrogen service. Vessels and associated structures are not designed to support higher density fluids. Density, liquid at saturation pressure at 2.17 °K (-271 °C) : 0.146 Kg/l.

For additional recommendations, consult Compressed Gas Association Pamphlets P-9, P-9.1, P-12, P-14 and Safety Bulletin SB-2.

Never carry a compressed gas cylinder or a container of a gas in cryogenic liquid form in an enclosed space such as a car trunk, van or station wagon. A leak can result in a fire, explosion, asphyxiation or a toxic exposure.

8. Exposure Controls, Personal Protection

EXPOSURE LIMITS[1]:

INGREDIENT	% VOLUME	PEL-OSHA[2]	TLV-ACGIH[3]	LD_{50} or OC_{50} Route/Species
Nitrogen FORMULA: N2 CAS: 7727-37-9 RTECS #: QW9700000	99.995 to 99.999	Simple Asphyxiant	Simple Asphyxiant	Not Available

[1] Refer to individual state of provincial regulations, as applicable, for limits which may be more stringent than those listed here.
[2] As stated in 29 CFR 1910, Subpart Z (revised July 1, 1993)
[3] As stated in the ACGIH 1994-1995 Threshold Limit Values for Chemical Substances and Physical Agents.

ENGINEERING CONTROLS:
Local exhaust to prevent accumulation of high concentrations so as to reduce the oxygen level in the air to less than 19.5%.

EYE/FACE PROTECTION:
Safety goggles or glasses as appropriate for the job. Faceshield recommended when handling cryogenic liquid material.

SKIN PROTECTION:
Protective gloves of material appropriate for the job. Insulated gloves recommended when handling cryogenic liquid material.

RESPIRATORY PROTECTION:
Positive pressure air line with full-face mask and escape bottle or self-contained breathing apparatus should be available for emergency use.

OTHER/GENERAL PROTECTION:
Safety shoes or other footwear as appropriate for the job.

9. Physical and Chemical Properties

PARAMETER	VALUE	UNITS
Physical state (gas, liquid, solid)	: Gas	
Vapor pressure	: Above critical temp.	
Vapor density (Air = 1)	: 0.967	
Evaporation point	: Not Available	
Boiling point	: -320.4	°F
	: -195.8	°C
Freezing point	: -345.9	°F
	: -209.9	°C
pH	: Not Applicable	
Specific gravity	: Not Available	
Oil/water partition coefficient	: Not Available	
Solubility (H20)	: Negligible	
Odor threshold	: Not Applicable	
Odor and appearance	: Colorless, odorless liquid	

10. Stability and Reactivity

STABILITY:
Stable

INCOMPATIBLE MATERIALS:
None

HAZARDOUS POLYMERIZATION:
Does not occur

11. Toxicological Information

Oxygen deficiency during pregnancy has produced developmental abnormalities in humans and experimental animals.

No data given in the Registry of Toxic Effects of Chemical Substances (RTECS) or Sax, Dangerous Properties of Industrial Materials, 7th ed.

12. Ecological Information

No data given.

13. Disposal Considerations

Do not attempt to dispose of residual waste or unused quantities. Return in the shipping container PROPERLY LABELED, WITH ANY VALVE OUTLET PLUGS OR CAPS SECURED AND VALVE PROTECTION CAP IN PLACE to BOC Gases or authorized distributor for proper disposal.

PRODUCT NAME: NITROGEN, REFRIGERATED LIQUID

14. Transport Information

PARAMETER	United States DOT	Canada TDG
PROPER SHIPPING NAME:	Nitrogen, refrigerated liquid	Nitrogen, refrigerated liquid
HAZARD CLASS:	2.2	2.2
IDENTIFICATION NUMBER:	UN 1977	UN 1977
SHIPPING LABEL:	NONFLAMMABLE GAS	NONFLAMMABLE GAS

15. Regulatory Information

SARA TITLE III NOTIFICATIONS AND INFORMATION

SARA TITLE III - HAZARD CLASSES:
Sudden Release of Pressure Hazard

16. Other Information

Compressed gas cylinders shall not be refilled without the express written permission of the owner. Shipment of a compressed gas cylinder which has not been filled by the owner or with his/her (written) consent is a violation of transportation regulations.

DISCLAIMER OF EXPRESSED AND IMPLIED WARRANTIES:
Although reasonable care has been taken in the preparation of this document, we extend no warranties and make no representations as to the accuracy or completeness of the information contained herein, and assume no responsibility regarding the suitability of this information for the user's intended purposes or for the consequences of its use. Each individual should make a determination as to the suitability of the information for their particular purpose(s).

Deconahol Safety Data Sheet

DECON-AHOL WFI® 70%

Sterile Pharmaceutical Clean Room Formula
USP Isopropyl Alcohol with Water for Injection (WFI)

MATERIAL SAFETY DATA SHEET

COMPLIES WITH OSHA HAZARD COMMUNICATION STANDARD 29 CFR 1910.1200
(Complies with Commission Directive 91/155/EEC amended by 2001/58/EC)



Veltek Associates, Inc.
15 Lee Boulevard, Malvern, PA 19355-1234
Tel: 610-644-8335 Fax: 610-644-8336
www.sterile.com

Material Safety Data Sheet

Material Name: DECON-AHOL® Sterile WFI Formula Aerosol **ID: VEL-104-AEROSOL**

*** Section 1 - Chemical Product and Company Identification ***

Chemical Name: USP Isopropyl Alcohol with USP Water for Injection Aerosol
Product Use: Decontaminant.
Manufacturer Information

Veltek Associates, Inc.
15 Lee Blvd.
Malvern, PA 19355-1234

Phone: 610-644-8335

Emergency # 24 Hr CHEMTREC U.S. (800) 424-9300

*** Section 2 - Hazards Identification ***

Emergency Overview

This product may be irritating to the eyes, skin, gastrointestinal tract and respiratory system. May cause central nervous system depression. Flammable liquid and vapor. Vapor may cause flash fire. Contents under pressure.

Potential Health Effects: Eyes

This product may cause irritation to the eyes. Direct contact with aerosol propellant may cause frostbite.

Potential Health Effects: Skin

This product may cause irritation to the skin. Direct contact with aerosol propellant may cause frostbite.

Potential Health Effects: Ingestion

Ingestion of this product may result in central nervous system effects including headache, sleepiness, dizziness, slurred speech and blurred vision.

Potential Health Effects: Inhalation

May cause irritation to the nose and respiratory tract. Inhalation may cause central nervous system depression with symptoms such as weakness, dizziness, confusion and drowsiness. Aerosol propellant can cause asphyxiation by oxygen displacement.

Medical Conditions Aggravated by Exposure

Persons with pre-existing skin, eye and respiratory disorders may be aggravated by exposure to isopropyl alcohol component.

HMIS Ratings: Health: 1 **Fire:** 3 **Physical Hazard:** 0
Hazard Scale: 0 = Minimal 1 = Slight 2 = Moderate 3 = Serious 4 = Severe * = Chronic hazard

*** Section 3 - Composition / Information on Ingredients ***

CAS #	Component	Percent
67-63-0	Isopropyl alcohol	70-91
7732-18-5	USP Water for Injection	9-30*
7727-37-9	Nitrogen Propellant, 90-130 psi	>0.1

Component Information/Information on Non-Hazardous Components

This product is considered hazardous under 29 CFR 1910.1200 (Hazard Communication). This product is a controlled product according to Canada's Controlled Product Regulation.

*Though the range is outside acceptable WHMIS limits, the range stated on this MSDS is the actual range within which this component varies between formulations of this product.

*** Section 4 - First Aid Measures ***

First Aid: Eyes

Immediately flush eyes with water for at least 15 minutes, while holding eyelids open. Seek medical attention at once.

First Aid: Skin

For skin contact flush with large amounts of water while removing contaminated clothing. Wash contaminated clothing before reuse. If irritation persists, get medical attention. If frostbite or freezing occurs, flush with lukewarm water. Do not use hot water! Get immediate medical attention.

Engineering Controls
Explosion proof exhaust ventilation should be used.
PERSONAL PROTECTIVE EQUIPMENT
Personal Protective Equipment: Eyes/Face
Wear chemical goggles; face shield (if splashing is possible).
Personal Protective Equipment: Skin
Use impervious gloves.
Personal Protective Equipment: Respiratory
Use a NIOSH approved HEPA filter, or supplied air respirators when exposures reach the OSHA established PELs.
Personal Protective Equipment: General
Eye wash fountain and emergency showers are recommended.

*** Section 9 - Physical & Chemical Properties ***

Appearance:	Clear, colorless liquid	**Odor:**	Mild alcohol
Physical State:	Liquid	**pH:**	7.0
Vapor Pressure:	28 torr @20°C	**Vapor Density:**	1.6 (air=1)
Boiling Point:	170°F (77°C)	**Melting Point:**	Not available
Solubility (H2O):	Complete	**Specific Gravity:**	0.8272-0.883 (H2O=1)
Freezing Point:	14°F (-10°C)	**Evaporation Rate:**	1.7 (Butyl Acetate=1)
Octanol/H2O Coeff.:	Not available	**Upper Flammability Limit (UFL):**	12% (% volume in air)
Lower Flammability Limit (LFL):	2% (% volume in air)	**Flash Point:**	70°F (22°C)
Auto Ignition:	Not Available		

*** Section 10 - Chemical Stability & Reactivity Information ***

Chemical Stability
Stable under normal conditions.
Chemical Stability: Conditions to Avoid
Keep away from heat, ignition sources and incompatible materials.
Incompatibility
Acids, metals, oxidizing materials, combustible materials, halogens, peroxides, bases, metal salts.
Hazardous Decomposition
Oxides of carbon, miscellaneous organic compounds.
Possibility of Hazardous Reactions
Will not occur.

*** Section 11 - Toxicological Information ***

Acute Dose Effects
A: General Product Information
Excessive inhalation or ingestion of this material may cause central nervous system depression. Symptoms include headache, dizziness, nausea and incoordination. May cause irritation of the eyes, skin, gastrointestinal tract or respiratory system.
B: Component Analysis - LD50/LC50
Isopropyl alcohol (67-63-0)
Inhalation LC50 Rat: 16000 mg/kg/8H; Oral LD50 Rat: 5045 mg/kg; Oral LD50 Mouse: 3600 mg/kg; Dermal LD50 Rabbit: 12800 mg/kg

Carcinogenicity
A: General Product Information
No carcinogenicity data available for this product.
B: Component Carcinogenicity
Isopropyl alcohol (67-63-0)
ACGIH: A4 - Not Classifiable as a Human Carcinogen
IARC: Monograph 71, 1999; Supplement 7, 1987; Monograph 15, 1977 (Group 3 (not classifiable))

Target Organ Effects

Central Nervous System (CNS), skin, eyes, respiratory system.

*** Section 12 - Ecological Information ***

Ecotoxicity

A: General Product Information

No information available for the product.

B: Component Analysis - Ecotoxicity - Aquatic Toxicity

Isopropyl alcohol (67-63-0)

Test & Species		Conditions
96 Hr LC50 fathead minnow (29 days old)	94900 mg/L	flow-through
96 Hr LC50 fathead minnow (31 days old)	61200 mg/L	flow-through
5 min EC50 Photobacterium phosphoreum	35390 mg/L	

*** Section 13 - Disposal Considerations ***

US EPA Waste Number & Descriptions

A: General Product Information

No additional information available.

B: Component Waste Numbers

No EPA Waste Numbers are applicable for this product's components.

Disposal Instructions

Do not puncture or incinerate pressurized containers. Dispose of waste material according to Local, State, Federal, and Provincial Environmental Regulations. If discarded, this product is considered a RCRA ignitable waste, D001.

See Section 7 for Handling Procedures. See Section 8 for Personal Protective Equipment recommendations.

*** Section 14 - Transportation Information ***

US DOT Information

Shipping Name: Aerosols, flammable
UN #: UN1950 **Hazard Class:** 2.1
Required Label(s): NONE

TDG Information

Shipping Name: Aerosols, flammable
UN #: UN1950 **Hazard Class:** 2.1
Required Label(s): NONE

IATA Information

Shipping Name: Aerosols, flammable
UN #: UN1950 **Hazard Class:** 2.1
Required Label(s): Flammable Gas

IMDG Information

Shipping Name: Aerosols, flammable
UN #: UN1950 **Hazard Class:** 2
Required Label(s): Flammable Gas

*** Section 15 - Regulatory Information ***

US Federal Regulations

A: General Product Information

All components are on the U.S. EPA TSCA Inventory List.

B: Component Analysis

This material contains one or more of the following chemicals required to be identified under SARA Section 302 (40 CFR 355 Appendix A), SARA Section 313 (40 CFR 372.65) and/or CERCLA (40 CFR 302.4).

Isopropyl alcohol (67-63-0)

SARA 313: 1.0 % de minimis concentration (only if manufactured by the strong acid process, no supplier notification)

State Regulations

A: General Product Information

Other state regulations may apply. Check individual state requirements.

B: Component Analysis - State

The following components appear on one or more of the following state hazardous substances lists:

Component	CAS	CA	MA	MN	NJ	PA	RI
Isopropyl alcohol	67-63-0	Yes	Yes	Yes	Yes	Yes	Yes
Nitrogen Propellant, 90-130 psi	7727-37-9	No	Yes	Yes	Yes	Yes	Yes

Canadian WHMIS Information

A: General Product Information

WHMIS Classification: B5- Flammable Aerosol, D2B- Toxic Material

B: Component Analysis - WHMIS IDL

The following components are identified under the Canadian Hazardous Products Act Ingredient Disclosure List:

Component	CAS #	Minimum Concentration
Isopropyl alcohol	67-63-0	1 % (English Item 904, French Item 1050)

Additional Regulatory Information

A: General Product Information

No additional information available.

B: Component Analysis - Inventory

Component	CAS #	TSCA	CAN	EEC
Isopropyl alcohol	67-63-0	Yes	DSL	EINECS
USP Water for Injection	7732-18-5	Yes	DSL	EINECS
Nitrogen Propellant, 90-130 psi	7727-37-9	Yes	DSL	EINECS

* * * Section 16 - Other Information * * *

Other Information

No additional information available.

MSDS History

New MSDS: 11 January 2005.

Key/Legend

ACGIH = American Conference of Governmental Industrial Hygienists; CERCLA = Comprehensive Environmental Response, Compensation and Liability Act; CFR = Code of Federal Regulations; CNS = Central Nervous System; DSL = Domestic Substances List; EINECS = European Inventory of Existing Commercial Chemical Substances; EPA = Environmental Protection Agency; HEPA = High Efficiency Particulate Air filters; IARC = International Agency for Research on Cancer; LC50 = Lethal Concentration 50%; LD50 = Lethal Dose 50%; NIOSH = National Institute for Occupational Safety and Health; NJTSR = New Jersey Trade Secret Registry; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = Permissible Exposure Limit; RCRA = Resource Conservation and Recovery Act; SARA = Superfund Amendments and Reauthorization Act; STEL = Short Term Exposure Limit; TDG = Transport Dangerous Goods TSCA = Toxic Substance Control Act; TWA = Time Weighted Average; WHMIS = Workplace Hazardous Materials Information System.

Contact: Arthur Vellutato, Sr., President

Contact Phone: 610-644-8335

This is the end of MSDS # VEL-104-AEROSOL

LabSolutions Powder Detergent Safety Data Sheet

Protecting your laboratory environment
LABCONCO.

8811 Prospect Avenue, K.C., MO 64132
(816) 333-8811, Fax (816) 363-0130, (800) 821-5525

Date of Issue: 10/31/1995	**Material Safety Data Sheet** (MSDS)	04334 04335 Date of Revision: 08/01/08

SECTION 1 - MANUFACTURER INFORMATION

Manufacturer Name:	Decon Laboratories, Inc.		
Trade Name:	**LabSolutions – Powder Detergent or Dri-Contrad®**	**Emergency Telephone** U.S.: (800) 424-9300 Canada: (703) 527-3887	
Chemical Name or Synonym:	N/A	**Info Telephone:**	610-755-0800
Mfg. Address:	460 Glennie Circle		
City:	King of Prussia	**State, Zip:** PA	19406

SECTION II - HAZARDOUS INGREDIENTS

CAS Number	Chemical Name	Percent	PEL	C	S	TLV	C	S	Units	313?
497-19-8	Sodium carbonate	<100							N/A	N
6834-92-0	Sodium metasilicate	<100							N/A	N
7758-29-4	Sodium tripolyphosphate	<100							N/A	N
51580-86-0	Sodium dichloroisocyanurate	<100							N/A	N

CAS Numbers beginning with letters are codes for items with no valid CAS assignments; "PEL" is OSHA Permissible Exposure Limit; "C" indicates the standard is a Ceiling value; "S" indicates the chemical has a "Skin Contact" notation; "TLV" is Threshold Limit Value; "313" indicates ingredient is reportable under SARA Title III, Section 313. NA-Not Applicable; NE-Not Established; UN-Unknown.

Additional Information:	Unidentified ingredients are not considered hazardous under the Federal Hazard Communication Standard (29 CFR 1910.1200)

SECTION III - PHYSICAL DATA

Boiling Point (°F):	N/A	**Specific Gravity:**	1.8 - 2.0
Vapor Pressure (mm of Hg):	N/A	**Melting Point:**	N/A
Vapor Density (Air=1):	N/A	**PH:**	>12 (10% solution)
Solubility:	Appreciable	**Evaporation Rate:**	N/A
Appearance:	White powder, mild odor of chlorine.	**% Volatiles:**	N/A

SECTION IV - FIRE AND EXPLOSION DATA

Flash Point:	N/A	LEL:	N/A	UEL:	N/A
Extinguishing Media:	Use media suitable for surrounding materials.				
Special Procedures:	No special procedures required.				
Unusual Fire / Expl Hazards:	None known.				

Additional Information:
Hazard Ratings
HMIS: Health = 3 Flammability = 0 Reactivity = 0 Personal Protection = B
NFPA: Health = 3 Flammability = 0 Reactivity = 0

LabSolutions – Powder Detergent or Dri-Contrad®

SECTION V - HEALTH HAZARD DATA				
Acute Health Effects/Symptoms:	Inhalation of dust may cause severe respiratory irritation. Skin contact may Cause irritation, possible burns. Eye contact is irritating and may cause Permanent damage. Pre-existing skin conditions may be aggravated by exposure.			
Chronic Health Effects / Symptoms:	None known.			
Carcinogenicity Data:	NTP: N	IARC: N	OSHA: N	OTHER: N/A

First Aid	
Eyes:	Immediately flush eyes with plenty of water for at least 15 minutes, while holding eyelids open. Seek prompt medical attention.
Skin:	Remove contaminated clothing. Wash thoroughly with soap and water. Seek medical attention if irritation persists. Wash clothing before reuse.
Ingestion:	Contact local Poison Control Center or physician immediately.
Inhalation:	Move victim to fresh air. Get medical attention if irritation persists.

SECTION VI - REACTIVITY DATA	
Stability:	Normally stable
Incompatibility:	Strong acids and phosphorus oxides.
Decomposition:	Carbon monoxide, carbon dioxide, chlorine gas.
Polymerization:	Will not occur.

SECTION VII - SPILL OR LEAK PROCEDURES	
Spill Procedures:	Wear personal protective equipment (see Section VIII). Carefully clean up spilled material and place in dry container for disposal. Avoid dust generation.
Waste Disposal Procedures:	Dispose in accordance with federal, state, and local regulations.

SECTION VIII - SPECIAL PROTECTION INFORMATION	
Respiratory:	If TLV is exceeded, or for symptoms of overexposure, wear a NIOSH approved dust / mist respirator.
Eyes:	If potential for eye contact exists, wear chemical goggles.
Clothing / Protective Gloves:	Wear chemical resistant gloves and clothing as needed to prevent skin contact.
Ventilation:	Not normally needed when use in this form. Specific needs should be addressed by supervisory or health / safety personnel.

SECTION IX - ADDITIONAL INFORMATION	
Safe Handling and Storage:	Store in a closed container in a dry area.

SECTION X – TRANSPORTATION INFORMATION	
DOT Hazard Class:	Non-Hazardous (Not Regulated)

The information contained herein has been developed based upon current available scientific data. New information may be developed from time to time which may render the conclusions of this report obsolete. Therefore, no warranty is extended as to the applicability of this information to the user's intended purpose or for the consequences of its use or misuse

LabSolutions Neutralizing Acid Rinse Safety Data Sheet

Protecting your laboratory environment
LABCONCO

8811 Prospect Avenue, K.C., MO 64132
(816) 333-8811, Fax (816) 363-0130, (800) 821-5525

Date of Issue: 01/01/1997	**Material Safety Data Sheet** (MSDS)	Date of Revision: 08/01/08

SECTION 1 - MANUFACTURER INFORMATION

Manufacturer Name:	Decon Laboratories, Inc.		
Trade Name:	**LabSolutions Neutralizing Acid Rinse or DeSCAL® Descaler Concentrate**	**Emergency Telephone** U.S.: (800) 424-9300 Canada: (703) 527-3887	
Chemical Name or Synonym:	N/A	**Info Telephone:**	610-755-0800
Mfg. Address:	460 Glennie Circle		
City:	King of Prussia	**State, Zip:**	PA 19406

SECTION II - HAZARDOUS INGREDIENTS

CAS Number	Chemical Name	Percent	PEL	C	S	TLV	C	S	Units	313?
7664-38-2	Phosphoric Acid, 75%	42%	1.0			1.0				Y

Additional Information:	Unidentified ingredients are not considered hazardous under the Federal Hazard Communication Standard (29 CFR 1910.1200)

SECTION III - PHYSICAL DATA

Boiling Point (°F):	>212	**Specific Gravity:**	1.18
Vapor Pressure (mm of Hg):	N/A	**% Volatiles:**	N/A
Vapor Density (Air=1):	N/A	**pH:**	1.00
Solubility:	Complete	**Evaporation Rate:**	>1 BuAc = 1
Appearance:	Clear liquid; no odor.		

SECTION IV - FIRE AND EXPLOSION DATA

Flash Point:	N/A	LEL: N/A	UEL: N/A
Extinguishing Media:	Use media suitable for surrounding materials.		
Special Procedures:	No special procedures required.		
Unusual Fire / Expl Hazards:	Protect firefighters from physical contact with product since it is a strong acid cleaner. The product is not anticipated to cause any contribution to the fire, but can cause chemical burns to the skin and eyes if not washed off and neutralized.		

Additional Information:
Hazard Ratings
HMIS: Health = 2 Flammability = 0 Reactivity = 0 Personal Protection = B
NFPA: Health = 2 Flammability = 0 Reactivity = 0

LabSolutions Neutralizing Acid Rinse or DeSCAL® Descaler Concentrate

SECTION V - HEALTH HAZARD DATA				
ACUTE HEALTH EFFECTS / SYMPTOMS: Inhalation of mist may cause respiratory irritation. Skin contact may cause reddening and irritation. Eye contact is irritating and may cause damage. Pre-existing skin conditions and detergent allergies may be aggravated by exposure.				
CHRONIC HEALTH EFFECTS / SYMPTOMS: Chronic health hazards are difficult to accurately assess for mixtures. Specific chronic effects can be studied from the individual hazardous chemicals as indicated under Section II as the best guess without extensive laboratory studies.				
Carcinogenicity Data:	NTP: None known	IARC: N	OSHA: N	OTHER: N/A

First Aid	
Eyes:	Immediately flush eyes with plenty of water for at least 15 minutes, while holding eyes open. Seek prompt medical attention. Fast response during first few seconds of exposure is vital to avoid permanent damage.
Skin:	Remove contaminated clothing. Wash thoroughly with soap and water. If irritation persists, seek medical attention. Wash clothing before reuse. Apply lotion to areas where irritation persists.
Ingestion:	Contact local Poison Control Center or physician immediately. Do not induce vomiting. Give water, milk, or milk of magnesia to help neutralize the acid.

SECTION VI - REACTIVITY DATA	
Stability:	Normally stable
Incompatibility:	Strong alkalis (bases).
Decomposition:	None known.
Polymerization:	Will not occur.

SECTION VII - SPILL OR LEAK PROCEDURES	
Spill Procedures:	Wear personal protective equipment (see Section VIII). Wash away with water. Neutralize using a weak alkali, e.g. Sodium Bicarbonate. Clean up with absorbent material.
Waste Disposal Procedures:	Dispose in accordance with federal, state, and local regulations.

SECTION VIII - SPECIAL PROTECTION INFORMATION	
Respiratory:	Under normal use, none is needed. If TLV is exceeded, or for symptoms of overexposure, wear a NIOSH approved dust / mist respirator.
Eyes:	Wear chemical goggles.
Clothing / Protective Gloves:	Wear latex, neoprene or other chemical resistant gloves. Wear lab coat to protect Garments.
Ventilation:	Local exhaust may be necessary under some handling / use conditions. Specific needs Should be addressed by supervisory or health / safety personnel.

SECTION IX - ADDITIONAL INFORMATION	
Safe Handling and Storage:	Store in a closed container. Do not freeze. Do not store above 100°F. Store separate from strong alkalis.

SECTION X – TRANSPORTATION INFORMATION		
DOT Hazard Class:	Product	Shipping by Ground
	1 Liter	ORM-D (DOT 49 CFR 173.154)

The information contained herein has been developed based upon current available scientific data. New information may be developed from time to time which may render the conclusions of this report obsolete. Therefore, no warranty is extended as to the applicability of this information to the user's intended purpose or for the consequences of its use or misuse.