FREEZE DRY

BENCHNOTES

APPROXIMATE FREEZE DRYING TIMES

Since freeze drying times are dependent upon the vapor pressure and temperature of the product, the following times are approximate:

SAFE TEMPERATURES AND DRYING TIMES FOR SELECTED MATERIALS

Product	Safe Temp °C	Collector Temp °C	Hours (approx)
Milk	-5	-40	10
Urea	-7	-40	10
Blood Plasma	-10 to -25	-40	16
Serum	-25	-40	18
Vaccinia	-30 to -40	-50	22
Influenza Vaccine	-30	-50	24
Human Tissue	-30 to -40	-50	48
Vegetable Tissue	-50	-80	60
Rose	-20	-50	48
Orchid	-20	-50	96
Fern	-20	-50	24
Fresh Water Algae	-30	-50	12-24
Rat Teeth	-70	-50	48

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FREEZE DRY
BENCHNOTES

Procedure for Freeze Drying Fish Samples for Volatile Substances Using a FreeZone® 4.5 Liter Freeze Dry System

Principle: *Lyophilization is used to remove moisture from biological samples, without loss of volatile substances (i. e. mercury).*

Procedure

A) Sample Preparation

1. Fish tissue, either whole body or segments, should be cut into pieces, to reduce drying time.

2. Place fish tissue in a 50 ml flask or in a petri dish for processing.

3. Pre-freeze the sample by either dipping them in liquid nitrogen or by placing them in a ultra low freezer (-70° C) for several hours.

B) Set up for FreeZone 4.5 Liter Freeze Dry System

1. Clean the rubber gasket on the lid and the condenser chamber to remove any dirt or contaminant that could cause a vacuum leak.

2. Check vacuum pump oil gauge to ensure proper level of oil and to ensure the oil is clear not cloudy or dirty. Cloudy oil should be replaced.

3. Turn on refrigeration. When temperature -40° C or lower is attained, turn on the vacuum pump. Make sure all the valves are in the VENT position.

4. Apply the samples when "no load" vacuum is around 25 microns (33×10^{-3} mBar) or less. Additional samples may be applied when vacuum is 100 microns (133×10^{-3} mBar) or less.

5. Samples are freeze dried to a constant weight. This is usually determined by weighing the sample several times over the course of the freeze drying process. NOTE: Remember to close the valve after the removal of each sample.

6. Freeze dried samples are stored in 50% nitric acid rinsed vials with Teflon caps, in either freeze dried form or they are homogenized by using a mortar and pestle.

Reference

U.S. Department of the Interior, Fish and Wildlife Service. National Fisheries Contaminant Research Center.

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6-02-A-5/96-100-R3

FREEZE DRY
BENCHNOTES

Extraction and Analytical Method for "Microcystin LR" in Cyanobacteria Samples

Principle: *Certain algae produces toxins that are harmful to mammals. By measuring these toxins, researchers can determine the drinkability of water.*

Procedure

A) Sample Preparation

1. Algae is removed from the stream in question (collect enough algae to approximate 1 gram of dry material).
2. Place in a graduated cylinder and gently bubble carbon dioxide through the sample for 10 minutes to remove zoo plankton.
3. Centrifuge and decant algal cells (these are in the supernatant) and transfer to freeze drying flask. Shell freeze samples as a thin shell using acetone/dry ice bath. Freeze dry for at least 24 hours.
4. Determine weight of dried cells.
5. Transfer 100 mg dried cells to a 15 ml centrifuge tube.
6. Add 5 ml 5% acetic acid and vortex.
7. Leave in the dark for at least one hour.
8. Vortex and sonicate for 3 x 30 seconds.
9. Leave in the dark for at least one hour.
10. Vortex and centrifuge.

B) Cartridge Preparation

11. Prepare HPLC (C18) cartridge by washing with 5 ml methanol and then rinsing with 5 ml water.
12. Apply sample to cartridge.
13. Wash cartridge in the following order:
    10 ml 5% acetic acid
    10 ml of water
    10 ml of 10% methanol in water
14. Elute with 5 ml methanol.
15. Remove methanol by evaporation.
16. Re-dissolve sample in 2 ml HPLC solvent (10 mM ammonium acetate: acetonitrile 76:24)
17. Inject 20 µl sample into the HPLC at 238 nm. Use U.V. detection at 238 nm.
18. Calculate and report microcystin LR concentration as µg LR per g dried biomass.

Reference

University of Alberta-Edmonton, Department of Health Service Administration

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6-03-A-5/96-100-R2

FREEZE DRY
BENCHNOTES

General Freeze Dry Application for Snake Venom and Other Small Volume Samples of 0.4 ml or Less

Principle: *Lyophilization allows a sample to retain its toxic and enzymatic effects.*

Procedure

A) Sample Preparation

1. Using a 0.2 µl ultra free MC filter (Millipore), filter the venom.
2. Centrifuge the sample in a microfuge for three minutes at 2000 g.
3. Discard the filter cup and plug the top of the collection tube with a "cigarette-type" filter used in the Pipetman P-5000 pipette (Rainin). This prevents the sample from escaping during lyophilization.
4. Start the lyophilizer and establish operating parameters.
5. Flash freeze the sample by dipping the collection tube into liquid nitrogen for about one minute. Immediately put the tube in a small freeze drying flask and place on the lyophilizer.
6. Lyophilize for at least two hours or until the individual tubes are at room temperature.
7. After freeze drying, cap the samples for storage at -20° C over a desiccant for future uses, or reconstitute to a set toxic level.
8. Inject the reconstituted samples into rabbits for antibody production.

Reference

University of Oklahoma

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6-04-A-5/96-100-R3

FREEZE DRY
BENCHNOTES

Freeze Drying Fungi

Principle: *The purpose of this standard operating procedure is to describe the freeze drying process of pure fungal cultures for long term storage.*

Procedure

A) Preparation of Wheat Straw Agar

1. Place about 20 grams wheat straw in a 100 ml beaker. Cover with water.
2. Autoclave the sample separately from the plain water agar.
3. Aseptically place 6-10 straws in clean petri dishes.
4. Pour plain water agar over the wheat straw.

B) Preparation of Cultures

1. Cultures are isolated from plant material.
2. Cultures must be single spored or hypha tipped on CA (carrot agar) or WSA (wheat straw agar).
3. Cultures must be positively identified.
4. Cultures are then single spored or hypha tipped again onto the wheat straw agar.
5. Cultures should be allowed to grow in pure culture 3-4 weeks before freeze drying.

C) Preparation of Ampules

1. Place a small amount of cotton into bottom of ampule.
2. Sterilize 5 ampules (7.5 mm) in a glass petri dish or sterilize 2 ampules (12.5 mm) upside down in a capped test tube.
3. Aseptically place 4-5 individual straws from culture dish into each ampule with forceps. Gently push down to bottom of ampule with a glass rod and label outside of ampule with sample names.

D) Preparation of Freeze Dry System

1. Clean the rubber gasket on the lid and the condenser chamber to remove any dirt or contaminant that could cause a vacuum leak.
2. Check vacuum pump oil gauge to ensure proper level of oil and to ensure the oil is clear not cloudy or dirty. Cloudy oil should be replaced.
3. Turn on refrigeration. When temperature of -40° C or lower is attained, turn on the vacuum pump. Make sure all the valves are in the VENT position.
4. Apply the samples when “no load” vacuum is around 25 microns (33×10^{-3} mBar) or less. Additional samples may be applied when vacuum is 100 microns (133×10^{-3} mBar) or less.
5. Gently insert ampules into manifold making sure they fit tightly.
6. Turn valves 180° to VAC. Ampules will feel very cold initially.
7. Freeze drying is complete when ampules feel warm (room temperature). For our cultures, this takes about 2-3 hours.
8. Use a sealing torch to melt and seal the ampule along the narrow neck. Do not allow the flame to melt the valve. Be very careful when removing the ampule stem from the valve due to sharp glass fragments.
9. When all the ampules have been sealed, turn off both vacuum and refrigeration switches.

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10. Attach ampule to 3x5 index card marked clearly with specimen number. Store in small filing cabinet in cold room.

E. Labels

1. Labels should be clearly marked with the specimen number and the date of freeze drying.

Reference

Plant Pathology Laboratory, Department of Entomology, Plant Pathology and Weed Science, New Mexico State University

FREEZE DRY

BENCHNOTES

General Freeze Dry Application for Monitoring Soil Samples

Application: *Analyzing soil samples for organic content.*

A core sample is taken either at a set depth or fraction of depths. (Procedure for obtaining a core sample can be found in any basic geological science textbook.) The sample is pre-frozen by placing it in a regular freezer overnight. Small holes are made in the sediment bag and placed in a freeze dry flask and attached to the freeze dry system with the condenser temperature at -50° C. It generally takes about one hour for every 1 cm^3 of sample or about 3-4 days when the flasks are loaded with 3 or 4 samples. The sample is stored or burned in a furnace for organic content.

Reference

University of Notre Dame, Department of Earth Science, Notre Dame, Indiana

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6-16-A-5/96-100-R3

FREEZE DRY
BENCHNOTES

Freeze Dry Applications for Monitoring Anti-Cancer Drugs

Application: *Anti-cancer drugs using radiolabeled Carbon 14*

Laboratory animals are dosed with a developmental compound labeled with C14. Urine, feces and tissue are taken from the animal to determine how much of the drug remains in its system or how much is excreted. The samples are freeze dried in order to concentrate the C14. The sample is then weighed and combusted by a sample oxidizer. The released C14 is then counted in a gamma counter and compared to a drug concentration curve.

Reference

ICI America Incorporated

Application: *Anti-cancer drugs using antibodies to DNA*

Commercially processed DNA is purified and mixed with the known antibodies, incubated for a set time (1-2 hours) and then placed in polyethylene Eppendorf* tubes with screw caps (15-50 ml are recommended). The sample is then pre-frozen in liquid nitrogen. Small needle holes are made in the caps to aid in freeze drying. The sample is placed in a freeze dry flask and connected to a freeze dry system to concentrate. After freeze drying, the sample is rehydrated with D-H_2O or TRIS buffer. It is analyzed for cleavage patterns by DNA electrophoresis. These patterns tell the researcher how the drug interacts. This process is used mainly in anti-cancer drugs.

*Eppendorf® is a registered trademark of Eppendorf-Netheler-Hinz GmbH.

Reference

California Institute of Technology, Department of Chemistry, Pasadena, California

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6-20-A-5/96-100-R3

FREEZE DRY

BENCHNOTES

Mango Powder Production by Freeze-Drying Process using a FreeZone® 4.5 Liter Freeze Dry System

Principle: *Mango (Mangifera indica L.) is the national fruit of the Philippines and the third most important export of the country. The challenges facing the Philippine mango industry are the seasonality in production and unstable market situations characterized by a period of supply glut. Freeze drying mango has helped to eliminate these market fluctuations and has advantages over other forms of processed mango. Freeze dried mango not only has a long shelf life due to considerable reduction of moisture content, it is economical in transportation, storage, packaging and handling because of reduced weight and space requirements.*

Apparatus

1. Freezer
2. FreeZone 4.5 Liter Freeze Dry System (or other collector capacity)
3. 300 milliliter Fast-Freeze® or Lyph-Lock® Flask

Procedure

A) Sample Preparation
1. Aseptically process mango puree.
2. Place mango puree in test tubes and pre-freeze at -65 °C for 6 hours.
3. Remove the puree from the freezer and place in a 300 milliliter flask.

B) FreeZone 4.5 Liter Freeze Dry System Set Up
1. Clean the rubber gasket on the lid and the collector chamber to remove any dirt or contamination that could cause a vacuum leak.
2. Check vacuum pump oil gauge to ensure proper level of oil and to ensure the oil is clear not cloudy or dirty. Cloudy oil should be replaced.
3. Turn on the refrigeration. When temperature reaches -40 °C or lower, turn on the vacuum pump. If in automatic mode, the vacuum pump will automatically turn on. Make sure all the valves are in the CLOSED position.
4. Apply one sample to the freeze dryer. When vacuum has recovered to 133 x 10^{-3} mbar or lower, add the next sample. Continue in this way until all samples are added.
5. Dry the samples until the moisture content of 2-3% is obtained (about 22.5 hours).
6. Samples can be stored in a dry room, shipped or reconstituted.

Reference

Central Luzon State University
Department of Agriculture-Bureau of Post-Harvest Research and Extension

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