

## SP004

### Lyophilization

#### Materials and Reagents:

1. Container suitable for lyophilization
2. Sample for lyophilization
3. Aluminum foil
4. Needle
5.  $-20^{\circ}\text{C}$  freezer
6.  $-80^{\circ}\text{C}$  freezer
7. Freeze dry flask, 1200 ml volume
8. Freeze dry flask lid with metal stem
9. Paper filter, round, 2 cm diameter
10. Lyophilizer
11. Lyophilizer sign-up sheet

#### Protocol:

1. \_\_\_\_ Read the notice on the lyophilizer to make sure sample is compatible with the machine (note 1).
2. \_\_\_\_ Transfer sample into a container suitable for lyophilization (notes 2 and 3).
3. \_\_\_\_ Cover container mouth with a piece of aluminum foil.
4. \_\_\_\_ Using a needle, poke several small holes in the foil.
5. \_\_\_\_ Place container in  $-20^{\circ}\text{C}$  freezer until frozen solid (note 4).
6. \_\_\_\_ Place container in  $-80^{\circ}\text{C}$  freezer for at least 1.5 hours (note 5).
7. \_\_\_\_ Remove freeze-dry flask and lid from drawers in B-204.
8. \_\_\_\_ Inspect freeze-dry lid to make sure it is suitable to use (note 6).
9. \_\_\_\_ Remove container from freezer and place in freeze-dry flask.
10. \_\_\_\_ Place rubber lid on freeze-dry flask (note 7).
11. \_\_\_\_ Insert the metal stem of the freeze-dry flask into an open port on the lyophilizer (note 8).
12. \_\_\_\_ Turn the port knob from vent to vacuum.
13. \_\_\_\_ Monitor the vacuum gauge to make sure the final reading is below  $100 \times 10^{-3}$  mbar (notes 9 and 10).
14. \_\_\_\_ Fill out the lyophilizer sign-up sheet.
15. \_\_\_\_ Inspect the lyophilizer daily to see when sample is completely dried.
16. \_\_\_\_ Turn port knob from vacuum to vent.
17. \_\_\_\_ Remove freeze dry flask from port (note 11).
18. \_\_\_\_ Fill out the lyophilizer sign up sheet.
19. \_\_\_\_ Return freeze dry flask and lid to storage area in B-204 (note 12).

**Notes:**

1. No organic solvents, phenol, glycerol or radioactivity may be placed on the lyophilizer.
2. Almost any type of container is suitable for lyophilization, including but not limited to 15 ml and 50 ml Falcon centrifuge tubes, Oakridge centrifuge tubes, 13 x 100 mm and 16 x 100 mm glass tubes, small round-bottom flasks, eppendorf centrifuge tubes, cryovials and 250 ml or smaller tissue culture filter units.
3. It is critical not to overfill the container for lyophilization because of expansion during the freezing process. It is advisable to fill plastic containers no more than 50% full, and glass containers no more than 30% full. Alternatively, samples may be shell-frozen using acetone containing dry ice; this will prevent expansion of the sample during the freezing process.
4. This usually takes 1 to 3 hours, depending on the sample composition.
5. 1.5 hours is the minimum amount of time needed to completely freeze the sample. Leaving the sample in the  $-80^{\circ}\text{C}$  for longer will only enhance the freezing process.
6. The lid must contain an intact, secure filter disc at the junction between the rubber lid and metal stem. If the filter is not correctly inserted, the metal stem must be removed and a new filter inserted. This is especially critical if the filter is ripped, as this can be sucked into the lyophilizer causing vacuum integrity to be lost.
7. The lid fits tightly onto the flask and can be difficult to put on. Make sure the lid forms a tight seal around the glass lip of the flask.
8. The stem fits tightly into the port and can be difficult to insert. Make sure the stem is entirely inserted, and the port fits tightly around the stem.
9. As soon as the vacuum is turned on to the flask, the gauge will read "HI." As the air is removed from the flask, the reading will eventually lower to around  $60 \times 10^{-3}$  millibars. If the vacuum does not reach a low level, the samples will thaw and be sucked into the lyophilizer. Also, all other samples on the lyophilizer will thaw. Therefore, it is critical that the vacuum gauge is monitored until an appropriate vacuum level has been reached. If the vacuum never reaches an acceptable level, then follow the protocol to remove the flask from the port, and try using a different port. Inform the person responsible for lyophilizer maintenance if a port is defective.
10. If the machine does not appear to be operating correctly, or the vacuum will not reach an appropriate level, immediately contact the lab personnel in charge of lyophilizer maintenance.
11. Do not remove the freeze dry flask from the lyophilizer until the flask has completely equilibrated with the room air pressure.
12. If the sample has escaped the container, clean the freeze dry flask and replace the filter in the lid before returning to storage area.