Instructions for Reviving Lyophilized Cultures

**A. Opening a double-vial**

1. Heat the tip of the outer vial under flame.
2. Squirt a few drops of water on the heated tip to crack glass.
3. Strike glass with file or pencil to remove tip.
4. Remove insulation and take out the inner vial.
5. With sterilized forceps, gently raise the cotton plug.

**B. Recovering culture from lyophilized specimen**

1. For recovering bacteria from lyophilized specimen, aseptically add 0.3-0.5 ml of appropriate liquid medium into the inner vial with a sterile pipette and mix thoroughly by pipetting up and down.
2. For recovering fungi or yeasts from lyophilized specimen, aseptically add 0.3-0.5ml of sterile distilled water into the inner vial with a sterile pipette and mix thoroughly by pipetting up and down.
3. Take 0.1-0.2 ml of resuspended culture and streak directly onto an appropriate agar plate or make a dilution and spread on medium to check for purity of the culture. Transfer the rest of the revived culture to an appropriate liquid medium and incubate at the required temperature.
4. If problems arise due to mishandling by BCRC, cultures will be replaced free-of-charge provided that the request is reported within one month after receiving the cultures.

**Note:**

· Keep freeze-dried cultures at 4℃ at all times before reviving it.
· To maintain the anaerobic condition of the liquid medium, please ensure a constant supply of carbon dioxide within the vial during culture maniuplation unless specific instructions are given.
· Ganoderma cultures, mushrooms, and certain species of fungi are usually shipped in the form of a slant. Please keep the slant at 24℃ before subculturing.
· Certain strains will have a longer lag phase after the freezed-dried process. These cultures should be given at least twice the normal incubation time and two stages of subculturing for proper growth before being considered as inviable.