

## INSTRUCTIONS FOR OPENING AND REVIVING FREEZE-DRIED CULTURES

### Bacteria, Fungi

Freeze-dried cultures and desiccates in artificial sand (fungi) are supplied in vacuum-sealed ampoules. Care should be taken when opening the ampoule. All instructions given below should be followed closely to ensure the safety of the person who opens the ampoule and to prevent contamination of the culture.

1. Check the number of the culture on the label inside the ampoule.
2. Make a file cut on the ampoule near the middle of the plug.
3. Disinfect the ampoule with an alcohol-dampened gauze/or alcohol-dampened cotton wool from just below the plug to the pointed end.
4. Apply a red-hot glass rod to the file cut to crack the glass and allow air to enter slowly into the ampoule.
5. Remove the pointed end of the ampoule into the disinfectant.
6. **Bacteria:** Add about 0.3 ml of appropriate broth to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid aerosol formation. Transfer the contents to one or more suitable solid and /or liquid media.

**Fungi:** Add about 0.3 ml of sterile distilled water to the dried suspension using a sterile Pasteur pipette and mix carefully to avoid creating aerosol. Transfer the suspension to a suitable solid medium.

In case of a desiccate in sand just remove the plug into a disinfectant, flame the mouth of the ampoule and empty the contents on a suitable solid medium.

7. Incubate the inoculated medium at appropriate conditions for several days, in case of filamentous fungi for at least one week.
8. Autoclave all the remains of the original ampoule.

**Notes:** Cultures should be grown on media and under conditions as recommended in the CCM Catalogue. Cultures may need at least one subculturing before they can be optimally used in experiments.

***Unopened ampoules should be kept in a dark and cool place.***

