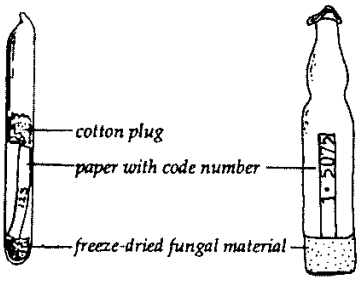
Instructions for reviving freeze-dried cultures of fungi, yeasts and bacteria

In case a material safety data sheet has been provided with the culture, read it carefully before starting to work! It is recommended to always work in a safety cabinet, and in case of pathogenic or toxigenic organisms this is compulsory!



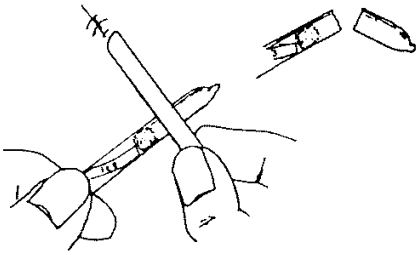
A freeze dried culture is supplied in a glass ampoule containing a dried pellet with the microbe. It may further contain a cotton plug, and a piece of paper with a code number or a label on the outside.

Opening procedure

1. *Start - all types of cultures:*
Disinfect the outer surface of the ampoule by cleaning it with 70% ethanol. Allow to dry.
Tap the ampoule gently on the work bench to loosen the frieze dried material from the glass.

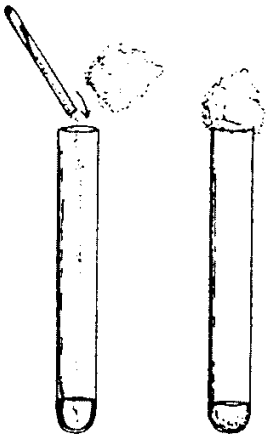
In case the freeze-dried material remains adhered to the glass wall or the pellet will not crumble, aseptically pipette a little sterile water or sterile malt peptone solution into the opened ampoule and gently stir with a sterile needle if necessary.

When breaking a glass ampoule wear something to protect your hands from cutting.



Open the glass ampoule by scoring the glass just above the cotton plug with a glass cutter or sharp file and breaking it at the scored mark. Ampoules with flat bottom lack a cotton plug, but have a pre-scored area at the constriction.
Flame the opening and remove the cotton plug gently with a sterile forceps.
Aseptic working and sterility are essential!

2. According to the type of organism, continue as follows:



Fungi (non-yeast)
Suspend the freeze-dried material by pouring the full content into a tube containing 1-2 ml of sterile water or sterile malt-peptone solution; shake gently and leave the tube at room temperature for 4-12 hrs.
Pour the suspension on a solid agar medium in a Petri dish or tube and incubate at a suitable temperature. The medium originally used is indicated on the label on the ampoule; the temperature only if room temperature (20-24 °C) is not suitable.
Allow sufficient time (2-3 wks) for reviving of freeze-dried fungal cultures.
Yeast
Suspend the freeze-dried material into 1 ml sterile water, shake gently and then poor ca. 0.5 ml of the suspension over a solid agar medium. If malt-peptone solution is used to suspend the material instead of water, the remaining suspension can be stored up to approximately two weeks for later use.

Bacteria
Wildtype strains: pour the content of the ampoule into a liquid medium, or to a tube containing a solid agar slant with 2-3 drops of sterile water. After incubation the culture can be used as inoculum for plate cultures.
Mutants and plasmid-bearing strains: Mutant and plasmid bearing strains should be transferred to 1 ml of a rich (non-selective) liquid medium (e.g. Luria Broth for *Escherichia coli*). Incubate this suspension for one hour at the appropriate temperature. Then add one drop of the suspension to an agar plate containing selective medium (e.g. with antibiotics) and streak for single colonies.

Cleaning up
Before discarding, sterilize all the remains in the original ampoule.