

## How to Revive Cultures?

### REVIVING FREEZE-DRIED CULTURES

Check each culture thoroughly upon receipt. If a culture is unsatisfactory, notify Bio-REV's technical support at [techserv@Bio-REV.com](mailto:techserv@Bio-REV.com) so that the strain in question can be investigated. Store freeze-dried cultures at 5° C or lower if they are not immediately rehydrated (except plant viruses, which should be stored at -20° C). Use the medium and incubation conditions specified in the catalog when first reviving strains to ensure optimal conditions for recovery.

#### Bacteria and Algae

Aseptically add to the freeze-dried material 0.3 to 0.4 ml (no more) of liquid medium with a Pasteur pipette and mix well. For bacteria, transfer the total mixture to a test tube of the recommended broth medium (5 to 6 ml). The last few drops of this suspension also may be transferred to an agar slant. Freeze-dried algae cultures must be initiated on agar plates.

Incubate cultures under the appropriate conditions. Given proper treatment and conditions, most freeze-dried cultures will grow out in a few days. However, some may exhibit a prolonged lag period and should be given twice the normal incubation time before discarding as nonviable.

#### Bacteriophages

Prepare an actively growing broth culture of the host before opening the phage specimen.

Rehydrate the freeze-dried phage specimen aseptically with 0.5 ml of appropriate broth (see maintenance conditions for host) and mix well. Use 0.1 ml of this mixture for the preparation of a new high-titer phage suspension. Preserve the remaining mixture in a sterile screw-capped vial at 2° to 10°C.

To prepare a high titer phage stock: using the appropriate medium, assay 0.1 ml of rehydrated phage by the agar layer method as described by M.H. Adams in *Bacteriophages* (1959, Interscience Publishers, Inc., New York). Add 10 to 20 ml of broth to the plate of the highest dilution showing confluent lysis. Scrape off soft agar layer with a sterile glass spreader. Extract the agar/broth mixture for 30 min in the refrigerator. Sediment agar and bacterial debris by centrifuging at low speed. Filter supernatant containing the phage through a 0.22 um filter or its equivalent. Store filtrate at 2° to 10° C or frozen.

#### Fungi

Use a Pasteur pipette to add approximately 0.3 ml sterile water to the inner vial of a double vial or to a serum vial (Preceptrol®). Then draw up the entire contents into the pipette and transfer to a test tube with about 5 ml sterile water.

Let the yeast or fungus rehydrate for at least a couple hours (overnight is not too long) before transferring to broth or solid agar. Incubate at the recommended temperature. Keep in mind that some cultures may exhibit a prolonged lag period and should be given twice the normal incubation time before discarding as nonviable.

Save the mixture of lyophilized material and water until you know you have growth. If not contaminated, it will keep several days in a refrigerator. If you do subsequently contaminate your culture, you can recover the desired microorganism by serial dilution and picking single colonies.

#### Plant Viruses

Plant viruses are usually distributed in freeze-dried plant tissues within single, sealed vials. For increased stability of the contents, vials should be stored at -20° C prior to use. Vials should be opened carefully, removing the metal retaining cap and the rubber stopper. If the vial has been flame-sealed, it may be opened according to directions on the product sheet.

For tissue reconstitution and inoculum preparation, the contents of the vial should be placed in a precooled (4° C) mortar with 2 to 3 ml of an inoculation buffer (e.g., 0.05 M sodium phosphate buffer, pH 7.0, with 10 mM sodium sulfite). A pestle is used to thoroughly triturate the tissue for inoculum preparation.

The inoculum may be rubbed onto host plants using a sterile cotton swab and a fine abrasive, such as 500 to 600 mesh carborundum (silicon carbide) or celite (diatomaceous earth). The abrasive may be added to the inoculum (50 to 100 mg/ml) or dusted onto the plants prior to inoculation. After inoculation, the plants should be sprayed with water to remove buffer salts and abrasive.

## **Cultures in Stopped Serum Vials**

Freeze-dried bacteria and fungi supplied in stoppered vials (most Preceptrol® cultures) should be rehydrated with 0.5 ml of appropriate broth or sterile water, then follow the directions above. Given proper treatment and conditions most freeze-dried cultures will grow out in a few days. However, some may exhibit a prolonged lag period and should be given twice the normal incubation time before discarding as nonviable.

## **THAWING FROZEN CULTURES (other than cell lines)**

Frozen cultures should be processed immediately upon arrival. If that is not possible, store vials in liquid nitrogen vapor. Alternatively, the frozen material can be stored in a mechanical freezer between -70 and -80°C for short periods (1 to 5 days). Frozen cultures are stable in the long term only when stored at -130°C or below. Viability of cultures stored between -80 and -130°C will vary with the nature of the cryoprotective agent and the strain and can only be determined empirically.

One of the most common mistakes when storing frozen material is to place it at -20°C (the freezer compartment of a conventional refrigerator-freezer). Never store frozen cultures in this manner. Storage at this temperature for even short periods will result in rapid loss of viability.

## **Cell Lines**

Consult the product sheet for specific instructions regarding your cell line. In general, cell lines should be thawed rapidly. Place the ampoule in a water bath at 37°C and agitate until its contents have thawed completely. Transfer the contents to a culture vessel with 10 volumes of appropriate medium. This ten-fold dilution will lower the concentration of cryoprotective agent to a level that does not necessitate its immediate complete removal, and fluid-changing the cultures after 24 hours will expedite the removal of the cryoprotective agent. Some cell lines have specific procedures for removing the cryoprotectant; refer to the product sheet for details.

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If continued storage of the frozen culture is necessary, store in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

## **Protists**

Immediately upon arrival, remove the frozen ampule from the dry ice and quickly transfer it to a 35°C water bath. Add the entire thawed contents to a single small culture vessel (T-25 tissue culture flask, 16x 125 mm screw-capped test tube, agar plate, etc.) of the recommended medium and incubate at the appropriate temperature.

## **MOLECULAR BIOLOGY MATERIALS**

Transfer cultures in test tubes to fresh medium as soon as possible. Cultures may be stored at 4°C for no longer than two weeks. For freeze-dried cultures, refer to the **Bacteria** section above.