INTRODUCTION

- All cultures should be regarded as potentially hazardous and should be opened by persons trained in microbiological techniques working in facilities with containment requirements appropriate for the biosafety level of the cultures.
- Work in a biological safety cabinet. If this is not possible, wear suitable eye protection. Hold vials away from face and over a can or tray.
- Wear gloves.
- Sterilize all empty vials and fragments before disposal.
Check each culture thoroughly upon receipt. If a culture is unsatisfactory, notify ATCC so that the strain in question can be investigated. Use the medium and incubation conditions specified on the product information sheet when first reviving strains to ensure optimal conditions for recovery.

If cultures are not revived immediately, store vials at 5°C or colder (plant viruses at -20°C or colder).

**BACTERIA AND ALGAE**

Aseptically add 0.5 mL of liquid medium to the freeze-dried material with a sterile Pasteur pipette and mix well. For bacteria, transfer the total mixture to a test tube containing 5 to 6 mL of the recommended broth medium. The last few drops of this suspension also may be transferred to an agar slant. 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**

1. Prepare an actively growing broth culture of the host before opening the phage specimen. The host should be 18 to 24 hours old prior to inoculation.

2. To propagate phage: Aseptically rehydrate the freeze-dried phage specimen with 1.0 mL of appropriate broth (see maintenance conditions for host) and mix well. Add 0.1 mL of this mixture to 0.9 mL of broth medium and serially dilute as desired. One drop of each dilution can be spotted onto prepared plates (three or four dilutions per plate). Lysis should be visible after overnight incubation.

3. To prepare a high-titer phage stock: Refer to the product sheet for instructions.

**FILAMENTOUS FUNGI AND YEAST**

Use a Pasteur pipette to add 0.5 to 1 mL sterile water to the freeze-dried pellet 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 a minimum of 2 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 for several days in a refrigerator. If you 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. Open the vials carefully, removing the metal retaining cap and the rubber stopper. If the vial has been flame-sealed, open according to directions on the other side of this sheet.

For tissue reconstitution and inoculum preparation, place the contents of the vial 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). Triturate the tissue thoroughly with a pestle to prepare the inoculum.

Rub the inoculum 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, spray the plants with water to remove buffer salts and abrasive.

**CULTURES IN STOPPERED SERUM VIALS**

Open serum vials carefully by aseptically removing the metal retaining cap and the rubber stopper, then follow instructions for the appropriate type of organism.

If you have questions, please contact a technical service representative at tech@atcc.org or 800-638-6597 (703-365-2700), or contact your local distributor. Details regarding our warranty can be found in the Material Transfer Agreement packed in your shipment or available at www.atcc.org.