GLYCEROL FREEZING BROTH

- For in vitro use only - Catalogue No. TG57

Our Glycerol Freezing Broth is used in the long-term frozen maintenance of bacterial cultures.

Freezing is a very common way of preservation and storage of microorganisms. Numerous researchers have reported the use of different additives to enhance survival of frozen bacteria. The addition of glycerol greatly enhances recovery of bacteria as reported by Costillo et al. Glycerol is a cryoprotective agent that is theorized to inhibit intracellular ice formation during rapid freezing.

Our Glycerol Freezing Broth consists of tryptic soy broth supplemented with glycerol; this medium has been described by the Centers for Disease Control (CDC), the American Society for Microbiology and the National Committee for Clinical Laboratory Standards (NCCLS).

Formula per Litre of Medium

Pancreatic Digest of Casein	17.0 g
Papaic Digest of Soybean Meal	3.0 g
Sodium Chloride	5.0 g
Dipotassium phosphate	2.5 g
Dextrose	6.0 g
Ascorbic Acid	5.0 g
Glycerol	150 mL

pH 7.3 ± 0.2

Recommended Procedure

Freezing Protocol

1. Allow the medium to warm to room temperature prior to inoculation.
2. Heavily inoculate the broth using a pure, fresh culture of the desired microorganism.
3. Place the inoculated cryovials in a freezer maintained at less than or equal to -50°C. The vials may also be stored in an ultra-low temperature freezer or vapor phase liquid nitrogen storage tank.

Thawing Protocol

1. When needed, remove a vial from the freezer or liquid nitrogen storage tank.
2. Thaw the contents rapidly by placing in luke-warm water. Do not exceed a 3-hour waiting period before using the thawed suspension; organism viability has shown to decrease dramatically after 3 hours for some fastidious organisms.
3. The contents of the vial can be used to prepare working control cultures or alternatively, the organism can be subcultured onto a non-selective medium such as blood agar to obtain isolated colonies after overnight incubation.

Interpretation of Results

Our Glycerol Freezing Broth can be used for the maintenance of a wide variety of fastidious and non-fastidious organisms. At -50°C, strains be kept for 1 year; strains may be kept indefinitely at temperatures less than -70°C. Once thawed, the cultures should be discarded after use; unused cell suspensions should never be refrozen due to decreased organism viability and possible contamination of the contents.

The NCCLS recommends that only up to three serial subcultures of the frozen stock culture be prepared prior to discarding the culture. Additional subcultures may be more prone to phenotypic alterations and may result in atypical results.

- *Microorganisms should not be left for more than 3 hours at room temperature in order to achieve highest recovery*
- *An increase in the initial cell concentration enhances the percentage of viable microorganisms after freezing*
- *Stock cultures should be checked annually for viability*

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Quality Control

After checking the medium for correct pH, colour, depth, and sterility, the following organisms are used to determine the performance of the completed medium. The inoculated vials are frozen at -50°C for 48 hours and sub-cultured onto Sheep Blood Agar.

Organism	Expected Results
Staphylococcus aureus ATCC 25923	Growth, good recovery
Escherichia coli ATCC 25922	Growth, good recovery

Storage and Shelf Life

Our Glycerol Freezing Broth should be stored in an upright position at 4°C to 8°C. Under these conditions the medium has a shelf life of 26 weeks from the date of manufacture.

References

1. Major CP, Dougal JD, Harrison Jr AP. The effect of the initial concentration upon survival of bacteria at -20°C. J Bacteriol 1955; 69:244-9.
2. Meryman HT, Hornblower M. Changes in red blood cells following rapid freezing with extracellular cryoprotective agents. Cryobiology 1972; 9:262-7.
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5. NCCLS. Quality assurance of commercially prepared microbiological media. 2nd ed. NCCLS document M22-A2. Wayne, PA: NCCLS, 1996.
6. Murray PR, Baron E, Pfaller M, Tenover F, Yolken R. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.