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Oxyrase CryoBroth™ Product Insert

Oxyrase CryoBroth™ is a sterile enzyme formulation that provides a protective anaerobic environment by utilizing Nature's Antioxidant® for preservation and enhanced recovery of organisms that have been stored cryogenically.

Precautions:
Oxyrase CryoBroth™ is a filter sterilized product and must be handled aseptically to maintain sterility. A **Safety Data Sheet** is available on our website.

Product Characteristics:
The formulation of Oxyrase CryoBroth™ combines cryo-protectants (to minimize damage from freezing / thawing) and Nature's Antioxidant® (to enhance the recovery of cells from the frozen state).

Freezing and thawing causes cell damage (3), Oxyrase CryoBroth™ has been used for the storage and recovery of anaerobes (including facultative anaerobes) and numerous other cell cultures (1). Oxyrase® Enzyme System enhances recovery of injured cells (2) and has been used to preserve cells frozen and stored in cryogenic conditions (3,4).

Limitations:
Oxyrase CryoBroth™ contains a penicillin binding protein that may interfere with penicillin and some related antibiotics.

Handling and Storage Instructions:
Oxyrase CryoBroth™ will arrive thawed but cold. Storage options are listed below:

1. Long Term Storage: Store the product at ≤ -20°C to maintain full activity. Oxyrase CryoBroth™ can be thawed and re-frozen five times without affecting its activity and performance. In cases where the product will be used infrequently and / or in small amounts, aseptically aliquot the product into smaller, individual, sterile containers (refer to short term storage, if needed).
2. Short Term Storage: Store the product at 2°C to 8°C for use within 30 days (a precipitant may form at this temperature).

When stored in this manner, the product will maintain its full activity to the printed expiration date on the label.

Thawing Oxyrase CryoBroth™:
A convenient way to thaw Oxyrase CryoBroth™ is to place it in the refrigerator overnight.

If necessary, the product can be thawed by warming. *Do **not** exceed a warming temperature of 37°C*. Only apply heat to the outside of the container while ice is still present inside the container. When all ice has melted, keep the product chilled by placing the container in ice until ready for use.

To ensure uniform activity within a thawed sample, *gently* mix the product before use or distribution (*do **not** agitate vigorously).* Vigorous agitation (i.e. shaking) causes foaming and denatures protein in the product, which may result in loss of activity.

In some cases, precipitate may be observed, but will not affect Oxyrase CryoBroth™ performance.

Instructions for Use:
Oxyrase CryoBroth™ is sterile and must be handled aseptically. It should not be agitated or vigorously shaken.

Solid materials are added directly to Oxyrase CryoBroth™ to avoid dilution. Thus, anaerobic bacteria would be added by suspending a colony or a loopful of confluent growth in Oxyrase CryoBroth™.

If the material to be stored is in a liquid, then add 1-part liquid to 2-parts Oxyrase CryoBroth™. Cell suspensions should be made at a high density (10/9 cfu/mL).

Place materials suspended in Oxyrase CryoBroth™ into a cryogenic freezer immediately after distribution.

After thawing Oxyrase CryoBroth™ samples, immediately transfer cells to a growth medium appropriate for cultivation.

Before committing a sample to storage in Oxyrase CryoBroth™ for the first time, a test should be made. Prepare and freeze the sample for 7-14 days. Then, thaw the sample and test for viability.

Quality Control:
The length of time that cells can remain viable in Oxyrase CryoBroth™ is dependent on numerous factors: nature, type of cell culture, and storage conditions of cryo-vials.

It is recommended that recovery of Oxyrase CryoBroth™ cell suspensions be scored and recorded each time that an Oxyrase CryoBroth™ vial is used. This history of cell viability, along with storage time, provides a useful record to determine optimal storage conditions.

Guarantee:
Oxyrase CryoBroth™ has a shelf-life of 12 months from date of manufacture, under recommended storage and use conditions.

If Oxyrase CryoBroth™ does not preserve and enhance recovery of organisms that have been stored cryogenically as specified under recommended storage and use conditions, Oxyrase, Inc. will refund your purchase price. To receive a product refund, write or call Oxyrase Inc. with the product lot number which is located on the Oxyrase CryoBroth™ label. Oxyrase, Inc. is available to answer any questions about this product and its applications.

1. Linda S.L. Yu and Daniel Y., C. Fung. *1990*. Effect of Oxyrase Enzyme on *Listeria Moncytogenes* and Other Facilitative Anaerobes. J. Food Safety 11:**3**, 163-175.
2. SM George, LLC Richardson, IE Pol, MW Peck. *1998.* Effect of Oxygen Concentration and Redox potential on Recovery of Sub-Lethally Heat-Damaged Cells of *E. coli* 0157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*. Appl. Microbiol. **84:5**, 903-909.
3. Mazur, Kathov, Katkova, and Critser. *2000*. The Enhancement of the Ability of Mouse Sperm to Survive Freezing and Thawing by the Use of High Concentrations of Glycerol and Presence of an *Escherichia coli* Membrane Preparation (Oxyrase) to Lower the Oxygen Concentration. Cryobiology. **40:4**, 187-209.
4. Koshimoto, Gamliel, Mazur. *2000*. Effect of Osmolality and Oxygen Tension on the Survival of Mouse Sperm Frozen to Various Concentrations of Glycerol and Raffinose. Cryobiology. **41:3**, 204-231.