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EC (and EC/NS) Oxyrase® Enzyme System Product Insert

Intended Use

EC (and EC/NS) Oxyrase[®] Enzyme System (Oxyrase) is a filtered enzyme additive used to produce anaerobic conditions in a wide variety of environments. It is available in a sterile (EC) and non-sterile (EC/NS) form.

Handling and Storage Instructions

The EC (and EC/NS) Oxyrase enzyme system has a shelf life of 18 months from the date of manufacture, when stored at a constant -20°C or colder. We guarantee that each order will have a minimum shelf life of 6 months when received. If needed, a longer useable shelf life needs to be requested when an order is placed. The Oxyrase enzyme system is shipped frozen, and will arrive partially thawed but cold. Upon receiving the product you have the following options:

- 1. <u>Short Term Storage:</u> Store the product at 4°C for use within 30 days. A precipitate may form at this temperature.
- 2. <u>Long Term Storage</u>: Store the product at a constant -20°C or colder until needed. You can thaw and re-freeze the EC (and EC/NS) Oxyrase five times without affecting its activity and performance. In cases where the product will be used infrequently and/or in small amounts, one can aseptically aliquot the product into smaller, individual, sterile containers. As needed, thaw each container once and discard after use. This minimizes the amount of freezing and thawing of EC (and EC/NS) Oxyrase. When stored in this manner, the product will maintain its full activity to the expiration date on the label.

Always handle EC (and EC/NS) Oxyrase aseptically. To prevent bacteria from growing never let EC/NS Oxyrase stand at temperature above 4°C.

Thawing Oxyrase

A convenient way to thaw the EC (and EC/NS) Oxyrase enzyme system is to place it in the refrigerator overnight. If necessary, you can also thaw the product by warming. When warming the product, always keep it cold to the touch. Do not exceed a warming temperature of 37°C. Only apply heat to the outside of the container while ice is still present inside. Frequently mix the product by gently swirling the contents. When all ice has melted, keep the product chilled by placing the bottle in ice until ready to use. To insure uniform activity of thawed samples, gently mix the product before use or distribution. Do not agitate vigorously. Vigorous agitation, such as shaking, causes foaming and denaturing of the proteins in the product which may result in a loss of activity.

Summary and Explanation

EC (and EC/NS) Oxyrase is an enzyme system derived from the cytoplasmic membrane of the microorganism, *Escherichia coli*. EC (and EC/NS) Oxyrase consists of a buffered suspension of particles, 0.2 microns or smaller. Although EC (and EC/NS) Oxyrase are made identically, EC Oxyrase is filtered sterilized and packaged aseptically; whereas, EC/NS Oxyrase is non-sterile, but has a bioburden less than 10³ cfu per milliliter. EC (and EC/NS) Oxyrase is active over a wide pH range. The rate of oxygen removal by EC (and EC/NS) Oxyrase increases with temperature. Above 55°C, EC (and EC/NS) Oxyrase is rapidly inactivated.

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Activity

One unit of EC (and EC/NS) Oxyrase activity will reduce dissolved oxygen (in 1 mL of air saturated 40 mM phosphate Buffer, pH 8.4, at 37°C) at the rate of 1% per second. Refer to the **Assay of Oxyrase Activity** for details.

EC (and EC/NS) Oxyrase enzyme system has a minimum activity of 30 units per milliliter when stored properly and used by the expiration date on the label.

Usage

The exact amount of EC (and EC/NS) Oxyrase and substrates needed to reduce oxygen in a given system are determined by a number of parameters including pH, temperature, kinds and amounts of substrates present, surface to depth ratio of the container, and head-space volume. Some experimentation may be necessary to optimize EC (and EC/NS) Oxyrase for a particular use. A suggested use level is a 1:100 dilution of the product. Oxyrase Inc. technical personnel are available to advise you in using Oxyrase for your application. Please feel free to contact us for more information.

Substrates

Lactic acid, succinic acid, formic acid, alpha-glycerol phosphate, or their salts, are substrates used for EC (and EC/NS) Oxyrase. Typically 10 mM to 20 mM of substrate is sufficient for EC (and EC/NS) Oxyrase activity in most applications. In some instances by adding more substrate or combinations of substrates, the rate of oxygen reduction is faster. The product of the reaction of EC (and EC/NS) Oxyrase with lactic acid is pyruvic acid; with formic acid it is carbon dioxide and water; with succinic acid it is fumaric acid; and with alpha-glycerol phosphate it is dihydroxyacetone phosphate.

Composition

EC (and EC/NS) Oxyrase consists of bacterial cell membrane fragments in particulate suspension in 20 mM Phosphate Buffer at a neutral pH. It contains 30 mg/ml or less dry weight solids. EC (and EC/NS) Oxyrase contains a substrate, L-Sodium Lactate at 100 mM to stabilize the enzyme during freeze-thaw cycles.

Precautions

Keep containers closed. When handling the product avoid getting on skin or in eyes. Do not ingest product. In accordance with good practices of personal cleanliness and hygiene, handle the product with due care and avoid and unnecessary contact with it. For more detailed information please refer to the **EC Oxyrase Material Safety Data Sheet.**

Limitations

Note for Microbiological uses only: EC (and EC/NS) Oxyrase contains a penicillin binding protein that may inactivate penicillin and some related antibiotics.

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