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

The Oxyrase® Enzyme System (Oxyrase) is obtained from the cytoplasmic membrane of the microorganism, *Escherichia coli*, used as Nature's Antioxidant® to produce anaerobic conditions in a wide variety of environments.

Oxyrase is available in a sterile (EC) and non-sterile (EC/NS) form. EC (and EC/NS) Oxyrase consist of a buffered suspension of particles made identically. EC Oxyrase is filtered sterilized and aseptically packaged; whereas EC/NS Oxyrase is non-sterile but has a bioburden less than 10<sup>3</sup> cfu per milliliter.

### **Precautions:**

Oxyrase is for In-Vitro Use only. EC Oxyrase is a filter sterilized product and must be handled aseptically to maintain sterility.

To prevent bacteria from growing within EC/NS Oxyrase, use and store EC/NS Oxyrase at a steady temperature colder than 8°C (refer to handling and storage instructions, if needed).

Some microorganisms produce significant amounts of gas during growth. To prevent buildup of pressure due to gas production, lids / caps can be loosely affixed to allow for the escape of gas and will not compromise the anaerobic conditions maintained in a medium containing EC (and EC/NS) Oxyrase. A **Material Safety Data Sheet** is available on our website.

### **Product Performance:**

EC (and EC/NS) Oxyrase consists of bacterial cell membrane fragments in 20 mM Phosphate Buffer at a neutral pH. Dry weight solids are 30 mg/mL or less.

Substrates for EC (and EC/NS) Oxyrase include lactic acid, succinic acid, formic acid or their salts, and alpha-glycerol phosphate in addition to oxygen. EC (and EC/NS) Oxyrase also contains L-Sodium Lactate at 100 mM to stabilize the Oxyrase during freeze-thaw cycles. This amount of substrate is diluted to extinction upon use. Substrate needs to be added (in the 10 - 20 mM range), to activate the enzyme system.

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. The rate of oxygen removal increases with temperature; however, above 55°C, EC (and EC/NS) Oxyrase is rapidly inactivated. Refer to the **Assay of Oxyrase Activity** for details. EC (and EC/NS) Oxyrase is active over a wide pH range of 6.8 to 8.4.

*(Under temperature and pH conditions beyond those specified the addition of more product / substrates and / or allowance for more time may be required to achieve complete anaerobiosis.)*

### **Limitations:**

EC (and EC/NS) Oxyrase contains a penicillin binding protein that may interfere with penicillin and some related antibiotics.

EC (and EC/NS) Oxyrase is not a substitute for nutrients or gasses required for growth of anaerobic microorganisms. For reduced environments, a chemical reducing agent may be required.

### **Handling and Storage Instructions:**

*Oxygen irreversibly inactivates Oxyrase in the absence of substrate.*

*Oxyrase loses activity at a rate of:*

*0.5 units/hr at 5°C*

*1.5 units/hr at 20°C*

*2.38 units/hr at 37°C*

*To protect Oxyrase activity, keep container headspace to a minimum. The headspace contains 28 times more oxygen than an equivalent volume of saturated water. Optionally, you can protect Oxyrase activity by putting a blanket of CO<sub>2</sub> over an Oxyrase solution to block O<sub>2</sub> intrusion. Be aware that solubilized CO<sub>2</sub> drives pH down. Protect Oxyrase activity by adding substrates to the solution. Remember, it takes 2 moles of substrate (L-lactate) to reduce 1 mole of O<sub>2</sub> to H<sub>2</sub>O. Refer to the Oxyrase Website Technical Page for a Lactate calculator and more information on this subject. Finally, Oxyrase activity can be protected by keeping Oxyrase solutions frozen when not in use.*

EC (and EC/NS) Oxyrase will arrive thawed but cold. The following storage options are listed below:

1. **Long Term Storage:** Store the product at a constant -20°C or colder to maintain full activity.

EC (and EC/NS) Oxyrase 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, **air-tight** containers **with minimum headspace** (refer to short term storage, if needed). To minimize the amount of freezing and thawing of the product, thaw each container once and discard after use.

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 a minimum activity of 30 units per mL to the printed expiration date on the label.

### **Thawing EC (and EC/NS) Oxyrase:**

A convenient way to thaw EC (and EC/NS) Oxyrase 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 EC (and EC/NS) Oxyrase performance.

### **Instructions for Use:**

The exact volume 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; a suggested use level is a 1:100 dilution of the product.

### **User Quality Control:**

The confirmation of EC (and EC/NS) Oxyrase activity may be verified by using a methylene blue test (refer to the **Assay of Activity** for details).

### **Guarantee:**

EC (and EC/NS) has a shelf-life of 18 months under recommended storage and use conditions in an unopened bottle. We guarantee a minimum of 6 months shelf-life from shipment date. If a longer shelf-life is needed, this should be arranged at the time your order is placed.

If EC (and EC/NS) does not maintain a minimum activity of 30 units per mL 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 EC (and EC/NS) label. Oxyrase, Inc. is available to answer any questions about this product and its applications.