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## Oxyrase® for Broth (OB) Product Insert

Oxyrase® for Broth is a sterile enzyme formulation that uses Nature's Antioxidant® to produce anaerobic conditions in a wide variety of bacteriological broth media.

### Precautions:

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

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

### Product Performance:

The formulation of OB combines substrates and Oxyrase®.

OB creates an anaerobic environment when added to bacteriological broth medium by keeping oxygen from intruding into the broth; eventually removes oxygen from head space of the sealed container.

When used as recommended within a bacteriological broth medium, OB reduces the oxygen concentration to less than 10 ppB (parts per billion) within 30 minutes and maintains an anaerobic environment within the bacteriological broth medium for at least 16 days at 35°C to 37°C in a sealed vessel incubated without shaking or mixing. OB should only be used with broth media that has an initial pH range of 6.8 to 8.4. (*OB works under temperature and pH conditions beyond those specified. However, you may need to add more product / substrates and / or allow for more time to achieve complete anaerobiosis.*)

OB works as specified in many types of broth medium, including the following list of commercially prepared media:

Columbia Broth (CB)	Brain Heart Infusion Broth (BHI)
Schaedler Broth	Brucella Broth
Nutrient Broth (NB)	Trypticase Soy Broth (TSB)
Eugon Broth	Meuller-Hinton Broth

Oxyrase® for Broth is not a substitute for nutrients or gases required for growth of anaerobic microorganisms. For reduced environments, lower than achieved by complete oxygen removal, a chemical reducing agent is required.

A thin layer of sterile mineral oil placed on top of the broth seals off the broth from oxygen in the headspace, assuring an anaerobic environment in the broth. This is helpful for stringent anaerobes.

### Limitations:

OB contains a penicillin binding protein that may interfere with penicillin and some related antibiotics.

OB is not a substitute for nutrients or gases required for growth of anaerobic microorganisms. For reduced environments, a chemical reducing agent may be required.

### Handling and Storage Instructions:

OB 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.

OB 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). 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 but does not affect product performance).

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

### Thawing Oxyrase® for Broth:

A convenient way to thaw OB 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 OB performance.

### Instructions for Use:

Aseptically, add 0.1 mL of OB to each 1.0 mL of *prepared, sterile* broth medium (if using a disposable transfer pipette, one drop of OB per mL of broth is a sufficient measure). *Do **not agitate vigorously** or **aerate** the suspension.* Incubate at 35°C to 37°C. Broth will become completely anaerobic in 30 minutes or less. Minimize the area of liquid surface exposed to air when possible. The specific gravity of the OB solution is calculated at 1.0345 g/mL.

### User Quality Control:

The performance of OB may be verified by inoculating one broth medium tube (BHI or TSB) with OB, and one tube without OB. Using anaerobic control microorganisms (i.e. *B. fragilis* - ATCC 25285 and *F. nucleatum* - ATCC 25586), inoculate mediums with a culture adjusted to a McFarland Turbidity Standard. Incubate both tubes aerobically **without agitation** at 35°C to 37°C for 48 to 72 hours.

If the OB is performing as specified, the OB treated broth will show heavy turbidity, whereas the aerobic control will remain clear.

### Guarantee:

OB has a shelf-life of 18 months under recommended storage and use conditions. 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 OB does not create an anaerobic environment 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® for Broth label. Oxyrase, Inc. is available to answer any questions about this product and its applications.