



**Oxyrase, Inc.**  
P. O. Box 1345  
Mansfield, Ohio 44901  
www.oxyrase.com  
Ph: 419-589-8800 Fax: 419-589-9919

---

### **Oxyrase® for Broth Product Insert**

Oxyrase for Broth is a sterile enzyme additive used to produce anaerobic conditions in a wide variety of bacteriological agar medium.

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

Oxyrase for Broth will arrive thawed but cold. You have the following storage options:

1. Long Term Storage: Store the product at a constant -10 C to -20 C temperature. You can thaw and re-freeze Oxyrase for Broth 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 Oxyrase. When stored in this manner, the product will maintain its full activity to the expiration date on the label.
2. Short Term Storage: Store the product at 4 C for use within 30 days.

#### **Thawing Oxyrase for Broth**

A convenient way to thaw Oxyrase for Broth 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 ensure 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 protein in the product which will result in loss of activity.

#### **Instructions for Use**

1. Aseptically add 0.1 ml of Oxyrase for Broth to each 5.0 ml of *prepared, sterile* broth medium. Alternatively, it may be convenient to use a disposable transfer pipet to distribute Oxyrase for Broth to numerous tubes. One drop of Oxyrase for Broth per ml of broth is a sufficient measure.
2. Incubate at 35 C to 37 C. The broth medium will be completely anaerobic within 30 minutes. When working with Oxyrase for Broth, use a screw cap tube, bottle or other closed vessel that minimizes the area of liquid surface exposed to air in the head space.

#### **Product Performance**

Oxyrase for Broth creates an anaerobic environment when added to bacteriological broth medium. Oxyrase for Broth keeps oxygen from intruding into the broth medium and removes oxygen from the trapped air space inside the tube or bottle.

Oxyrase for Broth should only be used with broth media that has an initial pH range of 6.8 to 8.4. When using the recommended concentrations and when incubated at 35 C to 37 C, the broth media will become completely anaerobic in 30 minutes or less.

You can incubate culture vessels, containing media supplemented with Oxyrase for Broth aerobically. Oxyrase for Broth keeps oxygen from intruding into the broth medium.

Oxyrase for Broth maintains an anaerobic environment in bacteriological broth medium for at least 16 days at 35 C to 37 C in a vessel incubated without shaking or mixing.

Oxyrase for Broth will not add visible turbidity to the standing bacteriological broth media for up to 16 days at 35 C to 37 C when used as instructed. A light precipitate may settle in the bottom of the tube with time. This precipitate will not interfere with observing growth in the tube.

## Summary and Explanation

Oxyrase for Broth is a formulated product that contains The Oxyrase Enzyme System and its substrates. When used as recommended with bacteriological broth media, Oxyrase for Broth reduces oxygen concentration to less than 10 ppBillion within 30 minutes and maintains this level of anaerobiosis for at least 16 days without contributing visible turbidity to the standing medium.

Oxyrase for Broth is for broth media with an initial pH of 6.8 to 8.4.

Oxyrase for Broth works as specified in the following commercially prepared media:

Columbia Broth	Brain Heart Infusion	Schaedler Broth
Brucella Broth	Nutrient Broth	Trypticase Soy Broth
Eugon Broth	Meuller-Hinton Broth	

We expect that Oxyrase for Broth will work in other broth media as well.

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

Oxyrase for Broth works under temperature and pH conditions beyond those specified. However, you may need to add more product and/or time to achieve complete anaerobiosis.

## Precautions

Oxyrase for Broth is for In Vitro Use only. Oxyrase for Broth is for use in bacteriological broth media. Oxyrase for Broth is a filter sterilized product and must be handled aseptically to maintain sterility. Some microorganisms produce significant amounts of gas during growth. Caps can be loosely affixed to allow for the escape of gas and to prevent build up of pressure due to gas production. Loose fitting caps will not compromise anaerobiosis in a medium containing Oxyrase for Broth. A **Material Safety Data Sheet** is available upon request.

## Limitations

Oxyrase for Broth contains a penicillin binding protein that may inactivate penicillin and some related antibiotics.

## User Quality Control

The performance of broth medium supplemented with Oxyrase for Broth may be verified by streaking the medium with pure cultures of stable, anaerobic control microorganisms shown to grow in that medium. Recommended anaerobic microorganisms for testing the media include: *Bacteriodes fragilis* ATCC 25285 and *Fusobacterium nucleatum* ATCC 25586. Recommended medium include Brain Heart Infusion Broth and Trypticase Soy Broth. Other important points to remember when testing the media are listed below.

- Follow instructions for using Oxyrase for Broth.
- Use only fresh cultures that are 18 to 24 hours old.
- Inoculate a 1:1000 dilution of a suspension of microorganisms equal to a McFarland No. 0.5 turbidity standard.
- Incubate without agitation at 35 C to 37 C for 24 hours in an aerobic incubator.

As an aerobic control, inoculate broth media that does not contain Oxyrase for Broth and incubate at 35 C to 37 C in an aerobic incubator. Incubate both plates aerobically at 35 C to 37 C for 48 to 72 hours.

If the Oxyrase for Broth is performing as specified, the inoculated culture in the media supplemented with Oxyrase for Broth will show heavy turbidity where as the aerobic control will remain clear.

## Guarantee

Oxyrase for Broth has a shelf life of 18 months under recommended storage and use conditions. We guarantee that each order will have a minimum shelf life of 6 months when shipped. Longer useable shelf life needs to be requested when an order is placed. If Oxyrase for Broth does not create an anaerobic environment in a bacteriological broth medium as specified and 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 bottle label. Oxyrase, Inc is available to answer any questions about this product and its applications.