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Oxyrase® for Agar & OxyDish System
Product Insert

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

Handling and Storage Instructions

Oxyrase for Agar 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 Agar five times without affecting its activity and performance. 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 Agar

A convenient way to thaw Oxyrase for Agar 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 Filling, Drying, and Storing Plates

1. Prepare agar medium using 10% less water than usual and autoclave. This volume will be recovered when adding Oxyrase for Agar.
2. Using a water bath, bring the temperature of sterile agar medium to 45 C - 48 C.
3. Thaw Oxyrase for Agar according to the instructions listed above in the "Handling and Storage" section.
4. Warm thawed Oxyrase for Agar to room temperature, just before adding it to the warm, sterile agar medium. *Adding cold Oxyrase for Agar to the medium will cause premature gelling of agar.*
5. Add the Oxyrase for Agar to the medium by pouring or pipetting it *down the side* the flask. One OA-0050 is used with 500 ml of medium. One OA-0100 for 1000 ml of medium. For other volumes, use a *1:10 dilution* of Oxyrase for Agar into the medium.
6. Swirl the flask to mix the Oxyrase for Agar into the medium. *Avoid foaming.*
7. Begin distributing the medium immediately after adding the Oxyrase for Agar.
8. Place an OxyDish™ in an upright position on a level surface. Remove the dish top and place it to the side.
9. Deliver 22 - 24 ml of molten agar medium into each dish base. Deliver more agar for plates that will be stored. An autopippetor is suggested.
10. When distributing the media, *retain a small amount of agar in the pipette.* This *avoids delivering foam and bubbles into the dish*, which will in turn interfere with the dish seal. If a bubble does get into the agar in the dish base, draw it up with the pipette.
11. If the filled dish base is to be moved before the agar solidifies, do so before placing the dish top over the base.
12. Allow the agar to completely solidify in the dish base.
13. Prior to use, the plate must be dried so that water does not condense within the dish during incubation or storage. Three simple methods for drying the plates are 1) Place the open plate in a laminar flow hood for less than 60 minutes or 2) Place the uncovered plate in a clean, dry incubator at 40 C to 45 C for 45 minutes or 3) Leave the covered, but not sealed plate in an upright position at room temperature for 2-4 hours. *It is very important that the plate surface dries evenly so that an effective seal may be formed.* You may need to modify these times and temperature to accommodate to your laboratory equipment and local environment.

Instructions for Filling, Drying, and Storing Plates (cont)

14. After drying, replace the dish top. Invert the assembled dish to form a seal. The plate may be stored at this point or used immediately.
15. Store plates in a closed container in a refrigerator. Depending on the media type and its intended use, plates can be stored for up to 14 days.

Use of Oxyrase for Agar with the Pour Plate Method

Prepare the agar media as described above. Make the media as usual, *do not make with 10 % less water*. Distribute the agar into the appropriate bottles or tubes. Autoclave the media before storage or use. Prior to use, melt the agar and bring to 45 C to 48 C. Just before making the pour plate, add a *1:30 dilution of Oxyrase for Agar* to the prepared medium. Pour the medium into the base of a standard petri dish containing the inoculum. Mix the inoculum into the medium by gently swirling the dish base. After the medium has solidified overlay the media in the dish base with agar medium that does or does not contain the Oxyrase for Agar. This depends on the organism you are growing, for strict anaerobes use media supplemented with Oxyrase. Use approximately 6 ml to 8 ml of agar media for the overlay. Incubate the plates aerobically at 35 C to 37 C.

Product Performance

Oxyrase for Agar creates an anaerobic environment when added to bacteriological agar medium. Oxyrase for Agar keeps oxygen from intruding into the agar medium. When used with the OxyDish, Oxyrase for Agar will also remove oxygen from the trapped air space inside of the sealed OxyDish. OxyDishes containing media supplemented with Oxyrase for Agar, are incubated aerobically at temperature up to 37 C. *OxyPlates may be opened and closed up to 3 times after incubating the agar surface with bacteria.* After the OxyPlate has been opened and closed, the plate will reestablish an anaerobic environment. OxyPlates may be staked to conserve incubator space. If necessary, humidify the incubator to prevent over-drying the plates.

Summary and Explanation

Oxyrase for Agar is a formulated product that contains The Oxyrase Enzyme System and its substrates. When used as recommended with bacteriological agar media, Oxyrase for Agar creates and maintains an anaerobic environment. *Oxyrase for Agar is for media with a pH range of 6.8 to 8.4*

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

Columbia CNA	Anaerobic Laked Blood	Brain Heart Infusion
Brucella agar	CDC-ANA Blood	Columbia Agar
Eugon Agar	Meuller-Hiton Agar	Nutrient Agar
Schaedler Agar	TSA	Wilkins Chalgren

We expect that Oxyrase for Agar will work in other agar media as well. Oxyrase for Agar can be used with whole blood or blood fractions in bacteriological media.

Oxyrase for Agar 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 Agar 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 Agar is for In Vitro Use only. Oxyrase for Agar is for use in bacteriological agar media. Oxyrase for Agar is a filter sterilized product and must be handled aseptically to maintain sterility. **Do not autoclave Oxyrase for Agar; this will inactivate the enzyme system!** A *Material Safety Data Sheet* is available upon request.

Limitations

Oxyrase for Agar contains a penicillin binding protein that may inactivate penicillin and some relates antibiotics.

User Quality Control

The performance of medium supplemented with Oxyrase for Agar 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 *Peptostreptococcus anaerobisis* ATCC 27337. Recommended medium include Brain Heart Infusion Agar and Brucella Agar. Other important points to remember when testing the media are listed below.

- Follow instructions for using Oxyrase for Agar.
- Use only fresh broth cultures that are 18 to 24 hours old.
- Streak plate with loopful of microorganisms from the above cultures.

As an aerobic control, streak a standard petri plate or an open OxyPlate. Incubate both plates aerobically at 35 C to 37 C for 48 to 72 hours. If the Oxyrase for Agar is performing as specified, the anaerobic plate will contain colonies of the test microorganism; the aerobic plate will not contain colonies.

OxyDish Features

The OxyDish is very similar to a standard culture dish however, there are some very *significant differences*. There features are pointed out below.

OxyDish Base: The base is the same size as a regular petri dish. It contains an isomark.

OxyDish Top: The top has a number of unique features. It contains a sealing ring. The top is designed to set over the dish base after it has been filled, without the sealing ring touching the molten agar. *When the base is filled with solidified agar and the dish inverted, the agar surface will come to rest on this sealing ring.* In this configuration, the Oxyrase for Agar in the medium will remove the Oxygen that is trapped in the space between the surface of the agar and the dish top.

Handling OxyPlates

To Open:

1. Place the OxyPlate on the bench with lid facing up.
2. Gently squeeze opposite sides of the lid to release the seal.
3. Lift the lid off.

To Close:

1. Simply align the base, with the agar facing down, over the lid, with the ring in the lid facing up.
2. Drop the base into the lid.
3. A seal will form in 10 to 20 seconds.

Guarantee

Oxyrase for Agar 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 Agar does not create an anaerobic environment in a bacteriological agar 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.