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OxyDish™* / Oxyrase® for Agar Product Insert

* - US Patent #6,204,051

The patented OxyDish™ is specially designed to create a seal that maintains anaerobiosis, after Oxyrase® for Agar (OA) has been added to a poured agar medium.

The OxyDish™ is very similar to a standard culture dish; however, there are some very *significant differences:* 1) The base is the same size as a regular petri dish, but contains an isomark useful for automation purposes. 2) The lid contains a sealing ring to set over the dish base after it has been filled. When the base is filled with solidified agar and the dish inverted, the agar surface will come to rest on this sealing ring.

Oxyrase® for Agar is a formulation containing Nature's Antioxidant®, the Oxyrase® Enzyme System. The Oxyrase® Enzyme System produces anaerobic conditions by removing oxygen from within the agar and from the confined head space above the agar surface. This creates and maintains a stand alone anaerobic environment without the need for bags, chambers, or jars. The use of the OxyPlate™ simplifies working with anaerobes *(refer to OxyPlate™ Product Insert - LAB.0017) for more details)*.

Precautions:

Oxyrase® for Agar is for In-Vitro Use only. Oxyrase® for Agar is a filter sterilized product and must be handled aseptically to maintain sterility. A **Material Safety Data Sheet** is available on our website.

Product Performance:

Oxyrase® for Agar combines substrates and Oxyrase with an agar medium that has a pH range of 6.8 to 8.4, and may include whole blood or blood factions. The following list of commercially prepared media have been used with Oxyrase® for Agar:

Columbia (CNA)	Anaerobic Laked Blood	Brain Heart Infusion
Brucella Agar	CDC-ANA Blood	Columbia Agar
Eugon Agar	Trypticase Soy (TSA)	Nutrient Agar
Schaedler Agar	Mueller-Hinton Agar	Wilkins Chalgren

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.

Do ***not*** autoclave Oxyrase® for Agar. This will inactivate the enzyme.

Limitations:

Oxyrase® for Agar contains a penicillin binding protein that may interfere with penicillin and some related antibiotics.

Handling and Storage Instructions:

Oxyrase® for Agar 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. Oxyrase® for Agar can thaw and re-freeze five times without affecting its activity and performance.

2. Short Term Storage: Store the product at 2°C to 8°C for use within 30 days (a precipitant may form, but does not affect product).

   When stored in this manner, the product will completely remove oxygen in about 5 minutes at a pH of 8.4 (TCOR - time for complete oxygen removal), until the printed expiration date on the label. Test TCOR by using a Methylene Blue Assay (refer to Assay of Oxyrase Activity - pdf).

Thawing Oxyrase® for Agar:

A convenient way to thaw OA 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* swirl 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.

Instructions for Use: *(OxyDish™ - for surface plating [1:10 dilution])*

1. Prepare media using 10% less water than usual, and autoclave.
2. Bring the sterile media to 45°C - 48°C, and add OA (thawed to room temperature) by pouring / pipetting OA *down the side* the flask (use a *1:10 dilution* of Oxyrase for Agar into the agar medium).
3. Gently, swirl the OA into the media (***avoid foaming***), and begin distributing the OA media mixture into each OxyDish™.
4. Place an OxyDish™ in an upright position on a level surface, remove the dish top and place it to the side.
5. Deliver 22 - 24 mL of molten OA / medium mixture into each dish base (add more volume to plates for longer storage times).
6. When distributing the media, avoid delivering bubbles / foam into the agar, which may interfere with the dish seal (draw bubbles up with a pipette, if needed).
7. Allow agar to dry / solidify completely before replacing the dish top over the base *(it is very important that the plate surface solidifies evenly so that an effective seal may be formed. Plates are dried and so water does **not** condense within the dish during incubation or storage).*:
   a) Place open plate in laminar flow hood for less than 15 minutes to dry. ***or***
   b) Remove and invert lid from plate base. Invert base, and place base over lid at an angle in a clean incubator at 40°C - 45°C for 45 min or less to dry.
8. Once dry, slide base onto inverted lid to close plate and form seal (*do **not** press or squeeze the lid and base togther when closing plate, handle the plate from the sides - refer to OxyPlate™ Product Insert for more info)*.
9. Depending on the media type and its intended use, store poured plates in a closed position for up to 14 days, in a closed container at 2°C to 8°C.

(For use with standard petri dish - for pour plating [1:30 dilution])

1. Make media as usual, distribute into containers, and sterilize.
2. Melt sterile media and bring to 45°C - 48°C in a water bath.
3. Distribute 19 mL of media into sterile tube *(1 tube per plate)*. Add 1 mL of OA to each tube, and gently mix tube(s).
4. Add inoculum directly to each tube.
5. Gently mix tube, and pour contents of tube into a sterile petri dish. Allow agar mixture to solidify.
6. Add additional 5 mL of media (*from step 2*) as an overlay, let overlay cool to solidify. For ***strict*** anaerobes: use an overlay supplemented with OA.
7. Invert plates and incubate aerobically at the desired temperature.

or

2 b. Add OA to sterilized medium. Use mixture for both base and overlay.

User Quality Control:

The performance of agar supplemented with OA may be verified by using pure anaerobic cultures of microorganisms (ie. *B. fragilis* - ATCC 25285; *P. anaerobius* - ATCC 27337; *F. nucleatum* - ATCC 25586) with that agar. As an aerobic control, use a plate without OA or an OxyPlate incubated in the open position. Incubate both plates aerobically at 35°C to 37°C for 48 to 72 hours. The anaerobic plate will contain colonies; the aerobic plate will not.

Guarantee:

Oxyrase® for Agar 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 OA does not maintain a TCOR in approximately 5 minutes at a pH of 8.4 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 Agar label. Oxyrase, Inc. is available to answer any questions about this product and applications.