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Product Information

Oxyrase® Enzyme System for Agar

Catalog Number **SAE0058**
Storage Temperature –20 °C

Product Description

The Oxyrase® Enzyme System is a mixture of membrane bound monooxygenases and dioxygenases that removes dissolved oxygen from aqueous and semi-solid (e.g., agar) environments. This creates truly oxygen-free conditions for study of anoxia in cells and tissues.[1] For anaerobic studies, the Oxyrase Enzyme System has been used as an alternative means of establishing anaerobic environments without the use of CO_2.[2]

The Oxyrase Enzyme System for Agar (OA) is a formulation specifically designed for use with agar plates to produce anaerobic conditions. By removing oxygen from both within the agar and the confined head space above the agar surface, OA creates and maintains a standalone anaerobic environment without the need for bags, chambers, or jars. OA combines substrates and the Oxyrase Enzyme System in an agar medium with a pH range of 6.8–8.4. OA contains a penicillin-binding protein that may interfere with penicillin and some related antibiotics.

The following commercially prepared media have been used with OA:

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

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

OA may be used in conjunction with the OxyDish™ culture dish (Product No. SAE0060). The OxyDish culture dish is specially designed to create a seal that maintains anaerobiosis, after OA has been added to a poured agar medium.

Preparation Instructions

OA may be thawed in a refrigerator overnight.

If necessary, the product can be thawed by warming, but at no higher than 37 °C. Thawing at slightly elevated temperatures is recommended while ice is still present inside the container.

When thawed, keep OA chilled on ice until ready for use. To ensure uniform activity within a thawed sample, gently swirl the OA before use or distribution. Do not agitate vigorously, as this causes foaming and risks denaturing of protein in the product, which may result in loss of activity.

Do **not** autoclave the Oxyrase Enzyme System for Agar. Autoclaving will inactivate the enzymes.

Storage/Stability

Long-term storage: The recommended long-term storage temperature for the product is ≤ –20 °C, to maintain full activity. OA can be thawed and re-frozen 5× without affecting its activity and performance.

Short-term storage: Store the product at 2–8 °C for use within 30 days. A precipitant may form, which does not affect product performance.

When stored properly, OA will remain active until the assigned expiration date for the specific batch. It will completely remove oxygen, with a TCOR (Time for Complete Oxygen Removal) of about 5 minutes at pH 8.4. TCOR may be tested with a Methylene Blue assay.[3]

OA is a sterile-filtered product. OA must be handled aseptically to maintain sterility.

Procedures

A. OxyDish culture dish - for surface plating [1:10 dilution]

1. Prepare 90% of the total desired volume of medium. Autoclave the medium.
2. Bring the sterile medium to 45–48 °C. Add OA (thawed to room temperature) by pouring/pipetting OA down the side the flask, for a final dilution of 1:10 of OA into the agar medium.
3. Gently swirl the OA into the medium, taking care to avoid foaming. Begin distributing the OA medium mixture into each OxyDish culture dish.
4. Place an OxyDish culture dish in an upright position on a level surface. Remove the dish top and place it to the side.
5. Deliver 22–24 mL of liquid OA/medium mixture into each dish base. (More volume may be added to plates for longer storage times.)
6. When distributing the medium, avoid creating bubbles/foam in 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 forms. Plates are dried, so water does not condense within the dish during incubation or storage.
   a. Place the open plate in a laminar flow hood to dry (<15 minutes).
   b. Remove and invert lid from plate base. Invert base, and place base over lid at an angle in a clean incubator at 40–45 °C for 45 minutes or less to dry.
8. Once dry, slide the base onto the inverted lid to close the plate and form a seal. Do not press or squeeze the lid and base together when closing the plate, as it is best to handle the plate from the sides.
9. Depending on the medium type and its intended use, store poured plates in a closed position for up to 14 days at 2–8 °C.

B. Standard petri dish, for pour plating [1:30 dilution]

1. Prepare the standard medium. Distribute into containers, and sterilize.
2. Bring the sterile medium to 45–48 °C in a water bath. (Alternatively, add OA to sterilized medium. Use the mixture for both base and overlay.)
3. Distribute 19 mL of medium into each sterile tube, with 1 tube per plate. Add 1 mL of OA to each tube, and gently mix.
4. Add inoculum directly to each tube.
5. Gently mix tube, and pour contents of tube into a sterile petri dish. Allow the agar mixture to solidify.
6. Add additional 5 mL of medium (from Step 2) as an overlay. Allow the overlay to solidify. For strict anaerobes, use an overlay supplemented with OA.
7. Invert the plates. Incubate aerobically at the desired temperature.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

References

1. Joseph, J.K., *et al*., *J. Am. Soc. Nephrol*., **1(5)**, 837-840 (1990).
2. Spangler, S.K., and Appelbaum, P.C., *J. Clin. Microbiol.*, **31(2)**, 460-462 (1993).
3. https://www.oxyrase.com/upload/documents/assay_of_oxyrase_activity.pdf

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