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Product Information

Oxyrase® Enzyme System for Broth

Catalog Number **SAE0013**
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 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 Broth (OB), a formulation of the Oxyrase Enzyme System with substrates, is specifically designed for use with bacteriological broth media to produce anaerobic conditions.[3] By removing oxygen both from within the broth medium and the head space above the medium, OB creates and maintains a standalone anaerobic environment.

The following commercially prepared media have been used with OB:

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

OB has been used to cultivate such species as *Shigella sonnei*[4] and *Shewanella oneidensis*.[5] OB has also been utilized to generate anaerobic conditions for cultivation of such species as *Escherichia coli*,[6] *Staphylococcus aureus*,[6] and *Pseudomonas aeruginosa*.[7] The use of OB with aged media has been reported.[8]

OB is not a substitute for nutrients or gasses required for growth of anaerobic microorganisms. For reduced environments, lower than those achieved by complete oxygen removal, a chemical reducing agent is required. OB contains a penicillin-binding protein that may interfere with penicillin and some related antibiotics.

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.

Preparation Instructions

OB 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 OB chilled on ice until ready for use. To ensure uniform activity within a thawed sample, gently swirl the OB 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 Broth. 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. OB can be thawed and re-frozen 5× without affecting its activity and performance. OB may be aliquotted aseptically into individual sterile containers for future use, to minimize freeze-thaw cycles.

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

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

Procedure

1. Add 0.1 mL of OB aseptically to each 1.0 mL of prepared sterile broth medium. The broth medium should have an initial pH range of 6.8–8.4.
2. If using a disposable transfer pipette, adding one drop of OB per 1 mL of broth is sufficient.
3. Incubate at 35–37 °C. The broth should become completely anaerobic in ≤30 minutes.

Notes: OB may be used at temperatures and pH values outside of the suggested ranges. However, more OB and/or more time may be needed in such instances to achieve complete anaerobiosis.

The suspension should **not** be agitated vigorously nor aerated. Exposure of the liquid broth surface to air should be minimized.

References

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5. Brennan, C.M. *et al.*, *BMC Microbiology*, **13**, 33 (2013).
6. Bradford, P.A. *et al.*, *Antimicrob. Agents Chemother.*, **49(9)**, 3903-3909 (2005).
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