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OxyFluor™ Product Insert

OxyFluor™ (OF) is an enzyme formulation that has been designed for use in fluorescence microscopy to prevent photo-bleaching and photo-damage due to oxygen. OF, together with a substrate, reduces oxygen to water.

OF also continues to keep oxygen out of the specimen being observed over extended periods of time.

All Oxyrase products are made sterile and packaged in sterile sealed containers. Oxyrase, Inc. is only responsible for the sterility of sealed, unopened products.

Due to the enzyme's ability to support microbial growth, it is the user's responsibility to maintain the sterility of the product. Oxyrase enzymes must be handled and kept in controlled conditions to prevent the occurrence of contamination (refer to section "Handling and Storage Instructions" for further clarification)

Precautions:

OF is for In-Vitro Use only. To prevent bacteria from growing within OF, use and store OF at a steady temperature colder than 8°C and handle it aseptically (refer to handling and storage instructions, if needed).

Use care in handling and disposing of this product. A **Material Safety Data Sheet** is available on our website.

Product Performance:

OxyFluor™ requires a substrate to reduce oxygen. Add 10 to 20 mM of DL-Lactate and / or DL-succinate substrates to the medium containing OF.

One unit of OxyFluor™ activity will reduce dissolved oxygen (in 1 mL of air saturated 40 mM phosphate Buffer, pH 8.4, at 37°C) at the rate of 1% per second. The rate of oxygen removal increases with temperature; however, above 55°C, OF is rapidly inactivated. OF is active over a wide pH range of 6.8 to 8.4.

Limitations:

Some fluorescent dyes may not be affected by oxygen. OF would not be expected to protect those dyes.

Handling and Storage Instructions:

OxyFluor™ will arrive (in 5 mL vials) 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. OF can be thawed and re-frozen five times without affecting its activity and performance. To minimize the number of freezing and thawing events, aliquot the product. Freeze and thaw each aliquot container once and discard after use.
2. Short Term Storage: Store the product at 2°C to 8°C for use within 30 days (a precipitant may form at this temperature).

Some selected references using OxyFluor™ :

1. Watermann-Storer, C.M., J.W. Sanger, and J.M. Sanger. 1993. Dynamics of Organelles in the Mitotic Spindles of Living Cells: Membrane and Microtubule Interactions. *Cell motility and the Cytoskeleton*. **26:** 19-39.
2. Mikhailov, A.V. And Gundersen, G.G.. 1995. Centripetal Transport of Microtubules in Motile Cells. *Cells Motility and the Cytoskeleton*. **32:** 173-186.
3. Watermann-Storer, C.M., D.Y., Wber, K.L., Keech, J., Cheney, R.E., Salmon, E.D., and Bement, W.M. 2000. Microtubules Remodel Actomyosin Networks in Xenopus Egg Extracts Via Two Mechanism of F-actin Transport. *J Cell Biol.* **150:** 276-361.

When stored in this manner, the product will maintain its full activity to the printed expiration date on the label.

Thawing OxyFluor™:

A convenient way to thaw OxyFluor™ 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 bath until ready for use.

To ensure uniform activity within a thawed sample, *gently* mix 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.

In some cases, precipitate may be observed, but will not affect OxyFluor™ performance

Instructions for Use:

The exact volume of OxyFluor™ and substrates needed to reduce oxygen in a given system are determined by a number of parameters: including pH, temperature, kinds and amounts of substrates present, surface to depth ratio of the container, and head-space volume. Some experimentation may be necessary; a suggested use level is a 1:100 dilution of OF plus substrate (DL-Lactate) at 10 mM.

User Quality Control:

The confirmation of OxyFluor™ activity may be verified by referring to the **Assay of Activity** listed on COA.

Guarantee:

OxyFluor™ 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.

Oxyrase Inc. maintains sealed, unopened retains for each Oxyrase product sold. OF is formulated so that a minimum activity of 30 units per mL remains if it is kept under recommended storage and use conditions. Retains are kept to verify remaining activity at expiration.

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