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### AnaSelect®\* for Broth Product Insert

\* - US Patent #7,374,905

AnaSelect® for Broth is a sterile enzyme formulation that uses Oxyrase® Enzyme System to produce anaerobic conditions in broth.

AnaSelect® enhances the recovery of anaerobes from mixed cultures by preferentially limiting growth of aerobic and facultative microorganisms without affecting the growth of anaerobes.

#### Precautions:

AnaSelect® for Broth is for In-Vitro Use only. AnaSelect® for Broth is a filter sterilized product and must be handled aseptically to maintain sterility.

Some microorganisms produce significant amounts of gas during growth. To prevent build up of pressure due to gas production, caps can be loosely affixed to allow for the escape of gas and will not compromise the anaerobic conditions maintained in a medium containing AnaSelect® for Broth.

AnaSelect® for Broth contains a low concentration of sodium azide. Use care in handling and disposing this product. A **Material Safety Data Sheet** is available on our website.

#### Product Performance:

The formulation of AnaSelect® for Broth combines the Oxyrase® Enzyme System with substrates and inhibitors.

AnaSelect® for Broth enhances the isolation of anaerobes by preferentially limiting growth of aerobes and facultatives with little to no effect on anaerobes. It creates an anaerobic environment when added to bacteriological broth media.

When used as recommended within a bacteriological broth medium, AnaSelect® for Broth reduces the oxygen concentration to less than 10 ppB (parts per billion) within 30 minutes and maintains an anaerobic environment within the bacteriological broth medium for days.

#### Limitations:

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

*(Selectivity for a microbe is affected by the number of microbes in the specimen. Should facultatives be overwhelming the system, the specimen may need to be diluted further.)*

AnaSelect® for Broth is not a substitute for nutrients or gasses required for growth of anaerobic microorganisms. For reduced environments, a chemical reducing agent may be required.

#### Handling and Storage Instructions:

AnaSelect® for Broth 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.
2. AnaSelect® for Broth can thaw and re-freeze five times without affecting its activity and performance. In cases where the product will be used infrequently and / or in small amounts, aseptically aliquot the product into smaller, individual, sterile containers (refer to short term storage, if needed). To minimize the amount of freezing and thawing of the product, thaw each 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, but does not affect product performance).

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

#### Thawing AnaSelect® for Broth:

A convenient way to thaw AnaSelect® for Broth 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* 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 AnaSelect® for Broth performance.

#### Instructions for Use:

Aseptically, add 0.1 mL of AnaSelect® for Broth to each 1.0 mL of medium (if using a disposable transfer pipet, one drop of AnaSelect® for Broth per mL of medium is a sufficient measure). *Do not agitate vigorously or aerate the suspension*, and incubate at 35°C to 37°C (broth will become completely anaerobic in 30 minutes or less). Minimize the area of liquid surface exposed to air when possible.

#### User Quality Control:

The performance of AnaSelect® for Broth may be verified by inoculating broth media, with and without AnaSelect® for Broth, using anaerobe(s) (*B. fragilis* - ATCC 25285 and/or *F. nucleatum* - ATCC 25586), and a facultative anaerobe (*E. Coli* - ATCC 25922). Adjust a 24 hr. broth (sample) culture to a McFarland turbidity standard.

Make an anaerobic mixture by inoculating a 5.0 mL broth tube with 0.1 mL of the adjusted anaerobes sample. Make a facultative mixture by inoculating a 5.0 mL broth tube with 0.1 mL of a 1:100 dilution of the adjusted facultative sample.

Incubate all test tubes, without agitation, at 35°C to 37°C for 24 to 72 hours and compare.

The anaerobe will grow to heavy turbidity in the AnaSelect® for Broth sample tube, and not grow in the sample tube lacking AnaSelect® for Broth. Growth will start at the bottom of the tube.

The facultative will grow lightly, if at all, in the sample tube containing AnaSelect® for Broth. The facultative will grow to heavy turbidity in the control sample tube lacking AnaSelect® for Broth.

#### Guarantee:

AnaSelect® for Broth has a shelf-life of 9 months under recommended storage and use conditions. We guarantee a minimum of 3 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 AnaSelect® for Broth does not create an anaerobic environment 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 AnaSelect® for Broth label. Oxyrase, Inc. is available to answer any questions about this product and its applications.

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