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## OxyPRAS Plus® Bile Bacteroides Esculin / KV Laked Blood Agar (BBE / KVL) BiPlate Product Insert

OxyPRAS Plus® BBE (used for the isolation and cultivation of anaerobic bacteria within the *Bacteriodes sp.*) / KVL (use Kanamycin and Vancomycin) Agar BiPlate are used for the isolation and cultivation of anaerobic bacteria from a variety of clinical and non-clinical sources.

### Precautions:

BBE/KVL BiPlate are for In-Vitro Use only. BBE/KVL BiPlate are packaged aseptically and must be handled aseptically to maintain sterility during use. A **Material Safety Data Sheet** is available on our website.

### Product Performance:

Bile Bacteroides Esculin agar with hemin is an enriched medium, useful for the selection and presumptive identification of the *Bacteriodes fragilis* group (1,2,3). Differentiation of *Bacteriodes sp.* is based on the hydrolysis of esculin and presence of catalase. Hemin provides nutrients for some strains of the pigmented *Bacteriodes* group, and enhance the growth of some *Bacteriodes sp.* (4,8). Gentamicin provides inhibition of *E. coli*.

Tryptic Soy Agar (TSA) medium with blood, vitamin K<sub>1</sub>, and hemin is an enriched, general purpose medium useful for the isolation of anaerobes (1,2,3). Vitamin K<sub>1</sub> and hemin provide nutrients for some strains of the pigmented *Bacteriodes* group, and enhances the growth of some *Bacteriodes sp.* and some gram-positive, non-spore forming anaerobes (4,5). Vancomycin and Kanamycin aid in the selective isolation of gram negative anaerobes, especially *Bacteriodes* (6). Kanamycin inhibits protein synthesis in susceptible microorganisms and Vancomycin inhibits gram-positive bacteria by interfering with cell wall synthesis (8). Laked blood improves pigmentation of the *Bacteriodes melaninogenicus* - *Bacteriodes asaccharolyticus* group (7).

The Oxyrase® Enzyme System used in OxyPRAS Plus® plates provides a reduced medium before sterilization and maintains the medium in a reduce state for storage and during use. The Oxyrase® Enzyme System prevents the formation of undesirable oxidation products in these PRAS plates. Growth of anaerobes on OxyPRAS Plus® plates require anaerobic incubation in jars, bags, or chambers.

### Media Formulation (per liter)

#### BBE - Initial pH: 7.5 (+/- 0.3)

Beef Extract	11.0 g
Gelatin	34.5 g
Ox bile	2.0 g
Esculin	1.0 g
Ferric ammonium citrate	0.5 g
Agar	15.0 g
Hemin	10.0 mg
Vitamin K <sub>1</sub>	1.0 mg
Gentamicin	2.0 mL
Oxyrase® Enzyme System - proprietary	
Deionized water (made to final volume)	

#### KVL - Initial pH: 7.5 (+/- 0.3)

Enzymatic Digest of Casein	15.0 g
Enzym. Digest of Animal Tissue	5.0 g
Yeast extract	5.0 g
Sodium Chloride	5.0 g
L-Cysteine	0.6 g
Agar	15.0 g
Hemin	5.0 mg
Vitamin K <sub>1</sub>	1.0 mg
Vancomycin	1.1 mL
Kanamycin	2.3 mL
Laked Sheep Blood	50.0 mL
Oxyrase® Enzyme System - proprietary	
Deionized water (made to final volume)	

This formula is typical. Production lots may be adjusted, to offset variances in raw materials in order to meet performance criteria.

### Limitations:

Plates may only allow for growth of select organisms. Additional testing may be required to identify various colony types grown.

The Oxyrase® Enzyme System contains a penicillin binding protein that may interfere with penicillin and some related antibiotics.

### Handling and Storage Instructions:

OxyPRAS Plus® BBE/KVL BiPlate will arrive at room temperature. The following storage options are listed below:

1. **Long Term Storage:** Store the product at 2°C to 8°C (cold temperature - CT). The expiration date of plates stored at this temperature is 6 months from the date of manufacture.
2. **Short Term Storage:** Store the product at 20°C to 25°C (room temperature - RT). The expiration date of plates stored at this temperature is 3 months from the date of manufacture.

If extended shelf life is not important, store plates at room temperature. Refer to plate / label for actual expiration date.

### Instructions for Use:

Before use, allow BBE/KVL BiPlate to warm to room temperature. Remove the plate from the protective pouch. Examine plates for contamination, evidence of oxidation / discoloration (i.e. plate is brown, instead of clear red), and the expiration date.

After inoculation is complete, invert plates and incubate in an anaerobic bag, jar, or chamber to maintain an anaerobic environment. Use an appropriate indicator (such as OxyBlue™) inside the plate, bag, jar, or chamber to test / confirm anaerobiosis.

### User Quality Control:

Oxyrase, Inc. certifies that samples of each lot were quality control tested and performed acceptably according to Oxyrase, Inc.'s specifications, which include Clinical and Laboratory Standards Institute (M22-A3: Quality Assurance for Commercially Prepared Microbiological Culture Media). The following tests were confirmed:

Organism	ATCC #	Results
<i>B. fragilis</i>	25285	growth; black colonies in 2-3 days
<i>P. melaninogenica</i>	13124	No growth in 2-3 days (for BBE)
<i>E. coli</i>	25922	No growth in 2-3 days
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<i>B. fragilis</i>	25285	growth in 2-3 days
<i>C. perfringens</i>	13124	No growth in 2-3 days
<i>S. aureus</i>	25923	No growth in 2-3 days (for KVL)
<i>E. coli</i>	25922	growth inhibited in 2-3 days

### Guarantee:

We guarantee 30 days of shelf-life for RT and 90 days of shelf-life for CT from shipment date. If a longer shelf-life is needed, this should be arranged at the time your order is placed.

If OxyPRAS Plus® BBE/KVL BiPlate fail to arrive with at least a 4 week shelf life, are contaminated and/or oxidized, or fail when used 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 printed directly on the plate in question (a return of defective product may be required for further investigation and evaluation). Oxyrase, Inc. is available to answer any questions about this product and its applications.

1. J.F. MacFaddin. 1986. Media for Isolation, Cultivation, Identification, Maintenance of Medical Bacteria. *J. Basic Microbiology*. 26(4): 240.  
2. Phillips, E., and P. Nash. 1985. Culture Media. *Manual of Clinical Microbiology*. 4: 1051-1092.  
3. Sutter, V.L., Citron, D.M., Edelstein, M.A.C., and Finegold, S.M. 1985, 4<sup>th</sup> ed. **Wadsworth Anaerobic Bacteriology Manual**. Star Publishing Co., Belmont, CA. pgs.: 85-89.  
4. Allen, S.D., Siders, T.A., and Marler, J.M. 1985. Isolation and Examination of Anaerobic Bacteria. *Manual of Clinical Microbiology*. 4: 413-433.  
5. Estevez, E.G. 1984. Bacterial Plate Media: Review of Mechanisms of Action. *Lab. Med.* 15: 258-262.  
6. Finegold, S.M., and Citron, D.M. 1980. Gram-Negative, Non-Spore Forming Anaerobic Bacilli. *Manual of Clinical Microbiology*. 3: 431-439.  
7. Finegold, S.M., Miller, A.B., and Posnick, D.L. 1965. Further Studies on Selective Media for *Bacteriodes* and Other Anaerobes. *Ernaehrungsfor.* 10: 517-528.  
8. Gibbons, R.J., and MacDonald, J.B. 1960. Hemin and Vitamin K Compounds as Required Factors for the Cultivation of Certain Strains of *Bacteriodes melaninogenicus*. *J. Bacteriol.* 80:164-170.