

**Oxyrase, Inc.**

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OxyPRAS Plus[®] BBE / KVL Bi-Plate Product Insert

Intended Use

The OxyPRAS Plus[®] BBE/KVL Bi-Plate is a prepared, ready-to-use, divided plate carrying two selective media. The Bile Bacteroides Esculin (BBE) side is intended for the in vitro isolation, cultivation, and presumptive identification of anaerobic bacteria of the Bacteroides fragilis group. The Kanamycin Vancomycin Laked Blood (KVL) side is intended for the in vitro isolation and cultivation of gram-negative anaerobic bacteria from clinical and non-clinical materials. Growth of anaerobes on OxyPRAS Plus[®] plates requires anaerobic incubation in a bag, jar, or chamber.

Intended Users

For use by trained laboratory personnel in clinical, reference, research, industrial, pharmaceutical, and veterinary microbiology settings.

Summary and Explanation

Bile Bacteroides Esculin agar with hemin is an enriched medium useful for the selection and presumptive identification of the Bacteroides fragilis group (1,2,3). Tryptic Soy Agar (TSA) medium with laked blood, vitamin K1, and hemin is an enriched, general-purpose medium useful for the isolation of anaerobes (1,2,3). OxyPRAS Plus[®] plates are supplied Pre-Reduced Anaerobically Sterilized (PRAS) using the Oxyrase[®] Enzyme System.

Principles of the Procedure

BBE side: differentiation of Bacteroides spp. is based on the hydrolysis of esculin and on the presence of catalase. Hemin provides nutrients for some strains of the pigmented Bacteroides group and enhances the growth of some Bacteroides spp. (4,8). Gentamicin inhibits E. coli and most gram-negative facultative anaerobes. KVL side: vitamin K1 and hemin provide nutrients for some strains of the pigmented Bacteroides group and enhance the growth of some Bacteroides spp. and some gram-positive, non-spore-forming anaerobes (4,5). Vancomycin and kanamycin aid in the selective isolation of gram-negative anaerobes, especially Bacteroides spp. (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 Prevotella melaninogenica and the Prevotella spp. group (7). The Oxyrase[®] Enzyme System used in OxyPRAS Plus[®] plates provides a reduced medium before sterilization and maintains the medium in a reduced state for storage and during use. The Oxyrase[®] Enzyme System prevents the formation of undesirable oxidation products in these PRAS plates.

Reagents

BBE side — Initial pH: 7.5 (+0.2 / -0.3)

Component	Amount
Beef Extract	11.0 g
Enzymatic Digest of 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 K1	1.0 mg
Gentamicin	2.0 mL
Oxyrase [®] Enzyme System	proprietary
Deionized water	to final volume

KVL side — Initial pH: 7.5 (+/- 0.3)

Component	Amount
Enzymatic Digest of Casein	15.0 g
Enzymatic Digest of Animal Tissue	5.0 g
Yeast Extract	5.0 g
Sodium Chloride	5.0 g
L-Cysteine	0.58 g
Agar	15.0 g

Component	Amount
Hemin	5.0 mg
Vitamin K1	1.0 mg
Vancomycin	1.1 mL
Kanamycin	3.82 mL
Laked Sheep Blood	51.0 mL
Sodium Bisulfite	0.1 g
Oxyrase [®] Enzyme System	proprietary
Deionized water	to final volume

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

Warnings and Precautions

- For In Vitro Diagnostic Use.
- OxyPRAS Plus[®] BBE/KVL Bi-Plates are packaged aseptically and must be handled aseptically to maintain sterility during use.
- Specimens and used culture plates may contain infectious agents; observe standard (universal) biohazard precautions when handling.
- Dispose of used plates as biohazardous waste; sterilize before disposal in accordance with applicable regulations.
- This product contains material of animal origin. Source animals are documented to be free of BSE/TSE.
- A Safety Data Sheet (SDS) is available on our website.

Storage and Handling

- OxyPRAS Plus[®] BBE/KVL Bi-Plates will arrive at room temperature.
- Store the product at 2°C to 8°C.
- The expiration date is 4 months from the date of manufacture; refer to the plate/label for the actual expiration date.

Specimen Collection and Handling

Collect, transport, and process specimens using standard techniques for the recovery of anaerobic bacteria. Prompt processing and protection of specimens from oxygen exposure maximize anaerobe recovery. Consult appropriate references for specimen selection, collection, transport, and storage.

Materials Required But Not Provided

Anaerobic bag, jar, or chamber with an appropriate oxygen-removal system; an anaerobic indicator (such as OxyBlue[™]); inoculating loops or swabs; an incubator capable of 35–37°C; and additional media, reagents, and equipment for Gram stain, subculture, and identification, as required.

Directions for Use

1. Before use, allow OxyPRAS Plus[®] BBE/KVL Bi-Plates to warm to room temperature.
2. Remove the plate from the protective pouch.
3. Examine the plate for contamination, evidence of oxidation or discoloration (i.e., the KVL side is brown instead of clear red), and the expiration date.
4. Inoculate and streak the plate using standard technique.
5. After inoculation is complete, invert the plate and incubate at 35–37°C in an anaerobic bag, jar, or chamber to maintain an anaerobic environment.
6. Use an appropriate indicator (such as OxyBlue[™]) inside the anaerobic bag, jar, or chamber to test and confirm anaerobiosis.
7. Examine the plate for growth after 2 to 3 days of incubation.

Quality Control

Oxyrase, Inc. certifies that samples of each lot were quality control tested and performed acceptably to Oxyrase, Inc. specifications, which incorporate CLSI M22-A3. The following tests were confirmed:

BBE side

Organism	ATCC No.	Result
<i>B. fragilis</i>	25285	Growth; black colonies in 2-3 days
<i>P. melaninogenica</i>	25845	No growth in 2-3 days
<i>E. coli</i>	25922	No growth in 2-3 days

KVL side

Organism	ATCC No.	Result
<i>B. fragilis</i>	25285	Growth in 2-3 days
<i>C. perfringens</i>	13124	No growth in 2-3 days
<i>P. melaninogenica</i>	25845	Growth in 2-3 days
<i>S. aureus</i>	25923	No growth in 2-3 days
<i>E. coli</i>	25922	Growth inhibited in 2-3 days

Physical appearance: uninoculated BBE medium is pale yellow and uninoculated KVL medium is bright red; both with no surface imperfections. Each laboratory should establish its own quality control program in accordance with applicable accreditation and regulatory requirements. The organisms listed above are recommended for user quality control.

Results and Interpretation

Examine both sides of the plate for growth following incubation. On the BBE side, members of the *Bacteroides fragilis* group grow as colonies surrounded by a black to brown coloration of the medium, indicating esculin hydrolysis. On the KVL side, colony morphology and pigmentation provide presumptive information. Interpret results together with other laboratory findings and confirm isolates by additional identification procedures.

Limitations of the Procedure

- Plates may only allow for growth of select organisms. Additional testing may be required to identify the various colony types grown.
- The Oxyrase® Enzyme System contains a penicillin-binding protein that may interfere with penicillin and some related antibiotics.
- *B. vulgatus* and *B. ovatus* may not hydrolyze esculin.
- Other organisms that may grow on the BBE medium include *Fusobacterium mortiferum*, *F. varium*, gentamicin-resistant *Enterobacteriaceae*, enterococci, pseudomonads, staphylococci, and yeast.

Product Guarantee

Oxyrase, Inc. guarantees a 28-day shelf life from the shipment date; arrange a longer shelf life at the time the order is placed. If OxyPRAS Plus® BBE/KVL Bi-Plates arrive with less than a 28-day shelf life, are contaminated or oxidized, or fail when used as specified, Oxyrase, Inc. will refund the purchase price. To request a refund, contact Oxyrase, Inc. with the lot number printed on the plate; return of the product may be required for investigation. Oxyrase, Inc. welcomes questions regarding this product and its applications.

Bibliography

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5. Estevez, E.G. 1984. Bacterial Plate Media: Review of Mechanisms of Action. *Lab. Med.* 15: 258-262.
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8. Gibbons, R.J., and MacDonald, J.B. 1960. Hemin and Vitamin K Compounds as Required Factors for the Cultivation of Certain Strains of *Bacteroides melaninogenicus*. *J. Bacteriol.* 80: 164-170.
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Key to Symbols

Symbol	Meaning
IVD	For in vitro diagnostic use
REF	Catalog number
LOT	Batch code / lot number
⌘ (hourglass)	Use-by / expiration date
2–8°C	Temperature limit (store at 2°C to 8°C)
⏏ (factory)	Manufacturer
① (book)	Consult instructions for use

Products

Catalog No. (REF): B-BCD-KV, B-BCD-10, M-BBK-2, M-BKBP-3

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